Effects of long-term action of high temperature and high light on the activity and energy interaction of both photosystems in tomato plants

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Abstract

The acclimation to high light, elevated temperature, and combination of both factors was evaluated in tomato (*Solanum lycopersicum* cv. M82) by determination of photochemical activities of PSI and PSII and by analyzing 77 K fluorescence of isolated thylakoid membranes. Developed plants were exposed for six days to different combinations of temperature and light intensity followed by five days of a recovery period. Photochemical activities of both photosystems showed different sensitivity towards the heat treatment in dependence on light intensity. Elevated temperature exhibited more negative impact on PSII activity, while PSI was slightly stimulated. Analysis of 77 K fluorescence emission and excitation spectra showed alterations in the energy distribution between both photosystems indicating alterations in light-harvesting complexes. Light intensity affected the antenna complexes of both photosystems stronger than temperature. Our results demonstrated that simultaneous action of high light intensity and high temperature promoted the acclimation of tomato plants regarding the activity of both photosystems in thylakoid membranes.

Additional key words: antenna complexes; electron transport rate; fluorescence; pigment-protein complexes; spillover.

Introduction

The structure and organization of pigment-protein complexes within thylakoid membranes are dynamic and flexible, thus allowing mutual reorganization of complexes within thylakoid membranes in response to changing environment and facilitating plant acclimation (Anderson *et al.* 2012). Although PSI, PSII, and their antenna complexes and the main light-harvesting complex LHCII are separately located in grana and stroma regions of thylakoid membranes, they can rearrange under different light conditions in order to balance the energy distribution and to maintain the optimal photosynthetic activity. In respect to changing environmental conditions and duration of treatments, a response of plants includes short- and long-term acclimations (Tikkanen *et al.* 2012a, Wientjes *et al.* 2013). The dynamics of thylakoid membrane structure and abilities of its components to rearrange allow plants to balance the energy supply of both photosystems and to control the electron transport rate when the changes of irradiance lead to an imbalance in reducing efficiencies of PSI and PSII (Tikkanen *et al.* 2012b). It is well-known that PSII is more susceptible to high-light damage than PSI. In order to protect PSII from light-induced injury, plants have developed different strategies – to decrease excitation energy to PSII and to increase photochemical de-excitation of reaction centre's chlorophylls (Chl) by enhancing electron transport. In terms of short-time acclimation (seconds to minutes), higher plants regulate the photosynthetic process by decreasing energy supply of PSII by process of a phosphorylation-dephosphorylation cycle of LHCII and PSII core proteins. Under low light, parts of LHCII and PSII core undergo phosphorylation and migrate to stroma thylakoids, which is accompanied by a decrease of thylakoid stacking (Tikkanen *et al.* 2008, Tikkanen *et al.* 2012a). The changes in response to light intensity seem to concern mainly PSII and its antenna, while PSI and LHCI are not considerably affected (Ballotari *et al.* 2007).

While the effects of long-term (hours, days) treatment

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Abbreviations: BQ – 1,4-benzoquinone; Car – carotenoids; Chl – chlorophyll; DCPIP – 2,6-dichlorophenol-indophenol; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_m – maximal fluorescence yield of the dark-adapted state; F_v – variable fluorescence; HL – high light intensity; HT – high temperature; MES – 2-(N-morpholino)ethanesulfonic acid; NT – normal temperature; NL – normal light intensity; R – recovery; Tricine – N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine.
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Research article

Tomato plants acclimate better to elevated temperature and high light than to treatment with each factor separately

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ABSTRACT

The influence of two factors – high temperature and high light intensity, acting separately or simultaneously on the pigment composition, fluorescent characteristics, membrane integrity and synthesis of protective substances was investigated in tomato plants (*Solanum lycopersicum* cv. M 82). Moderate elevated temperatures (38/29 °C) were applied under optimum or high light intensity for 2 and 6 days and after that the plants are allowed to recover for 5 days at optimum conditions. Parameters of chlorophyll fluorescence were used to evaluate the alterations of photosystem I and photosystem II activity and malondialdehyde content was determined as a measure of stress-induced peroxidation of membrane lipids. The response of treated plants to high light and elevated temperature was estimated by analyzing the accumulation of anthocyanins. Both stress factors exhibit different impact on studied parameters – high light intensity influences considerably quantum yield of photosystem II and photochemical quenching that is compensated to some extent when applied at elevated temperature. High temperature reduces strongly non-photochemical quenching. Data obtained show that after two days under particular conditions, the plants tend to acclimate, but this is achieved after longer treatment – 6 days. During the recovery period the activity of photosystem I and the quantum yield of photosystem II recover almost completely, while the values of non-photochemical quenching although slightly higher, did not reach the levels at the beginning of treatment.

1. Introduction

The photosynthetic process is very sensitive to extreme temperatures, excess light intensity, UV-radiation, drought etc. The primary photosynthetic reactions which take place in thylakoid membranes of chloroplasts include light absorption, electron transport, oxygen evolution and energy transduction are known to be extremely susceptible to environmental stress conditions (Ashraf and Harris, 2013). Under changed environment in many regions on Earth plants suffer adverse effect of high temperature very often combined with water deficit, high light intensity and drought. To cope with these unfavorable environmental conditions the plants developed various protective strategies through activation of enzymatic defense and accumulation of protective compounds. The influence of each individual factor – high or low temperature, high light intensity, salinity or drought on photosynthesis is intensively studied for variety of plant species and photosynthetic organisms – algae and cyanobacteria in respect to morphological and physiological changes, alterations of primary photosynthetic reactions and of biochemical processes (Wahid et al., 2007 and references herein). The development at elevated temperature causes alterations in structural organization of thylakoids (Karim et al., 1997) and loss of grana stacking or their swelling and formation of antenna depleted PSII (Zhang et al., 2005). Plants differ in respect to their heat tolerance and various threshold temperatures has been reported from different groups as the correct estimation is difficult due to the additional influence of other environmental factors (Wahid et al., 2007). Short time high temperature treatment (2 h at 45 °C) affects the functional activity of photosynthetic apparatus in respect to thermotolerance of two tomato cultivars by different manner (Camejo et al., 2005), the

Abbreviations: chl, chlorophyll; F_o, ground fluorescence in the dark-adapted state; F_v, variable fluorescence; F'_o, ground fluorescence in the light-adapted state; F_m, maximal fluorescence in dark-adapted state; F'_m, maximal fluorescence in light-adapted state; MDA, malonedialdehyde; NPQ, nonphotochemical quenching; PSI (II), photosystem I (II); q_p, photochemical quenching; Φ_{PSII}, quantum yield of photosystem II; ROS, reactive oxygen species; TBA, thiobarbituric acid; TCA, trichloracetic acid.

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The Lack of Lutein Accelerates the Extent of Light-induced Bleaching of Photosynthetic Pigments in Thylakoid Membranes of *Arabidopsis thaliana*

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ABSTRACT

The high light-induced bleaching of photosynthetic pigments and the degradation of proteins of light-harvesting complexes of PSI and PSII were investigated in isolated thylakoid membranes of *Arabidopsis thaliana*, wt and lutein-deficient mutant lut2, with the aim of unraveling the role of lutein for the degree of bleaching and degradation. By the means of absorption spectroscopy and western blot analysis, we show that the lack of lutein leads to a higher extent of pigment photobleaching and protein degradation in mutant thylakoid membranes in comparison with wt. The highest extent of bleaching is suffered by chlorophyll a and carotenoids, while chlorophyll b is bleached in lut2 thylakoids during long periods at high illumination. The high light-induced degradation of Lhca1, Lhcb2 proteins and PsbS was followed and it is shown that Lhca1 is more damaged than Lhcb2. The degradation of analyzed proteins is more pronounced in lut2 mutant thylakoid membranes. The lack of lutein influences the high light-induced alterations in organization of pigment–protein complexes as revealed by 77 K fluorescence.

INTRODUCTION

The effective performance of the photosynthetic process in higher plants relies on absorbing light energy that is transformed into chemical energy used to fix atmospheric CO_2 into sugars and to evolve oxygen. The absorbance of light is performed by photosynthetic pigments, chlorophylls and carotenoids that are bound to proteins thus forming the main pigment–protein complexes of thylakoid membranes (1). The major light-harvesting complex (LHCII) is the most common pigment–protein complex that exists in a monomeric or trimeric organization *in vivo* and is responsible for absorbing sun light, delivering it to the reaction centers of photosystem 2 (PSII) and photosystem 1 (PSI) or for dissipation of the excessive light as harmless heat (2). In the structural model of trimeric LHCII complex, in the monomer, the photosynthetic pigments are presented by eight chlorophylls a, six chlorophylls b and four xanthophylls—two luteins, one neoxanthin and one1 violaxanthin with respective binding sites designated as L1, L2, N1 and V1 (3,4). The light-harvesting complex of PSI-LHCI is composed of four different membrane proteins (Lhca1-4) and coordinates chlorophyll a, chlorophyll b and the carotenoids—β-carotene, violaxantine and lutein (5,6). It has been shown that LHCI protects PSI core from high light attack (7,8). The different light-harvesting proteins, of LHCII and LHCI, demonstrate high sequence similarity but nevertheless they show considerable variations in respect to their three-dimensional organization.

