Fluorescence Lifetime Imaging Microscopy of *Synechocystis* WT Cells — Variation in Photosynthetic Performance of Individual Cells in Various Strains of *sp. PCC 6803*

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Abstract: The FLIM (fluorescence lifetime imaging microscopy) technique allows picosecond fluorescence measurements at the level of the individual cell. Using this technique we were able to observe heterogeneity of cyanobacterial cells in a culture grown under controlled conditions and we were able to resolve structural variations within individual cells. It can be concluded that on the one hand the inhomogeneous distribution of photosynthetic pigments within the cell leads to variation of the fluorescence intensity, whereas on the other hand it is impossible to detect variation in the relative amounts of photosystem I and II throughout the cell. Different *Synechocystis sp. PCC 6803* strain lines were compared to each other and differences were observed in the average fluorescence lifetimes obtained for individual cells of the various cell lines. The differences can be traced back to variable efficiency of excitation energy transfer from the phycobilisome antenna to the photosystems. We could successfully demonstrate that there is heterogeneity inside individual cells, within individual cultures, and between various wild-type cell lines.
Brassinosteroids regulate the thylakoid membrane architecture and the photosystem II function

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ABSTRACT

Brassinosteroids (BRs) are plant steroid hormones known to positively affect photosynthesis. In this work we investigated the architecture and function of photosynthetic membranes in mutant Arabidopsis rosettes of BR gain-of-function (overexpressing the BR receptor BR INSENSITIVE 1 (BRI1), BRI1/PHB and loss-of-function (br1-16 with inactive BRI1 receptor, and constitutive photosynthesis and dwarfism (cpl) deficient in BR biosynthesis) mutants. Data from atomic force microscopy, circular dichroism, fluorescence spectroscopy, and polarization derelimitation of oxygen yields revealed major structural (altered thylakoids, smaller photosystem II supercomplexes) and functional (strongly inhibited oxygen evolution, reduced photosystem II quantum yield) changes in all the mutants with altered BR response compared to the wild type plants. The recorded thermal dependences showed severe thermal instability of the oxygen yields in the BR mutant plants. Our results suggest that an optimal BR level is required for the normal thylakoid structure and function.

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Hofmeister ions control protein dynamics

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ABSTRACT

Background: Recently, we have elaborated a thermodynamic theory that could coherently interpret the diverse effects of Hofmeister ions on proteins, based on a single physical parameter, the protein–water interfacial tension (DéR et al., Journal of Physical Chemistry B. 2007, 111, 5344–5350). This theory, implying a “liquid drop model”, predicts changes in protein conformational fluctuations upon addition of Hofmeister salts (containing either kosmotropic or chaotropic anions) to the medium.

Methods: Here, we report experimental tests of this prediction using a complex approach by applying methods especially suited for the detection of protein fluctuation changes (neutron scattering, micro-calorimetry, and Fourier-transform infrared spectroscopy).

Results: It is demonstrated that Hofmeister salts, via setting the hydrophobic/hydrophilic properties of the protein–water interface, control conformational fluctuations even in the interior of the typical membrane transport protein bacteriorhodopsin, around its temperature-induced, unusual α(3) → α(2) conformational transition between 60 and 90°C. We found that below this transition cosmotropic (COO-) ions increase structural fluctuations of bphII. It was also shown that, in each case, an onset of enhanced equilibrium fluctuations presages this phase transition in the course of the thermotropic response of bphII.

Conclusions: These results are in full agreement with the theory, and demonstrate that predictions based on protein–water interfacial tension changes can describe Hofmeister effects and interpret protein dynamics phenomena even in unusual cases.

General significance: This approach is expected to provide a useful guide to understand the principles governing the interplay between protein interfacial properties and conformational dynamics, in general.