In photosynthesis, carotenoids perform multiple functions of accessory light-harvesting pigments (9) playing an important role in the formation and stabilization of functional trimetric structure of light-harvesting complexes of cyanobacteria and higher plants (10) and for the assembly of the functional PSII (11), dissipate the excess absorbed light as heat nonphotochemical quenching (NPQ) (12), prevent peroxidation of thylakoid lipids via quenching of harmful triplets of chlorophyll and scavenging of stress-generated reactive oxygen species (13). The oxygen-free carotenoid, β-carotene, binds to the polypeptides of the reaction centers of PSI and PSII, while the oxygenated carotenoids, xanthophylls, are intrinsic component of light-harvesting complexes and their relative abundance is highly conserved suggesting that every particular species plays a specific role (14). It had been suggested that the mutants of *Arabidopsis* and *Chlamydomonas* that lack lutein and/or zeaxanthin are more photosensitive than the respective wt, but that lutein alone is unable to provide effective photoprotection (14).

Exposure of higher plants to high light intensity causes inactivation of PSII (damage of D1 protein) so-called photoinhibition and photobleaching of photosynthetic pigments and it is generally accepted that both processes are mediated by highly active oxygen radicals generated by excessive light. Formation of damaging singlet oxygen can occur either in the reaction center of PSII by transmitting excitation energy from triplet excited state of chlorophylls to molecular oxygen or in the LHCII as a result of intersystem crossing between singlet and triplet excited states of chlorophylls (15,16). Deactivation of singlet oxygen, generated in the reaction center of PSII is realized by β-carotene (17) while xanthophylls, lutein, zeaxanthin and violaxanthin, are involved in dissipation of excessive excitation energy as harmless heat in the light-harvesting complexes (15,16,18). Dissipation of excessive absorbed light, NPQ, especially its qE component, is dependent on the very precise macromolecular organization of LHCII (19) and any alteration in its macrostructure, found in carotenoid mutants, leads to reduction in qE (20).

Lutein is the most abundant xanthophyll in higher plants and has two binding sites to the light-harvesting proteins, L1 is common for all antenna proteins and L2 that is present in some of the antenna subcomplexes (16,21). The lack of lutein in lut2 mutant is compensated by increased amounts of xanthophylls from the β-carotene branch of pathway from lycopene (22). The binding of

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ALTERATIONS IN PRIMARY PHOTOSYNTHETIC REACTIONS OF *ARABIDOPSIS THALIANA*, wt and lut2, UNDER PHOTOINHIBITION in vivo

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(*Submitted by Academician D. Koumanov on March 4, 2015*)

Abstract

The role of lutein content, the most abundant xanthophyll in plants, for the primary photosynthetic reactions under conditions of high light illumination was investigated using a carotenoids mutant lut2 and in comparison with wild type *Arabidopsis thaliana*. The mutant lut2 does not contain lutein but its lack is compensated by an increase of the amount of β-carotene branch xanthophylls. The high light-induced alterations in the functioning of primary photosynthetic reactions of *Arabidopsis thaliana* were investigated. Photoinhibitory treatment was performed at room temperature on detached leaves. The lack of lutein leads to higher sensitivity of lut2 in comparison with wt towards photoinhibitory illumination expressed as: 1) the amount of photosynthetic pigments is lower after illumination due to degradation and/or bleaching; 2) with increase of time of illumination the maximum quantum yield of PSII declines faster in lut2 than in wt; 3) the energy delivery to PSI, caused by light-induced rearrangement of pigment-protein complexes within thylakoid membranes and/or a generation of fluorescence quencher in PSII is more pronounced in lut2; 4) analysis of flash oxygen yields show that photoinhibitory illumination significantly decreases the number of grana situated PSII reaction centres in lut2 and only moderately in wt. Results presented indicate that in vivo the lutein deficient mutant of *Arabidopsis thaliana* lut2 is more susceptible to photoinhibitory treatment at room temperature.

Key words: *Arabidopsis thaliana*, photoinhibition, carotenoids, 77K fluorescence, flash oxygen yields

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The intrinsically disordered protein LEA7 from *Arabidopsis thaliana* protects the isolated enzyme lactate dehydrogenase and enzymes in a soluble leaf proteome during freezing and drying

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ABSTRACT

The accumulation of Late Embryogenesis Abundant (LEA) proteins in plants is associated with tolerance against stresses such as freezing and desiccation. Two main functions have been attributed to LEA proteins: membrane stabilization and enzyme protection. We have hypothesized previously that LEA7 from *Arabidopsis thaliana* may stabilize membranes because it interacts with liposomes in the dry state. Here we show that LEA7, contrary to this expectation, did not stabilize liposomes during drying and rehydration. Instead, it partially preserved the activity of the enzyme lactate dehydrogenase (LDH) during drying and freezing. Fourier-transform infrared (FTIR) spectroscopy showed no evidence of aggregation of LDH in the dry or rehydrated state under conditions that lead to complete loss of activity. To approximate the complex influence of intracellular conditions on the protective effects of a LEA protein in a convenient in-vitro assay, we measured the activity of two Arabidopsis enzymes (glucose-6-P dehydrogenase and ADP-glucose pyrophosphorylase) in total soluble leaf protein extract (Arabidopsis soluble proteome, ASP) after drying and rehydration or freezing and thawing. LEA7 partially preserved the activity of both enzymes under these conditions, suggesting its role as an enzyme protectant in vivo. Further FTIR analyses indicated the partial reversibility of protein aggregation in the dry ASP during rehydration. Similarly, aggregation in the dry ASP was strongly reduced by LEA7. In addition, mixtures of LEA7 with sucrose or verbascose reduced aggregation more than the single additives, presumably through the effects of the protein on the H-bonding network of the sugar glasses.

1. Introduction

Anhydrobiosis or "life without water" is a phenomenon that has received much attention and although mechanisms responsible for cellular desiccation tolerance have been proposed (e.g. [1,2]), many functional aspects are still unresolved [3]. There is, however, widespread consensus that sugars and Late Embryogenesis Abundant (LEA) proteins can be major contributors to cell stability in the dry state, even in cells that naturally do not contain LEA proteins [4]. In addition, some organisms can achieve desiccation tolerance without the accumulation of sugars [5,6]. LEA proteins have been first identified in plant seeds during maturation, when the seeds attain desiccation tolerance [7], but were later also found in vegetative plant organs, in bacteria and various anhydrobiotic invertebrates [8,9].

The precise in vivo function of most LEA proteins remains unresolved, which may at least in part be due to their unstructured nature in solution, which has made functional predictions impossible. However, many of these proteins fold mainly into α-helices during drying [10]. Results from various in vitro assays suggest that some LEA proteins are involved in the stabilization of cellular constituents such as proteins and membranes, but other functions have also been proposed [8,9]. Only for the cold induced *Arabidopsis thaliana* LEA proteins COR15A and COR15B, membrane stabilization during freezing could be clearly established as their in vivo function, while enzyme stabilization could be excluded [11]. However, many in vitro investigations have shown that LEA proteins can effectively prevent inactivation of sensitive enzymes such as lactate dehydrogenase (LDH) during freezing or drying [9,12]. Under the appropriate drying conditions such enzymes aggregate, which may contribute to their inactivation. Aggregation can be prevented by many LEA proteins that are thought to function as "molecular shields" by preventing direct contact between enzyme molecules [13–15].

Abbreviations: AGPase, ADP-glucose-pyrophosphorylase; ASP, Arabidopsis soluble proteome; CF, carboxyfluoresceine; DTT, dithiothreitol; FTIR, Fourier-transform infrared; G6PDH, glucose-6-phosphate dehydrogenase; LDH, lactate dehydrogenase; LEA, late embryogenesis abundant; LG, β-lactoglobulin; PMSF, phenylmethylsulfonyl fluoride; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine; RNaseA, ribonuclease A.

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ORIGINAL ARTICLE

Functional characterization of selected LEA proteins from *Arabidopsis thaliana* in yeast and in vitro

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Abstract
Main conclusion **Expression of eight LEA genes enhanced desiccation tolerance in yeast, including two LEA_2 genes encoding atypical, stably folded proteins. The recombinant proteins showed enzyme, but not membrane protection during drying.**

To screen for possible functions of late embryogenesis abundant (LEA) proteins in cellular stress tolerance, 15 candidate genes from six *Arabidopsis thaliana* LEA protein families were expressed in *Saccharomyces cerevisiae* as a genetically amenable eukaryotic model organism. Desiccation stress experiments showed that eight of the 15 LEA proteins significantly enhanced yeast survival. While none of the proteins belonging to the LEA_1, LEA_5 or AtM families provided protection to yeast cells, two of three LEA_2 proteins, all three LEA_4 proteins and three of four dehydrins were effective. However, no significantly enhanced tolerance toward freezing, salt, osmotic or oxidative stress was observed. While most LEA proteins are highly hydrophilic and intrinsically disordered, LEA_2 proteins are "atypical", since they are more hydrophobic and possess a stable folded structure in solution. Because nothing was known about the functional properties of LEA_2 proteins, we expressed the three Arabidopsis proteins LEA1, LEA26 and LEA27 in *Escherichia coli*. The bacteria expressed all three proteins in inclusion bodies from which they could be purified and refolded. Correct folding was ascertained by Fourier transform Infrared (FTIR) spectroscopy. None of the proteins was able to stabilize liposomes during freezing or drying, but they were all able to protect the enzyme lactate dehydrogenase (LDH) from inactivation during freezing. Significantly, only LEA1 and LEA27, which also protected yeast cells during drying, were able to stabilize LDH during desiccation and subsequent rehydration.

Keywords *Arabidopsis* · Desiccation · Enzyme · Freezing · LEA · Yeast

Abbreviations

BSA	Bovine serum albumin
CFU	Colony-forming units
CD	Circular dichroism
CF	Carboxyfluorescein
EPC	Egg phosphatidylcholine
FTIR	Fourier transform infrared
GFP	Green fluorescent protein
GOI	Gene of interest
GRAVY	Grand average of hydropathy
IDP	Intrinsically disordered protein
LDH	Lactate dehydrogenase
LEA	Late embryogenesis abundant
POPC	1-Palmitoyl-2-oleoyl-phosphatidylcholine
RH	Relative humidity
TEN	Tes, EDTA, NaCl buffer

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UV-B induced alteration of oxygen evolving reactions in pea thylakoid membranes as affected by scavengers of reactive oxygen species

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Abstract

The effect of UV-B irradiation at temperatures of 22 and 4 °C on flash induced oxygen yields, photochemical activity, and energy transfer in pea thylakoid membranes in the absence and presence of scavengers of reactive oxygen species (ROS) was studied. Three different scavengers were used: dimethyl sulfoxide (DMSO), histidine (His), and *n*-propyl gallate (nPG). As result of the UV-B treatment of isolated membranes, the flash oxygen yields were considerably affected – the amplitudes decreased and the oscillation pattern was lost. The analysis of the flash oxygen yields and initial oxygen burst showed alterations of a number of oxygen evolving centers in the S_0 state as well as changes of decay kinetics of the oxygen burst under continuous irradiation. ROS scavengers exhibited more or less expressed protective effects, nPG being the most effective against UV-B induced damages of the flash oxygen yields. At both the temperatures, photosystem II (PS II) mediated electron transport was more sensitive to the UV-B treatment in comparison with photosystem I (PS I). The analysis of 77 K fluorescence spectra showed that the fluorescence ratio F735/F685 increased by the UV-B treatment probably due to a redistribution of excitation energy between both photosystems most likely caused by partial unstacking and due to a decrease of PS II fluorescence resulting from reaction center-type quenching. The nPG was the most powerful scavenger which protected the oxygen evolution capacity of PS II in the absence and presence of an exogenous electron acceptor to the highest extent.

Additional key words: 77 K fluorescence, DMSO, flash oxygen yields, histidine, initial oxygen burst, *n*-propyl gallate, oxygen evolution, *Pisum sativum*, ROS.

Introduction

UV-B radiation (280 - 320 nm) is known to be very harmful to all biological organisms and can induce damages of their structure and function. Photosynthesizing organisms and especially higher plants are particularly sensitive to increased UV-B. Plant response to increased UV-B involves an activation of a defense system by stimulation ROS scavenging enzyme activities and accumulation of UV-B absorbing compounds (Fedina *et al*. 2007, 2010). The UV-B induces structural alterations in chloroplast grana and stroma structures and in membrane lipids (Hollosy 2002). One of the most susceptible parts is the photosynthetic apparatus and specifically the pigment-protein complexes of photosystem (PS) II. UV-B induces the impairment of PS II mediated electron transport and degradation of D1 protein (Friso *et al*. 1994, Strid *et al*. 1994, Hideg and Vass 1996). UV-B exposure of *Spirulina platensis* results in alterations of fluorescence emission of pigment-protein complexes of thylakoids and a considerable decrease of PS II activity, whereas almost no effect on PS I (Rajagopal *et al*. 2000). *In vitro* studies have shown that the most sensitive component of the PS II electron transport is the water-oxidizing complex and particularly the manganese cluster (Renger *et al*. 1989, Hideg *et al*. 1993). Besides the manganese cluster, Renger *et al*. (1989) also suggested Q_A-Q_B-apoproteins as target, while later on, Szilard *et al*. (2007) showed a correlation between UV-B induced damages and the oxidation state of the water-splitting complex. Barbato *et al*. (1995) reported that degradation of D1 protein under UV-B irradiation occurs in the presence of the functional manganese cluster on the donor side. It is believed that

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Abbreviations: BQ - 1,4 benzoquinone; Chl - chlorophyll; DCMU - 3-(3,4dichlorophenyl)-1,1-dimethyl urea; DCPIP - 2,6-dichlorophenol-indophenol; DMSO - dimethyl sulfoxide; His - histidine; MDA - malondialdehyde; MES - 2-[N-morpholino] ethanesulfonic acid; nPG - *n*-propyl gallate; PS - photosystem; ROS - reactive oxygen species; S_i - redox states of water oxidizing complex; Tricine - *N*-[tris(hydroxymethyl) methyl] glycine; Tris - 2-amino-2-hydroxymethyl-propane-1,3-diol; UVB_{BE} - biological effectiveness of UV-B radiation.

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UV-B-INDUCED ALTERATIONS IN PRIMARY PHOTOSYNTHETIC REACTIONS IN ISOLATED THYLAKOID MEMBRANES OF *ARABIDOPSIS THALIANA* (C24)

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(*Submitted by Academician K. Koumanov on June 19, 2013*)

Abstract

The effect of UV-B irradiation on primary photosynthetic reactions of isolated thylakoid membranes from *Arabidopsis thaliana* (C24) has been investigated at low and room temperature. The energy distribution between the main pigment-protein complexes and oxygen evolving activity of PSII centres were strongly affected by UV-B treatment when irradiation was performed at room than at low temperature. The energy interaction in the pigment protein complex of PSII-core antenna is more affected by UV-B treatment at both temperatures than the energy distribution between both photosystems, as revealed by fluorescence rations of 77K spectra. The grana situated PSII centres are more sensitive to UV-B irradiation in respect to flash oxygen yields than PSII centres, situated in the stroma thylakoids.

Key words: 77K fluorescence, *Arabidopsis thaliana*, oxygen production reactions, UV-B radiation

Introduction. The sun light reaching the surface of the Earth can be divided into two spectral regions – the UV range (200–400 nm) and the visible light (400–700 nm) which is the photosynthetically active radiation. Although the UV radiation comprises only a small part (7%) of the sun electromagnetic radiation, it has a negative impact on living organisms. Absorbed UV-B radiation (280–320 nm) can affect all biological macromolecules as DNA, proteins, lipids [1].

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RESEARCH ARTICLE Open Access

Interactions of the amphiphiles arbutin and tryptophan with phosphatidylcholine and phosphatidylethanolamine bilayers in the dry state

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Abstract

Background: Water is essential for life, but some organisms can survive complete desiccation, while many more survive partial dehydration during drying or freezing. The function of some protective molecules, such as sugars, has been extensively studied, but much less is known about the effects of amphiphiles such as flavonoids and other aromatic compounds. Amphiphiles may be largely soluble under fully hydrated conditions, but will partition into membranes upon removal of water. Little is known about the effects of amphiphiles on membrane stability and how amphiphile structure and function are related. Here, we have used two of the most intensively studied amphiphiles, tryptophan (Trp) and arbutin (Arb), along with their isolated hydrophilic moieties glycine (Gly) and glucose (Glc) to better understand structure-function relationships in amphiphile-membrane interactions in the dry state.

Results: Fourier-transform infrared (FTIR) spectroscopy was used to measure gel-to-liquid crystalline phase transition temperatures (T_m) of liposomes formed from phosphatidylcholine and phosphatidylethanolamine in the presence of the different additives. In anhydrous samples, both Glc and Arb strongly depressed T_m, independent of lipid composition, while Gly had no measurable effect. Trp, on the other hand, either depressed or increased T_m, depending on lipid composition. We found no evidence for strong interactions of any of the compounds with the lipid carbonyl or choline groups, while all additives except Gly seemed to interact with the phosphate groups. In the case of Arb and Glc, this also had a strong effect on the sugar OH vibrations in the FTIR spectra. In addition, vibrations from the hydrophobic indole and phenol moieties of Trp and Arb, respectively, provided evidence for interactions with the lipid bilayers.

Conclusions: The two amphiphiles Arb and Trp interact differently with dry bilayers. The interactions of Arb are dominated by contributions of the Glc moiety, while the indole governs the effects of Trp. In addition, only Trp-membrane interactions showed a strong influence of lipid composition. Further investigations, using the large structural diversity of plant amphiphiles will help to understand how their structure determines the interaction with membranes and how that influences their biological functions, for example under freezing or dehydration conditions.