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TERPENOID PROFILE OF *ARTEMISIA ALBA* IS RELATED TO ENDOGENOUS CYTOKININS IN VITRO

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Abstract


Modifications of plant growth regulators supplied to *Artemisia alba* in vitro cultures have been previously shown to affect plant morphogenesis and terpenoid profile of the essential oil of aerial parts. We report here on the effects of plant growth regulators on the terpenoid profile, structure of photosystem II in vitro as well as on the endogenous cytokinin levels of both above- and underground parts. The contents of cytokinin bioactive forms (free bases and ribosides) were followed. It was revealed that the growth regulators-modified growth and development as well as the alterations of photosystem II structural organization were related to the cytokinin levels of *Artemisia alba*. Thus, elevated monoterpenoid levels were associated with a higher peripheral antennae aggregation and elevation of *trans*-zeatin riboside, dihydrozeatin and dihydrozeatin riboside as well as $\text{N}^\circ$-(2-isopentenyl) adenine in the aerials of the respective plant growth regulators treatments. These results imply of the role of exogenous factors such as cytokinins and auxins supplementation in affecting the terpenoid biogenesis in vitro by altering the levels of endogenous cytokinins and physiological status of the plant organism.
Calorimetry-based profiling of blood plasma from colorectal cancer patients

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ABSTRACT

Background: Differential scanning calorimetry (DSC), a highly sensitive technique for resolving thermally-induced protein folding/unfolding transitions, recently was recognized as a novel tool for disease diagnosis and monitoring. To further elaborate this approach we have applied DSC in a study of blood plasma from patients with colorectal cancer (CRC) at different stages of tumor development and localization.

Methods: Blood plasma from patients diagnosed with CRC was analyzed by DSC. The CRC thermograms were compared to those of healthy individuals and patients with gastric cancer and non-cancerous soft tissue inflammation. The thermodynamic parameters: excess heat capacity and enthalpy of the transitions corresponding to the most abundant plasma proteins, as well as transition and first moment temperatures were determined from the calorimetric profiles.

Results: The calorimetric profiles of blood plasma from CRC patients are found to be distinct from those of healthy individuals and those of patients with soft tissue, non-cancerous inflammation. Generally the CRC thermograms exhibit reduced heat capacity of the major albumin/globulin-assigned thermal transitions, lower enthalpy and a featureless high temperature region compared to healthy individuals.

Conclusions: A classification of blood plasma proteome from patients with colorectal cancer (CRC1, CRC2 and CRC3 groups, and subgroups within each group CRC1.1, CRC2.1 and CRC3.1) is proposed based on the derived thermodynamic parameters.

General significance: The presented data demonstrate a proof of principle and confirm that the DSC technique has a potential to monitor changes in the CRC blood plasma proteome. This study is a further step toward the validation of calorimetry as a diagnostic tool.

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Dynamic properties of photosystem II membranes at physiological temperatures characterized by elastic incoherent neutron scattering. Increased flexibility associated with the inactivation of the oxygen evolving complex

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Abstract Elastic incoherent neutron scattering (EINS), a non-invasive technique which is capable of measuring the mean square displacement of atoms in the sample, has been widely used in biology for exploring the dynamics of proteins and lipid membranes but studies on photosynthetic systems are scarce. In this study we investigated the dynamic characteristics of Photosystem II (PSII) membrane fragments between 280 and 340 K, i.e., in the physiological temperature range and in the range of thermal denaturation of some of the protein complexes. The mean square displacement values revealed the presence of a hydration-sensitive transition in the sample between 310 and 320 K, suggesting that the oxygen evolving complex (OEC) plays an important role in the transition. Indeed, in samples in which the OEC had been removed by TRIS- or heat-treatments (323 and 333 K) no such transition was found. Further support on the main role of OEC in these reorganizations is provided by data obtained from differential scanning calorimetry experiments, showing marked differences between the untreated and TRIS-treated samples. In contrast, circular dichroism spectra exhibited only minor changes in the exciton interactions below 323 K, showing that the molecular organization of the pigment-protein complexes remains essentially unaffected. Our data, along with earlier incoherent neutron scattering data on PSII membranes at cryogenic temperatures (Pieper et al., Biochemistry 46:11398–11409, 2007), demonstrate that this technique can be applied to characterize the

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Microcalorimetry of Blood Serum Proteome: A Modified Interaction Network in the Multiple Myeloma Case

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ABSTRACT: Here we report on a novel approach in the study of multiple myeloma (MM), namely, differential scanning calorimetry (DSC) combined with serum protein electrophoresis. Distinct thermodynamic signatures describe the DSC thermograms of MM blood sera, in contrast to the unique profile found for healthy individuals. The thermal behavior of MM sera reflects a complex interplay between the serum concentration and isotype of the M-protein and of albumin, and modified ligand-and/or protein-protein interactions, resulting in stabilization of globulins and at least a fraction of albumin. In all MM cases the 85 °C, transferrin-assigned transition is missing. A distinct feature of IgG isotype (κ and λ) DSC profiles only is the presence of a transition at 82 °C. A DSC-based classification of MM depicts two sets of melting patterns (MMt2 and MMt3 with two or three successive thermal transitions), and subsets within each set (MMt2 or MMt3, the subscript i = 1, 2 or 3 denotes the main transition being one of the three transitions). The results demonstrate the potential of DSC to monitor MM-related modifications of the serum proteome, even at low M-protein concentrations, Bence Jones and importantly nonsecretory multiple myeloma cases, and prove DSC as a versatile tool for oncohematology.
Digalactosyl-diacylglycerol-deficiency lowers the thermal stability of thylakoid membranes

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Abstract We investigated the effects of digalactosyl-diacylglycerol (DGDG) on the organization and thermal stability of thylakoid membranes, using wild-type Arabidopsis thaliana and the DGDG-deficient mutant, dgd1. Circular-dichroism measurements reveal that DGDG-deficiency hampers the formation of the chirally organized macromdomains containing the main chlorophyll a/b light-harvesting complexes. The mutation also brings about changes in the overall chlorophyll fluorescence lifetimes, measured in whole leaves as well as in isolated thylakoids. As shown by time-resolved measurements, using the lipophilic fluorescence probe Merocyanine 540 (MC540), the altered lipid composition affects the packing of lipids in the thylakoid membranes but, as revealed by flash-induced electrochromic absorbance changes, the membranes retain their ability for energization. Thermal stability measurements revealed more significant differences. The disassembly of the chiral macromdomains around 55°C, the thermal destabilization of photosystem I complex at 61°C as detected by green gel electrophoresis, as well as the sharp drop in the overall chlorophyll fluorescence lifetime above 45°C (values for the wild type—WT) occur at 4–7°C lower temperatures in dgd1. Similar differences are revealed in the temperature dependence of the lipid packing and the membrane permeability: at elevated temperatures MC540 appears to be extruded from the dgd1 membrane bilayer around 35°C, whereas in WT, it remains lipid-bound up to 45°C and dgd1 and WT membranes become leaky around 35 and 45°C, respectively. It is concluded that DGDG plays important roles in the overall organization of thylakoid membranes especially at elevated temperatures.

Keywords Arabidopsis mutants ·
Monitoring Photosynthesis in Individual Cells of *Synechocystis* sp. PCC 6803 on a Picosecond Timescale

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ABSTRACT Picosecond fluorescence kinetics of wild-type (WT) and mutant cells of *Synechocystis* sp. PCC 6803, were studied at the ensemble level with a streak-camera and at the cell level using fluorescence-lifetime-imaging microscopy (FLIM). The FLIM measurements are in good agreement with the ensemble measurements, but they (can) unveil variations between and within cells. The BE mutant cells, devoid of photosystem II (PSII) and of the light-harvesting phycobilisomes, allowed the study of photosystem I (PSI) in vivo for the first time, and the observed 6-ps equilibration process and 25-ps trapping process are the same as found previously for isolated PSI. No major differences are detected between different cells. The PAL mutant cells, devoid of phycobilisomes, show four lifetimes: ~20 ps (PSI and PSI), ~30 ps, ~40 ps, and 2.8 ns (all due to PSII), but not all cells are identical and variations in the kinetics are traced back to differences in the PSI/PSII ratio. Finally, FLIM measurements on WT cells reveal that in some cells or parts of cells, phycobilisomes are disconnected from PSII/PSII. It is argued that the FLIM setup used can become instrumental in unraveling photosynthetic regulation mechanisms in the future.
Differential scanning calorimetry of photosynthetic membranes: Resolving contributions of the major photosynthetic complexes to the sequential thermal transitions