Keywords: Amphiphiles, Arbutin, Desiccation, Fourier-transform infrared spectroscopy, Lipid phase transition, Model membranes, Tryptophan

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SENSITIVITY OF TWO ECOTYPES OF *ARABIDOPSIS THALIANA* (Cvi AND Te) TOWARDS UV-B IRRADIATION

Maya Velitchkova, Daniela Stanoeva, Antoaneta V. Popova

(*Submitted by Academician K. Koumanov on February 12, 2013*)

Abstract

The susceptibility of *Arabidopsis thaliana* towards the detrimental effect of UV-B irradiation was investigated using two ecotypes, Cvi and Te. The effect of UV-B treatment on primary photosynthetic reactions – energy interaction between the main pigment-protein complexes and oxygen evolution, was evaluated at low (4 °C) and at room (22 °C) temperature. UV-B-induced alterations of investigated photosynthetic reactions are better expressed at 22 °C than at 4 °C for Cvi. For Te ecotype the energy interaction was suppressed to higher extent at 22 °C, while oxygen evolving activity was affected similarly at both temperatures. At low and room temperature, the energy interaction in the complex PSII-core antenna is affected stronger by UV-B treatment than the energy distribution between both photosystems, as revealed by fluorescence ratios of 77 K spectra. The results presented indicate that the *Arabidopsis thaliana* ecotype Cvi (Cape Verde Islands) is less affected by UV-B irradiation in respect to the investigated primary photosynthetic reactions than the ecotype Te (Finland).

Key words: ecotypes of *Arabidopsis thaliana*, UV-B radiation, 77 K fluorescence, flash oxygen yields

Introduction. During their development, photosynthetic organisms – green algae, cyanobacteria, higher plants, are subjected to different environmental stress conditions as high and low temperature, high light intensity, salinity, dehydration, UV irradiation that influence negatively their growth and productivity. The sun electromagnetic radiation in the region of 200–400 nm (UV light) represents only seven per cent of the whole spectrum. The ozone layer in the stratosphere absorbs all of the solar UV-C (< 280 nm) and part of UV-B (280–320 nm) radiation, but

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EFFECTS OF 24-EPIBRASSINOLIDE PRE-TREATMENT ON UV-B-INDUCED CHANGES IN THE PIGMENT CONTENT OF PEA LEAVES

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(*Submitted by Academician K. Koumanov on December 15, 2012*)

Abstract

In the present work, the effects of 24-epibrassinolide (EBR) on the UV-B-induced changes in the pigment content of pea leaves were studied. Control (non-EBR-treated) and EBR-treated plants were irradiated with UV-B for 3 h and pigment analysis was performed after 24 and 48 h. The results show that EBR spraying of plants 48 h prior to UV-B exposure alleviates its detrimental effect on chlorophyll *a* and *b* (Chl *a* and Chl *b*) content in comparison with control pea leaves. An increase in carotenoids (Car) and UV-B absorbing compounds was also observed at low dose of UV-B radiation. For the first time, it is shown that UV-B damage effect on control leaves is accompanied by a significant (more than 50%) increase in their pheophytin *a* (Pheo *a*) content 48 h after the UV-B exposure and that the EBR pre-treatment prevents the increase of Pheo *a* content in UV-B irradiated leaves. In addition, it is demonstrated that EBR application modifies UV-B-induced alterations of energy distribution between the main pigment-protein complexes in pea thylakoid membranes.

Key words: brassinosteroids, UV-B radiation, photosynthetic pigments, pheophytin *a*, UV-B absorbing compounds, 77 K fluorescence

Introduction. Brassinosteroids are steroidal plant hormones involved in a wide range of developmental processes as well as in plant responses to environmental stresses via activation of different protective mechanisms (for review see [1]). To date the mechanisms by which these compounds are involved in plant stress responses are not clear. It has been shown that the EBR application exerts a direct role in the regulation of plant photosynthesis [2]. Several studies

CHAPTER EIGHT

Carotenoid–Lipid Interactions

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Contents

Abstract

Carotenoids are the most widely spread pigments, both in the plant and animal kingdom, that perform numerous important physiological functions. In photosynthetic organisms, carotenoids are involved in the processes of light harvesting, photoprotection, and electron transfer, serve as scavengers of reactive oxygen species, and perform a structural role in photosynthetic membranes. In animals and humans, carotenoids perform a number of functions, of which the best established are their provitamin A activity and antioxidant properties. A great number of investigations devoted to understanding the basic principles of carotenoid impact on membrane organization,

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dynamics, physical and mechanical properties, as also their antioxidant activities, have been performed using model membranes. In this review, the structural and spectral characteristics of carotenoids are discussed, as well as the most frequently applied methods of investigation, with special attention to pigment–lipid interactions. The carotenoid–lipid interactions are characterized in several aspects: degree of integration, orientation and conformation of the pigment molecules into lipid membranes, and the impact of carotenoids on the thermotropic phase behavior and dynamics of the lipid bilayer.

1. INTRODUCTION

Carotenoids are the most widely spread and important group of pigments, represented by more than 750 structurally related compounds. They are found in all photosynthetic organisms, green algae, cyanobacteria, and higher plants, as well as in the animal kingdom. Carotenoids are responsible for most of the yellow and red colors of fruits, flowers, birds, insects, and marine invertebrates [1]. In higher plants, carotenoids are synthesized and localized in the plastids and are biosynthetically related with other isoprenoids as tocopherols and chlorophylls [2]. Carotenoids are arranged in two big groups—apolar carotenes with main representatives—β-carotene and lycopene and such containing oxygen and named xanthophylls (zeaxanthin, lutein).

In photosynthetic organisms, carotenoids perform multiple important physiological functions such as light harvesting, photoprotection, electron transfer, scavengining, and structural [3,4 and references therein]. Carotenoids play the role of accessory photosynthetic pigments, covering efficiently the blue-green region of the electromagnetic spectrum that is not absorbed by chlorophylls [5]. The absorbed light is transferred to chlorophylls via singlet–singlet mechanism, thus broadening the spectral region of utilized sun light. This process is documented in detail in studies on reaction centers and light-harvesting complexes (LHCs) of photosynthetic bacteria [6]. The effectiveness of energy transfer from carotenoids to chlorophylls is dependent on a number of factors such as the type of the respective carotenoid and the stereochemical structure of its polyene chain, and the distance between the pigments and their organization [7]. For lutein, the most frequently found xanthophyll in the LHCs of photosynthetic membranes, the energy transfer is with 100% effectiveness, determined by the dense packing of the photosynthetic pigments [8].

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Influence of drying on the secondary structure of intrinsically disordered and globular proteins

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ABSTRACT

Circular dichroism (CD) spectroscopy of five Arabidopsis late embryogenesis abundant (LEA) proteins constituting the plant specific families LEA_5 and LEA_6 showed that they are intrinsically disordered in solution and partially fold during drying. Structural predictions were comparable to these results for hydrated LEA_6, but not for LEA_5 proteins. FTIR spectroscopy showed that verbascose, but not sucrose, strongly affected the structure of the dry proteins. The four investigated globular proteins were only mildly affected by drying in the absence, but strongly in the presence of sugars. These data highlight the larger structural flexibility of disordered compared to globular proteins and the impact of sugars on the structure of both disordered and globular proteins during drying.

1. Introduction

LEA proteins have been found in plants, invertebrates and bacteria and have been associated with cellular dehydration tolerance. Most LEA proteins have been predicted to be IDPs, i.e. to have no stable structure under physiological, fully hydrated conditions [1,2]. The genome of *Arabidopsis thaliana* contains 51 genes encoding LEA proteins [3]. Three of these proteins have been shown to be unstructured in solution, while they fold into α-helices upon drying [4,5], similar to other LEA proteins [1]. Here we report the characterization of the secondary structure of five additional Arabidopsis LEA proteins forming the two small plant specific families LEA_5 (LEA20 and LEA35, Pfam PF00477) and LEA_6 (LEA15, LEA16 and LEA17, Pfam PF10714).

The first LEA_6 gene (*PvLEA18*) was characterized as drought induced in the common bean (*Phaseolus vulgaris*) [6]. Apart from the fact that the protein does not stabilize enzymes during desiccation in vitro [7] it is functionally and structurally uncharacterized. Of the three homologous genes in Arabidopsis, *LEA15* is expressed specifically in seeds and strongly induced by the phytohormone ABA. *LEA16* is induced under salt stress in leaves, while *LEA17* is only highly expressed in flower buds, but not regulated by any stress treatments [3].

The LEA_5 group in Arabidopsis comprises only two genes. *LEA20* (*EM6*) is expressed constitutively in all investigated tissues, but is induced in leaves under salt stress and after ABA treatment, while *LEA35* (*EM1*) is seed specific [3]. LEA_5 proteins from other plant species are unstructured in solution [8–11] and protect the enzyme lactate dehydrogenase against inactivation during desiccation [12].

Due to the lack of a stable secondary structure, IDPs are generally more flexible than globular proteins [13], but the structure of globular proteins can also be influenced by drying and sugars can stabilize the structure and function of globular proteins [14,15]. The influence of sugars on the structure of dry LEA proteins has only rarely been reported. To investigate the differences in the structural responses of globular proteins and IDPs to drying in the absence or presence of sugars, we used CD and FTIR spectroscopy to compare the secondary structures of the five Arabidopsis LEA proteins of the LEA_5 and LEA_6 families with those of four globular proteins known to contain different amounts of α-helices and β-sheets.

Abbreviations: ABA, abscisic acid; BSA, bovine serum albumin; CD, circular dichroism; FTIR, Fourier-transform infrared; GRAVY, grand average of hydropathy; IDP, intrinsically disordered protein; LEA, late embryogenesis abundant; LG, β-lactoglobulin; RFO, raffinose-family oligosaccharide; RNaseA, ribonuclease A; Suc, sucrose; Thau, thaumatin; Ver, verbascose.

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Penetration of Lysozyme and Cytochrome C in Lipid Bilayer: Fluorescent Study

Ivaylo Zlatanov · Antoaneta Popova

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Abstract Lysozyme and cytochrome c (CytC) are well-investigated proteins. Their specific interactions with lipid membranes, however, keep surprising secrets. Lysozyme destroys bacterial membrane; CytC binds hydrophobically to alkyl chains of the membrane lipid tails, indicating that both proteins are able to interact directly with the inner membrane components, especially with the fatty acyl chains of membrane lipids. The degrees of integration, depth of localization in the hydrophobic interior of different types of model membranes, and the type of interaction of lysozyme and CytC with surrounding lipids were investigated by fluorescent spectroscopy. Three different fluorescent markers, located at approximately 6.5, 9, and 18 Å into the lipid bilayer, were used. In addition, liposomes were designed as electrically neutral or positively or negatively charged to unravel the importance of the net electrical charge for lipid/protein interaction. CytC penetrates deeper into the lipid bilayer in comparison with lysozyme, and data are discussed in the terms of Stern–Volmer quenching of fluorescence.

Keywords Cytochrome c · Fluorescent probes · Liposomes · Lysozyme · Protein penetration · Quenching of fluorescence

Liposomes are widely used as models for investigation of biological membranes (New 1999). In pharmacology liposomes are often applied as drug targets and drug transporters (Tashjian et al. 2008). Among the various types of liposomes, unilamellar bilayer vesicles are preferred as model systems because they have flexible, cell-like surfaces and are suitable for numerous applications (Stamouli et al. 2003; Richter et al. 2007).

Our first step was to create a handle procedure for preparation of stable liposomes with controlled fluorescent and electrical properties. The procedure was based on the work of Hub et al. (1982). Small amounts of fluorescent lipids were added to the initial lipid mixture for liposome formation. Obtained vesicles were visible by fluorescent microscope. Using the technique of fluorescent spectroscopy it is possible to observe fine changes in the membrane surrounding of the fluorophores under the influence of different physical factors or chemical agents.

An important factor for the physical and chemical properties of the liposomes is their net electrical charge. In order to obtain different degrees of positive or negative electrical charge, we added different amounts of positively or negatively charged phospholipids or fatty acids before the liposome formation.

The next step was to obtain stable liposomes. For this purpose an apparatus for extrusion of the vesicles was constructed on the basis of the original ideas of Subbarao et al. (1991) and MacDonald et al. (1991). A detailed description of the apparatus is given below.

Experiments were carried out with lysozyme and CytC to investigate their interactions with the model membranes. Lysozyme, a cationic protein with a small molecular weight of 14.6 kDa, has been completely characterized in respect to its primary, secondary, and tertiary structure (Blake et al. 1965). It hydrolyzes the glycosidic linkage between *N*-acetylmuramic acid and *N*-acetylglucosamine in bacterial cell wall and destroys the structural integrity of the membrane (Salton 1957). Lysozyme is able also to

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CHAPTER TEN

β-Carotene–Lipid Interactions in Liposomes with Different Lipid Composition

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Contents

Abstract

Carotenoids perform light harvesting, photoprotection, electron transfer, and structural role in photosynthetic membranes. To unravel the β-carotene contribution to the stability of membranes, liposomes with different lipid composition (resembling the photosynthetic membranes, containing mainly galactolipids with a high degree of unsaturation, and egg phosphatidylcholine) were used. The aim was to gain insight into the mechanism of β-carotene–lipid interactions with a special focus on the fluidity of the bilayer. Data from absorption, pyrene fluorescence, and resonance Raman spectroscopy revealed that the degree of lipids' unsaturation regulates the penetration of β-carotene molecules into the membrane, thus modifying the lipid–pigment interactions.

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RESEARCH ARTICLE Open Access

Thermotropic phase behavior and headgroup interactions of the nonbilayer lipids phosphatidylethanolamine and monogalactosyldiacylglycerol in the dry state

Antoaneta V Popova[1,2] and Dirk K Hincha[1*]

Abstract

Background: Although biological membranes are organized as lipid bilayers, they contain a substantial fraction of lipids that have a strong tendency to adopt a nonlamellar, most often inverted hexagonal (H_{II}) phase. The polymorphic phase behavior of such nonbilayer lipids has been studied previously with a variety of methods in the fully hydrated state or at different degrees of dehydration. Here, we present a study of the thermotropic phase behavior of the nonbilayer lipids egg phosphatidylethanolamine (EPE) and monogalactosyldiacylglycerol (MGDG) with a focus on interactions between the lipid molecules in the interfacial and headgroup regions.

Results: Liposomes were investigated in the dry state by Fourier-transform Infrared (FTIR) spectroscopy and Differential Scanning Calorimetry (DSC). Dry EPE showed a gel to liquid-crystalline phase transition below 0°C and a liquid-crystalline to H_{II} transition at 100°C. MGDG, on the other hand, was in the liquid-crystalline phase down to -30°C and showed a nonbilayer transition at about 85°C. Mixtures (1:1 by mass) with two different phosphatidylcholines (PC) formed bilayers with no evidence for nonbilayer transitions up to 120°C. FTIR spectroscopy revealed complex interactions between the nonbilayer lipids and PC. Strong H-bonding interactions occurred between the sugar headgroup of MGDG and the phosphate, carbonyl and choline groups of PC. Similarly, the ethanolamine moiety of EPE was H-bonded to the carbonyl and choline groups of PC and probably interacted through charge pairing with the phosphate group.

Conclusions: This study provides a comprehensive characterization of dry membranes containing the two most important nonbilayer lipids (PE and MGDG) in living cells. These data will be of particular relevance for the analysis of interactions between membranes and low molecular weight solutes or soluble proteins that are presumably involved in cellular protection during anhydrobiosis.

Background

Biological membranes are composed of a wide range of lipids with different physicochemical properties. While the major components are usually bilayer forming lipids, most cellular membranes also contain a significant complement of lipids that can adopt nonbilayer structures such as an inverted hexagonal phase (H_{II}) [1,2]. Some biologically important functions such as membrane fusion [3,4] are related to formation of H_{II} structures. Therefore, the formation of H_{II} has been the subject of considerable interest and different molecular models have been developed that reflect the physical and chemical properties governing the transition from lamellar to H_{II} phase [5-7]. The transition dependends on different internal (molecular shape, degree of unsaturation and length of the fatty acyl chains, charge and hydration of the headgroup) and external factors (water content, temperature, pH, ionic strength, solutes) [6-10]. In addition, H-bonding between lipid molecules also contributes to their phase behavior [11].

Cellular membranes are primary sites of damage during environmental stresses such as freezing or drying. In

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Structural transitions in the intrinsically disordered plant dehydration stress protein LEA7 upon drying are modulated by the presence of membranes

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ABSTRACT

Dehydration stress-related late embryogenesis abundant (LEA) proteins have been found in plants, invertebrates and bacteria. Most LEA proteins are unstructured in solution, but some fold into amphipathic α-helices during drying. The Pfam LEA_4 (Group 3) protein LEA7 from the higher plant *Arabidopsis thaliana* was predicted to be 87% α-helical, while CD spectroscopy showed it to be largely unstructured in solution and only 35% α-helical in the dry state. However, the dry protein contained 15% β-sheets. FTIR spectroscopy revealed the β-sheets to be largely due to aggregation. β-Sheet content was reduced and α-helix content increased when LEA7 was dried in the presence of liposomes with secondary structure apparently influenced by lipid composition. Secondary structure was also affected by the presence of membranes in the fully hydrated state. A temperature-induced increase in the flexibility of the dry protein was also only observed in the presence of membranes. Functional interactions of LEA7 with membranes in the dry state were indicated by its influence on the thermotropic phase transitions of the lipids and interactions with the lipid headgroup phosphates.

1. Introduction

LEA proteins comprise a large and heterogeneous group of proteins that were first found to accumulate in plant seeds during maturation [1]. Their accumulation often coincides with the onset of desiccation tolerance in seeds [2,3]. In addition to their presence in plant seeds, LEA proteins are accumulated in response to dehydration stress in vegetative plant organs (e.g. [4]) and in some species of bacteria [5], nematodes [6], rotifers [7], insects [8] and cyanobacteria [9]. Recently, systematic *in silico* investigations [4,10] identified 51 LEA protein encoding genes in the genome of the model plant *Arabidopsis thaliana* that were divided into nine phylogenetically unrelated groups according to amino acid sequence similarity. Most of these proteins were predicted to be intrinsically disordered (IDPs) [4], i.e. proteins without a stable secondary structure in solution [11]. Experimental evidence for this lack of stable secondary structure has been obtained for several LEA proteins from diverse biological sources (see [12] for a recent review).