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ABSTRACT
Differential scanning calorimetry is an efficient tool to study and thermodynamically describe biological macromolecules, proteins and lipids, and complex biological systems. It has been applied in investigations of thylakoid membranes and membrane fragments since the 80s, but only recently an attempt has been made to describe the sequence of the thermally induced events. Herein, we further elaborate this approach by reviewing the results obtained on intact thylakoids from wild-type plants and the chlorophyll b-less mutant *chlorina* fli, isolated lamellar aggregates of the major light-harvesting complex of photosystem II, granal and stroma membranes. The comparison of the revealed thermal transitions allowed the assignment of the calorimetric features associated with the melting of the major photosynthetic complexes in thylakoid membranes. This basic knowledge will be of great help to follow and compare the thermal stability of photosynthetic complexes under stress conditions and in a variety of mutants. The thermal behavior of another type of an energy-producing membrane, the purple membrane of Halobacteria, is also reviewed. Although it represents a much more simple membrane system, it exhibits a complex melting profile that resembles the one found for the major light-harvesting complex of photosystem II.

KEYWORDS: differential scanning calorimetry, thylakoid membranes, *chlorina* fli, major light-harvesting complex of photosystem II, photosystem I, photosystem II, purple membrane, bacteriochlorophyll

INTRODUCTION
Biological membranes are unique inhomogeneous spatial structures, consisting of proteins embedded in a lipid matrix, with architecture and stability being strongly related to their functions. In this review we deal with photosynthetic membranes, which are evolutionary-optimized for high photochemical efficiency and high thermal stability, especially regarding the purple membrane (PM) from *Halobacterium salinarum*. In particular the thermodynamic behavior of the two types of membranes capable of energy transduction by two different mechanisms, both of which however are light-driven (i.e. the processes of photosynthesis in thylakoid membranes (TM) and proton pumping in PM), is discussed.

Purple membranes (Fig. 1A) contain only one protein, the lipid–membrane–embedded retinal-containing 7-trans helix protein bacteriochlorophyll (bR) [1]. bR operates as a light energy converter and unidirectionally pumps protons upon absorption of light [2, 3]. bR molecules are packed in trimers, dividing the lipid bilayer into two discontinuous compartments forming a 2D hexagonal membrane crystal array [4, 5] (Fig. 1A). bR has an open, naturally stable
Temperature dependence of the lipid packing in thylakoid membranes studied by time- and spectrally resolved fluorescence of Merocyanine 540

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ABSTRACT
The lipid packing of thylakoid membranes is an important factor for photosynthetic performance. However, surprisingly little is known about it and it is generally accepted that the bulk thylakoid lipids adopt the liquid–crystalline phase above ~30 °C and that a phase transition occurs only above 45 °C. In order to obtain information on the nature of the lipid microenvironment and its temperature dependence, steady-state and time-resolved fluorescence measurements were performed on the fluorescent probe Merocyanine 540 (MC540) incorporated in isolated spinach thylakoids and in model lipid systems (dipalmitoyl phosphatidylcholine and dioleoyl phosphatidylethanolamine) adopting different phases. It was demonstrated that the degree and way of incorporation differs for most lipid phases – upon selective excitation at 570 nm, the amplitude of the fluorescence component that corresponds to membrane-incorporated MC540 is about 20% in gel-, 60% in rippled gel-, and 90% in liquid-crystalline and inverted hexagonal phase, respectively. For thylakoids, the data reveal hindered incorporation of MC540 (amplitude about 30% at 7 °C) and marked spectral heterogeneity at all temperatures. The incorporation of MC540 in thylakoids strongly depends on temperature. Remarkably, above 25 °C MC540 becomes almost completely extruded from the lipid environment, indicating major rearrangements in the membrane.

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Phase behavior of phosphatidylglycerol in spinach thylakoid membranes as revealed by $^{31}$P-NMR

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Abstract

Non-bilayer lipids account for about half of the total lipid content in chloroplast thylakoid membranes. This lends high propensity of the thylakoid lipid mixture to participate in different phases which might be functionally required. It is for instance known that the chloroplast enzyme violaxanthin de-epoxidase (VDE) requires a non-bilayer phase for proper functioning in vitro but direct evidence for the presence of non-bilayer lipid structures in thylakoid membranes under physiological conditions is still missing.