The expression of many LEA proteins has been linked to the acquisition of desiccation tolerance in seeds, pollen and anhydrobiotic organisms (reviewed in [13]), but the functional role of LEA proteins in cellular stress protection is still largely unresolved. Based on in-vitro studies, a number of functions have been proposed, such as water binding, ion sequesteration and stabilization of DNA, RNA, proteins and membranes ([12,13] and references therein). Some LEA proteins are able to prevent the inactivation of enzymes during freezing or drying by preventing protein aggregation [14–17]. A LEA protein from the desiccation tolerant nematode *Aphelenchus avenae* showed anti-aggregation activity even under fully hydrated conditions when the protein was introduced into desiccation sensitive mammalian cells [16] and two plant LEA proteins showed chaperone function towards sensitive enzymes during heat treatment [18].

Some LEA proteins acquire secondary, mainly α-helical, structure during drying [7,19–24]. Structural modeling and FTIR spectroscopy indicated that four different LEA proteins interact with membranes in the dry state by folding into amphipathic α-helices and these interactions lead to the stabilization of membranes in the dry state [7,22,24,25].

However, a shortcoming of these previous studies is that protein structural determinations were performed on isolated proteins without taking a possible influence of the interaction with membranes on protein structure into account. Here we present a detailed FTIR spectroscopy investigation of dehydration-induced structural

Abbreviation: CD, circular dichroism; EPE, egg phosphatidylethanolamine; EPG, egg phosphatidylglycerol; FTIR, Fourier-transform infrared; GRAVY, grand average of hydropathy; IDP, intrinsically disordered protein; LEA, late embryogenesis abundant; LG, β-lactoglobulin; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine; T_m, gel to liquid-crystalline phase transition temperature

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Integration of β-carotene molecules in small liposomes

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Abstract. The most typical feature of carotenoids is the long polyene chain with conjugated double bonds suggesting that they can serve as conductors of electrons, acting as ‘‘molecular wires’’, important elements in the molecular electronic devices. Carotenoids are essential components of photosynthetic systems, performing different functions as light harvesting, photoprotection and electron transfer. They act also as natural antioxidants. In addition they perform structural role stabilizing the three-dimensional organization of photosynthetic membranes. Carotenoids contribute to the stability of the lipid phase, preserving the membrane integrity under potentially harmful environmental conditions. Carotenoids can be easily integrated into model membranes, facilitating the investigation of their functional roles. In carotenoid-egg phosphatidylcholine (EPC) liposomes *β*-carotene is randomly distributed in the hydrocarbon interior of the bilayer, without any preferred, well defined orientation and retains a substantial degree of mobility. Here we investigate the degree of integration of *β*-carotene in small unilamellar EPC liposomes and the changes in *β*-carotene absorption and Raman spectra due to the lipid-pigment interaction. All observed changes in *β*-carotene absorption and Raman spectra may be regarded as a result of the lipid-pigment interactions leading to the polyene geometry distortion and increasing of the environment heterogenety in the liposomes as compared to the solutions.

1. Introduction

Carotenoids are widespread natural molecules, which play multiple important physiological functions.The most typical feature of carotenoids is the long polyene chain with conjugated double bonds [1]. The long chain of conjugated bonds acts like a wire, allowing the electrical energy to move from one side of the molecule to the other, so called ‘‘molecular wire’’ [2]. Molecular wires, which would allow electron flow to take place between different components, are important elements in the design of molecular devices. Carotenoids are essential components of biological membranes performing multiple functions. In photosynthetic membranes they play role in light-collecting, photoprotection and electron transfer as well as stabilizing the three-dimensional integrity of bacterial and plant antenna complexes and for the assembly of functional photosystem II [3,4]. The structural role of carotenoids is probably not restricted only to the photosynthetic membranes but can be

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Interaction of two intrinsically disordered plant stress proteins (COR15A and COR15B) with lipid membranes in the dry state

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ABSTRACT

COR15A and *COR15B* form a tandem repeat of highly homologous genes in *Arabidopsis thaliana*. Both genes are highly cold induced and the encoded proteins belong to the Pfam LEA_4 group (group 3) of the late embryogenesis abundant (LEA) proteins. Both proteins were predicted to be intrinsically disordered in solution. Only COR15A has previously been characterized and it was shown to be localized in the soluble stroma fraction of chloroplasts. Ectopic expression of *COR15A* in Arabidopsis resulted in increased freezing tolerance of both chloroplasts after freezing and thawing of intact leaves and of isolated protoplasts frozen and thawed in vitro. In the present study we have generated recombinant mature COR15A and COR15B for a comparative study of their structure and possible function as membrane protectants. CD spectroscopy showed that both proteins are predominantly unstructured in solution and mainly α-helical after drying. Both proteins showed similar effects on the thermotropic phase behavior of dry liposomes. A decrease in the gel to liquid-crystalline phase transition temperature depended on both the unsaturation of the fatty acyl chains and lipid headgroup structure. FTIR spectroscopy indicated no strong interactions between the proteins and the lipid phosphate and carbonyl groups, but significant interactions with the galactose headgroup of the chloroplast lipid monogalactosyldiacylglycerol. These findings were rationalized by modeling the secondary structure of COR15A and COR15B. Helical wheel projection indicated the presence of amphipathic α-helices in both proteins. The helices lacked a clear separation of positive and negative charges on the hydrophilic face, but contained several hydroxylated amino acids.

1. Introduction

Although it is generally assumed that protein function is based on a stable three-dimensional structure, it has more recently been recognized that a substantial part of all cellular proteomes consists of proteins that either completely lack stable structure or that have large unstructured domains [1]. These proteins are now mainly referred to in the literature as intrinsically disordered proteins (IDPs). Many of these proteins perform essential cellular functions, e.g. in signal transduction and the regulation of transcription [2,3]. The ability of IDPs to bind to their target molecules, such as RNA, DNA and other (structured) proteins [4] is of crucial importance for their function and in many cases it has been observed that binding induces increased secondary structure in IDPs [5,6], indicating their ability to fold under the appropriate conditions.

Late embryogenesis abundant (LEA) proteins were first described almost 30 years ago as a group of proteins that are accumulated in cotton seeds during the late stages of development, when the embryo becomes desiccation tolerant [7]. Subsequently, related proteins were found not only in plant seeds, but also in other plant tissues, in some bacterial species and in animals such as nematodes, rotifers and brine shrimp (see [8] for a recent review). In all these cases the occurrence of the proteins was related to environmental stress conditions such as freezing, drought, or desiccation. Some LEA proteins have independently been described in the model plant species *Arabidopsis thaliana* as cold regulated or COR proteins because they are highly induced upon cold treatment [9]. While a large body of knowledge is now available about the cold regulation of the genes encoding COR proteins [10–12], the analysis of their structure and function is lagging far behind.

One of the best characterized Arabidopsis COR proteins is COR15A. Its gene was first cloned and described as cold and drought induced

Abbreviations: CD, circular dichroism; COR, cold regulated; DLnPC, 1, 2-dilinolenoyl-sn-glycero-3-phosphatidylcholine; DLnPE, 1, 2-dilinolenoyl-sn-glycero-3-phosphatidylethanolamine; EPE, egg phosphatidylethanolamine; FTIR, Fourier-transform infrared; H_{II}, hexagonal II phase; IDP, intrinsically disordered protein; LDH, lactate dehydrogenase; LEA, late embryogenesis abundant; MGDG, monogalactosyldiacylglycerol; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine; T_{Hex}, bilayer to hexagonal II phase transition temperature; T_m, gel to liquid-crystalline phase transition temperature

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INVOLVEMENT OF REACTIVE OXYGEN RADICALS IN PHOTOINHIBITION OF PRIMARY PHOTOSYNTHETIC REACTIONS – EFFECT OF TEMPERATURE AND OXYGEN RADICAL SCAVENGERS

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ABSTRACT

Exposure of leaves or chloroplasts to high light intensity leads to inactivation of photosynthesis. Two processes are observed - inhibition of photochemical activity of both photosystems and photobleaching of pigments. Oxygen evolving complex, located at the oxidizing side of Photosystem II, is the most sensitive component of photosynthetic apparatus to environmental stress factors. In the present work the effect of high light treatment at room and low temperatures on kinetic parameters of flash oxygen yields and oxygen evolution were studied. Isolated thylakoid membranes were subjected to high light illumination for different periods of time at room (22^0C) and low (4^0C) temperature. Flash oxygen yields were determined using fast oxygen rate electrode. Photochemical activity of photosystem II was measured by Clark oxygen electrode using artificial electron acceptor. Data presented show that the damaging effect of high light treatment on oxygen evolution is lower at 4^0C than at 22^0C. When high light treatment was carried out in the presence of histidine and DMSO - scavengers of oxygen radicals, the inhibition process was retarded. Data are discussed in terms of different production rate and mobility of oxygen radicals at room and low temperature.