In this work, we used phosphatidylglycerol (PG) as an intrinsic bulk lipid label for $^{31}$P-NMR studies to monitor lipid phases of thylakoid membranes. We show that in intact thylakoid membranes the characteristic lamellar signal is observed only below 20 °C. But at the same time an isotropic phase is present, which becomes even dominant between 14 and 28 °C despite the presence of fully functional large membrane sheets that are capable of generating and maintaining a transmembrane electric field. Tris-washed membranes show a similar behavior but the lamellar phase is present up to higher temperatures. Thus, our data show that the location of the phospholipids is not restricted to the bilayer phase and that the lamellar phase co-exists with a non-bilayer isotropic phase.

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Keywords: Non-bilayer lipid phase; Isotropic lipid phase; Phosphatidylglycerol; $^{31}$P-NMR; Thylakoid membranes
Kinetic nature of the thermal destabilization of LHCII macroaggregates

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Abstract

The main light-harvesting chl a/b pigment–protein complex of photosystem II (LHCII) in isolated state forms macroaggregates with different ultrastructure and lipid content [J. Simidjiev, V. Barza, L. Mustardy, G. Gambl, Anal. Biochem. 250 (1997) 169–175]. The thermodynamic stability of highly delipitated tightly bound LHCII macroaggregates is studied by differential scanning calorimetry and fluorescence spectroscopy. The calorimetric profile of LHCII is asymmetric, the denaturation transition is taking place at around 72 °C. A shoulder, which overlaps with the main denaturation transition, appears around 58 °C. The denaturation temperature strongly depends on the scanning rate indicating the kinetic nature of the thermal destabilization of LHCII macroaggregates. The fluorescence data prove that the thermal denaturation of LHCII is an irreversible and kinetically controlled process.

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Keywords: Light-harvesting chlorophyll a/b pigment–protein complex; Differential scanning calorimetry; Denaturation transition; Calorimetric enthalpy; Fluorescence emission
Structural Rearrangements in Chloroplast Thylakoid Membranes Revealed by Differential Scanning Calorimetry and Circular Dichroism Spectroscopy. Thermo-optic Effect

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ABSTRACT. The thermo-optic mechanism in thylakoid membranes was earlier identified by measuring the thermal and light stabilities of pigment arrays with different levels of structural complexity [Cseh, Z., et al. (2000) Biochemistry 39, 15250–15257]. (According to the thermo-optic mechanism, fast local thermal transients, arising from the dissipation of excess, photosynthetically not used, excitation energy, induce elementary structural changes due to the “built-in” thermal instabilities of the given structural units.) The same mechanism was found to be responsible for the light-induced trimer-to-monomer transition in LHClI, the main chlorophyll a/b light-harvesting antenna of photosystem II (PSII) [Garab, G., et al. (2002) Biochemistry 41, 15121–15129]. In this paper, differential scanning calorimetry (DSC) and circular dichroism (CD) spectroscopy on thylakoid membranes of barley and pea are used to correlate the thermo-optically inducible structural changes with well-discernible calorimetric transitions. The thylakoid membranes exhibited six major DSC bands, with maxima between about 43 and 87 °C. The heat sorption curves were analyzed both by mathematical deconvolution of the overall endotherm and by a successive annealing procedure; these yielded similar thermodynamic parameters, transition temperature and calorimetric enthalpy. A systematic comparison of the DSC and CD data on samples with different levels of complexity revealed that the light-induced disassembly of chirally organized macromdomains contributes profoundly to the first endothermic event, a weak and broad DSC band between 43 and 48 °C. Similarly to the main macromdomain-associated CD signals, this low enthalpy band could be diminished by prolonged photoinhibitory preillumination, the extent of which depended on the temperature of preillumination. By means of nonnaturating, “green” gel electrophoresis and CD fingerprinting, it is shown that the second main endotherm, around 60 °C, originates to a large extent from the monomerization of LHClI trimers. The main DSC band, around 70 °C, which exhibits the highest enthalpy change, and another band around 75–77 °C relate to the dismantling of LHClI and other pigment–protein complexes, which under physiologically relevant conditions cannot be induced by light. The currently available data suggest the following sequence of events of thermo-optically inducible changes: (i) unstacking of membranes, followed by (ii) lateral disassembly of the chiral macromdomains and (iii) monomerization of LHClI trimers. We propose that thermo-optical structural reorganizations provide a structural flexibility, which is proportional to the intensity of the excess excitation, while for their localized nature, the structural stability of the system can be retained.