Keywords: Oxygen evolution, oxygen radical scavengers, photoinhibition, temperature

Introduction

It is well known that exposure of plants to high light intensities leads to inactivation of the photosynthetic process (8). Although the detailed mechanisms of photoinhibition are not really known, it is widely accepted that the main target of photoinactivation is the photosystem II complex (PSII), which undergoes inhibition of electron transport activity followed by degradation and removal of the D1 protein (known as secondary quinone [QB]-binding protein and 32 kD protein) (1, 3). In the recent years, it has been demonstrated that PSI is also photoinhibited, particularly at low temperature, in chilling sensitive plants (4). Oxygen evolving complex is the most sensitive component of photosynthetic apparatus to environmental stress factors - high temperature, high light intensities, UV-radiation, etc. The damaging process is believed to be mediated by active oxygen radicals like singlet oxygen, superoxide radicals, hydroxyl radicals and hydrogen peroxides.

For photosynthetic production of one oxygen molecule, cooperation of five oxidizing equivalents, generated by four successive photoreactions in one reaction center, is required (Kok's non-cooperative model) (5). The intermediate states, S_0 to S_4 differ in their stability and redox state. S_0 state is the most reduced and stable one, while the more oxidized states, S_2 and S_3, revert to S_1 in dark within few minutes. Complex nature of oxygen evolution and some rare phenomena led to the idea of existing of two different mechanisms of oxygen evolution. It has been proposed that in addition to non-cooperative mechanism of oxygen evolution that takes place in grana situated PSII centers (PSII), cooperation between oxidizing intermediates from different oxygen-evolving centers can occur (10, 12). This so-called cooperative mechanism of oxygen evolution takes place mainly in PSII centers situated in non-appressed thylakoids PSII centers (2).

In the present research, we studied the effect of high light treatment on the parameters of flash oxygen evolution and the effect of histidine and dimethyl sulfoxide (DMSO) – scavengers of singlet oxygen and hydroxyl radicals, respectively. Isolated thylakoid membranes were subjected to high light illumination for different time periods in the absence or presence of histidine or DMSO. The effect of temperature during high light treatment was also studied. The

Research Article

Response of isolated thylakoid membranes with altered fluidity to short term heat stress

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ABSTRACT

The effect of alterations of lipid phase order of thylakoid membranes on the thermosensitivity of photosystem I (PS I) and photosystem II (PS II) was studied. Plant sterols stigmasterol and cholesterol were applied to decrease the fluidity in isolated membranes. After sterol treatment, a decrease of the temperature of 50 % inhibition of PSII activity was observed. Heat stress-induced stimulation of PSI-mediated electron transport rate was registered for control, but not for sterol-treated membranes. Effect of altered lipid order on oxygen evolving complex was evaluated by means of flash oxygen yields revealing changes in the stoichiometry of $PSII_{\alpha}$ and $PSII_{\beta}$ centers. The effect of sterol incorporation on the changes in the thermotropic behavior of the main pigment-protein complexes was studied by differential scanning calorimetry (DSC). DSC traces of control thylakoids in the temperature range 20-98 ^{o}C exhibited several irreversible endothermic transitions. Incorporation of cholesterol and stigmasterol results in superimposition of the transitions and only two main bands could be resolved. While high temperature band peaks at the same temperature after treatment with both sterols, the band that combines low temperature transitions shows different melting temperature (T_m) : 70 ^{o}C for stigmasterol- and 65 ^{0}C for cholesterol-treated membranes. The data presented here emphasise the crucial role of lipid order for the response of thylakoids to high temperatures, mediated not only by changes in the fluidity of bulk lipid phase as result of sterol incorporation but also by changes in the thermotropic properties of pigment-protein complexes. **[Physiol. Mol. Biol. Plants 2009; 15(1) : 43-52]** *E-mail : mayav@bio21.bas.bg*

Key words : *Cholesterol, Fluidity, Heat stress, Oxygen flash yields, Thylakoid membrane, Stigmasterol*

INTRODUCTION

The fluidity and permeability of membrane lipid phase play an important role in controlling light reactions of photosynthesis, for effective electron and energy transfer (Siegenthaler and Tremolieres, 1998). The lateral separation of the main pigment-protein complexes in thylakoid membranes, participation of mobile electron carriers in the electron transport reactions and regulation of energy distribution between PSI and PSII through physical movement of the light-harvesting chlorophyll *a/b* complex emphasize the role of lipid matrix and in particular, of its fluidity for the effectiveness of the photosynthetic process – linear electron transport, capture and transmitting of light energy. Several studies on this topic, including artificially manipulated thylakoids (incorporation of cholesterol or cholesteryl hemisuccinate, catalytic hydrogenation) or by using membranes from lipid mutants (genetically modified membrane fluidity) discuss the effects of altered properties of lipid matrix on functional characteristics of photosynthetic apparatus (Siegenthaler and Tremolieres, 1998; Williams, 1998). The importance of membrane fluidity is clearly evident in respect to plant response to changes of environmental conditions. Changes of lipid saturation level inevitably reflect membrane fluidity, which is well-characterized phenomenon of plant and algal acclimation to temperature and light conditions (Raison *et al.,* 1982; Klyachko-Gurvich *et al.,* 1999). To date, a number of studies have been reported on the influence of the degree of fatty acid unsaturation on the extent of low temperature photoinhibition of some cyanobacteria (Kanavero *et al,.* 1997) and tobacco transgenic plants (Moon *et al.,* 1995).

Photosynthetic reactions exhibit different heat sensitivity. Exposure of isolated chloroplast or leaves of higher plants to elevated temperatures leads to considerable changes in structural organization of thylakoid membranes and their photosynthetic activities

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in plant signaling and alteration of gene expression has only been demonstrated for phosphatidic acid, which is produced through the activity of phospholipase D (*21*). Therefore, further research on LPC generation and signaling can hopefully tell us more about the evolution of response regulation in plants and mammals, including that in the development of the AM symbiosis.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5848/265/DC1
Materials and Methods
Figs. S1 to S5
Table S1

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Functional Divergence of Former Alleles in an Ancient Asexual Invertebrate

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Theory suggests it should be difficult for asexual organisms to adapt to a changing environment because genetic diversity can only arise from mutations accumulating within direct antecedents and not through sexual exchange. In an asexual microinvertebrate, the bdelloid rotifer, we have observed a mechanism by which such organisms could acquire the diversity needed for adaptation. Gene copies most likely representing former alleles have diverged in function so that the proteins they encode play complementary roles in survival of dry conditions. One protein prevents desiccation-sensitive enzymes from aggregating during drying, whereas its counterpart does not have this activity, but is able to associate with phospholipid bilayers and is potentially involved in maintenance of membrane integrity. The functional divergence of former alleles observed here suggests that adoption of asexual reproduction could itself be an evolutionary mechanism for the generation of diversity.

Bdelloid rotifers (Rotifera, Bdelloidea) have survived for tens of millions of years without sexual reproduction and meiotic recombination (*1*–*4*). Male bdelloid rotifers have never been observed, and the genetic evidence is consistent with fully asexual reproduction by thelytoky. Long-lasting asexual lineages are thought to be rare because their apomictic nature does not allow the accumulation of favorable, or the elimination of detrimental, mutations through genetic exchange (*5*–*7*). However, one consequence of apomixis is that the sequence homogeneity of gene copies that previously were alleles in sexual ancestors is no longer maintained by recombination. This allows the former alleles to accumulate mutations and become divergent—a phenomenon referred to as the Meselson effect (*8*). Thus, in sexually reproducing monogonont rotifers (Rotifera, Monogononta) alleles differ very little from each other at synonymous sites (by up to 2.4% for *hsp82*), but corresponding gene copies in individual bdelloid clones can differ by as much as 49% (*1*). In principle, this effect should allow independent evolution of former alleles through which they can acquire different functions.

We looked for evidence of functional divergence among former alleles in a gene set associated with desiccation tolerance in bdelloid rotifers (*9*, *10*). cDNAs representing ~100 dehydration-induced genes from the bdelloid rotifer *Adineta ricciae* were identified, one of which encoded a polypeptide related to the group 3 late embryogenesis abundant (LEA) proteins characterized in plant seeds. LEA proteins are linked with desiccation tolerance in plants, invertebrates, and microorganisms (*11*). We identified two similar but distinct sequences and named them *Ar-lea-1A* and *Ar-lea-1B*. Both genes contain nine small introns (Fig. 1A), although there is a major structural difference in exon 2, which in *Ar-lea-1A* contains a 132–base pair (bp) segment with no counterpart in *Ar-lea-1B*. Aligned coding sequences show 13.5% synonymous site divergence (K_s) over the whole gene. This divergence is much greater than that observed between alleles of sexual animals, but is within the range of values observed in bdelloids for former allele pairs (*1*, *3*, *4*, *8*).

To confirm the presence of two *lea* gene copies in the *A. ricciae* genome, Southern hybridization experiments were performed with probes from both the 5′ and 3′ ends of *Ar-lea-1B*, which cross-hybridize to the corresponding regions of *Ar-lea-1A* (Fig. 1B). Both genes reside on ~5.0-kb Dra I genome fragments, but these could be distinguished by double digestion with either Eco RI or Nde I; a restriction map of each gene was constructed accordingly (Fig. 1, A and B). As further confirmation of *lea* gene copy number, fluorescence in situ hybridization (FISH) was carried out on *A. ricciae* embryo nuclei. Cytogenetic analysis shows 12 chromosomes in this species (Fig. 1C, left), as in the related species, *A. vaga* (*12*). Hybridization with a fluorescent probe corresponding to the whole of *Ar-lea-1A* produced two signals in interphase nuclei, consistent with detection of *lea* genes on two separate chromosomes (Fig. 1C, right). Our cloning and hybridization data show two related, but divergent, *lea* genes on different chromosomes in *A. ricciae*, and we interpret these to be former alleles that have diverged by the Meselson effect. Other interpretations are possible, for

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Effects of Cholesterol on Dry Bilayers: Interactions between Phosphatidylcholine Unsaturation and Glycolipid or Free Sugar

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ABSTRACT Cholesterol and other sterols are important components of biological membranes and are known to strongly influence the physical characteristics of lipid bilayers. Although this has been studied extensively in fully hydrated membranes, little is known about the effects of cholesterol on the stability of membranes in the dry state. Here, we present a Fourier transform infrared spectroscopy study on the effects of cholesterol on the phase behavior of dry liposomes composed of phosphatidylcholines with different degrees of fatty acid unsaturation or of mixtures of phosphatidylcholine with a plant galactolipid. In addition, we have analyzed the H-bonding of cholesterol, galactose, and a combination of the two additives to the P=O and C=O groups in dry phosphatidylcholine bilayers. The data indicate a complex balance of interactions between the different components in the dry state and a strong influence of fatty acid unsaturation on the interactions of the diacyl lipids with both cholesterol and galactose.

INTRODUCTION

Cholesterol (Chol) and other sterols are essential constituents of the membranes of eukaryotic cells, and the plasma membranes of mammalian cells contain up to 50 mol % Chol. Sterols perform different roles in membranes with perhaps the most important one being to modulate the physicochemical properties of the lipid bilayer (see Ohvo-Reikila et al. (1) and Yeagle (2) for reviews), including the formation of liquid-ordered domains, or rafts (3–5). Incorporation of Chol into phosphatidylcholine (PC) bilayers results in a broadening of the cooperative gel to liquid-crystalline phase transition by disordering the gel phase and ordering the liquid-crystalline phase of the host lipids. At a sufficiently high Chol content, the phase transition may be completely abolished (6). These effects are strongest with lipids containing completely saturated fatty acyl chains and become progressively weaker with an increasing degree of unsaturation, being completely absent with lipids containing polyunsaturated, long-chain fatty acids (7–9).

Interestingly, it has been shown that in freeze-dried liposomes made from fully saturated PC, the addition of Chol leads to a marked reduction in the gel to liquid-crystalline phase transition temperature (T_m) (10). Investigations into the effects of Chol on the stability of liposomes during drying have only rarely been reported so far, and in these studies no clear effects on solute leakage were found (11,12). However, the available data suggest that the membrane-stabilizing effect of the lyoprotectant trehalose is modulated by the presence of Chol in the bilayers (10,13).

Chol could influence the interactions of phospholipids with sugars in two opposing ways, namely by increasing the lipid spacing due to its intercalation between the lipids, thereby disrupting intermolecular interactions between phospholipids (14) and presumably increasing the ability of sugars to interact with lipid headgroups and by competing with the sugars for H-bonding interactions with the lipid headgroups.

Chol is situated in a lipid bilayer with its rigid sterol ring structure positioned between the fatty acyl chains and its acyl chain extending toward the bilayer center, parallel to the fatty acyl chains of the diacyl lipids (see Yeagle (2) for a review). In contrast, the Chol OH group is localized in the membrane interfacial region, allowing interactions with the ester carbonyl (15,16), the phosphate and choline groups, as well as with water molecules (15). Recent molecular dynamics simulations indicate that in the fully hydrated state there is no apparent competition between trehalose and Chol for interaction sites on PC molecules (17).

Although a modulation of the effect of trehalose on the phase behavior of fully saturated PC bilayers by Chol in the dry state has been shown (10,13), possible H-bonding interactions of Chol with dry lipids and the influence of sugars on such interactions have not been reported. Also, the effects of lipid unsaturation and of the presence of glycolipids have so far not been investigated. Here, we have used Fourier transform infrared spectroscopy (FTIR) to characterize the effects of Chol on the gel to liquid-crystalline phase transition of dry membranes containing different molecular species of PC or the plant glycolipid digalactosyldiacylglycerol (DGDG). In addition, we have analyzed the interactions of Chol and galactose (Gal) with different parts of PC headgroups separately and in combination, providing the first description of this complex interaction network to our knowledge.

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Effect of Membrane Fluidity on Photosynthetic Oxygen Production Reactions

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The effect of changes of membrane fluidity on the oxygen evolving capability of isolated thylakoids was investigated. Alteration of the lipid phase fluidity was achieved by incorporation of the plant sterol stigmasterol. Incorporation of stigmasterol in the lipid bilayer of thylakoid membranes results in rigidization of the hydrophobic phase of thylakoid membranes and decreases the degree of packing of the lipid head groups. These changes of lipid order are accompanied by a reduction of oxygen evolution, measured with 1,4-benzoquinone as an electron acceptor, and by a more pronounced inhibition of PSI-mediated electron transport. By analysis of the parameters of oxygen flash yields and oxygen burst under continuous illumination it was shown that after treatment with stigmasterol: 1.) the number of active oxygen-evolving centres decreased; 2.) the remaining active oxygen-evolving centres were not affected in respect to the oscillation pattern; 3.) the contribution of the slow oxygen-evolving centres in oxygen burst yield was increased. The effect of stigmasterol was compared with the well-studied effect of cholesterol. Results were discussed in terms of determining the role of lipid order for the organization and functioning of the photosynthetic machinery.

Key words: Thylakoid Membrane Fluidity, Oxygen Evolution, Stigmasterol

Introduction

Lateral separation of main pigment-protein complexes in thylakoid membranes of higher plants, involvement of mobile electron carriers in the electron transport chain and physical movement of the light-harvesting chlorophyll *a/b* complex during state I-state II transitions emphasize on the role of the lipid matrix and, in particular, of its fluidity for the effectiveness of the photosynthetic process – linear electron transport, capture and transmitting of light energy. Several studies on this topic that use thylakoid membranes with artificially manipulated lipid phase (incorporation of cholesterol or cholesteryl hemisuccinate) or lipid mutants (genetically altered membrane fluidity) discuss the importance of fluidity of the lipid matrix on functional characteristics of the photosynthetic apparatus, located in thylakoid membranes (Ford and Barber, 1983; Siegenthaler and Tremolieres, 1998; Yamamoto *et al.*, 1981).

Although sterols are mainly found in plasma membranes of animals and higher plants, and only in very low concentration in intracellular membranes, they could be used for artificial alteration of thylakoid membrane fluidity. Sterols are essential constituents of eukaryotic membranes and play multiple roles in membrane organization, dynamics, function and sorting (Lindsey *et al.*, 2003). Intermolecular interactions between sterols and membrane lipids modulate the physical state of the bilayer via restricting the mobility of the fatty acyl chains and in turn regulate the membrane fluidity and permeability (Hartmann, 1998). Sterols modulate also the activity of membrane-bound proteins and enzymes by affecting either their conformation or protein activity by direct protein-sterol interactions (Cooke and Burden, 1990). It has been widely reviewed that cholesterol exhibits an ordering effect on the packing of phospholipids in their liquid-crystalline state and an disordering effect below the chain melting transition temperature – in the gel phase. Incorporation of cholesterol in pure bilayers of naturally occurring phospholipids induces the formation of liquid-ordered

Abbreviations: 1,4 BQ, 1,4-benzoquinone; chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenolindophenol; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, dipalmitoylphosphatidylcholine; MES, 2-(morpholino)ethanesulfonic acid; MV, methyl viologen; PSI (II), photosystem I (II); TMA-DPH, trimethylammonium-diphenyl-DPH; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine.

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CHAPTER 6

Effects of Sugars on the Stability and Structure of Lipid Membranes During Drying

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Contents

Abstract

Most plants, microbes, and animals that can survive complete dehydration (anhydrobiotes) accumulate high concentrations of sugars in their cells during desiccation. Since cellular membranes are a primary target of desiccation damage in cells, liposomes have been widely used as comparatively simple model systems to study the effects of sugars on the properties of membranes during drying. In addition, there is a pronounced technical interest in the stabilization of liposomes with encapsulated medical drugs in the dry state for pharmaceutical purposes. Most sugars induce a depression of the membrane phase transition temperature of the dry lipid and form a glass (vitrify) during drying. In this paper, we critically review the current state of knowledge about the physical mechanisms that may lead to membrane stabilization in dry systems, with a special emphasis on possible interactions between membrane lipids and sugars in the dry state. For this purpose we compare the effects of soluble disaccharides, oligosaccharides, and polysaccharides, and also the effects of lipid-bound sugars on the stability and physical behavior of liposomes.

1. THE ROLE OF SUGARS IN THE DESICCATION TOLERANCE OF CELLS AND ORGANISMS

For most living organisms, water is an essential prerequisit of survival and even a moderate loss of cellular water can lead to physiological damage and ultimately death. Several species of microbes, plants, and animals, however, have evolved

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