

## Chlorophyll *a* Self-assembly in Polar Solvent–Water Mixtures†

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### ABSTRACT

The conversion of chlorophyll *a* (Chl *a*) monomers into large aggregates in six polar solvents upon addition of water has been studied by means of absorption, fluorescence spectroscopy and fluorescence lifetime measurements for the purpose of elucidating the various environmental factors promoting Chl *a* self-assembly and determining the type of its organization. Two empirical solvent parameter scales were used for quantitative characterization of the different solvation properties of the solvents and their mixtures with water. The mole fractions of water  $f_{1/2}$  giving rise to the midpoint values of the relative fluorescence quantum yield were determined for each solvent, and then various solvent–water mixture parameters for the  $f_{1/2}$  values were compared. On the basis of their comparison, it is concluded that the hydrogen-bonding ability and the dipole–dipole interactions (function of the dielectric constant) of the solvent–water mixtures are those that promote Chl *a* self-assembly. The influence of the different nature of the non-aqueous solvents on the Chl aggregation is manifested by both the different water contents required to induce Chl monomer  $\rightarrow$  aggregate transition and the formation of two types of aggregates at the completion of the transition: species absorbing at 740–760 nm (in methanol, ethanol, acetonitrile, acetone) and at 667–670 nm (in pyridine and tetrahydrofuran). It is concluded that the type of Chl organization depends on the coordination ability and the polarizability (function of the index of refraction) of the organic solvent. The ordering of the solvents with respect to the  $f_{1/2}$  values—methanol < ethanol < acetonitrile < acetone < pyridine < tetrahydrofuran—yielded a typical lyotropic (Hofmeister) series. On the basis of this solvent ordering and the disparate effects of the two groups of solvents on the Chl *a* aggregate organization, it is pointed out that the mechanism of Chl *a* self-assembly in aqueous media can be considered a manifestation of the Hofmeister effect, as displayed in the lipid-phase behavior (Koynova *et al.*, *Eur. J. Biophys.* 25, 261–274, 1997). It relates to the solvent ability to modify the bulk

structure and to distribute unevenly between the Chl–water interface and bulk liquid.

### INTRODUCTION

The process of photosynthesis in various organisms (plants, algae, photosynthetic bacteria) starts with the high efficient light collection by so-called antenna complexes. Chlorophyll *a* (Chl *a*)‡ and bacteriochlorophyll *c* (Bchl *c*) are the major antenna pigments in higher plants and in green photosynthetic bacteria, respectively. Their organization as high efficient light collectors is of crucial importance for the functioning of the overall photosynthetic unit. In contrast to green photosynthetic bacteria, where strong pigment–pigment interactions determine the organization of chlorosomal Bchl *c* and *d* (1), in higher plants the polypeptide amino acid residues bind and organize the antenna Chl *a* and *b* by specific and nonspecific interactions (2). Despite the fact that the structure of several Chl–protein complexes is known at near atomic resolution, the molecular mechanism for their assembly and structural stability remains unclear.

One of the reasons is that the mechanism for Chl self-assembly in aqueous media is also unclear. That such knowledge is of importance is evident by the same effects that medium composition has on both the chlorosomes (3) and Bchl *c* and *d* aggregates in aqueous media (4) or on chloroplast fragments (5) and Chl *a* aggregates in aqueous solvents (6). While the formation of Chl aggregates in various organic solvent–water mixtures is well documented (6–18), little is known about the contribution of the structure and properties of the medium for the assembly and stabilization of different Chl aggregate organizations in aqueous media. A study on the relationship between the Chl aggregation and solvent structure and properties can lead to an elucidation of the mechanism for Chl assembly and dissolution in aqueous media and consequently in the photosynthetic antenna complexes.

Despite the well-documented formation of Chl *a* aggregates in organic solvent–water mixtures, there are only a few studies aimed at clarifying and quantifying the dependence of the process of Chl *a* aggregation on the properties of the



## Detection of the metastable rippled gel phase in hydrated phosphatidylcholine by fluorescence spectroscopy

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### Abstract

Steady-state and time-resolved emission spectroscopy of 1-anilinonaphthalene-8-sulfonic acid (ANS) have been used for characterization of the metastable rippled gel phase,  $P_{\beta}'(\text{mst})$ , formed in fully-hydrated dipalmitoylphosphatidylcholine (DPPC) upon cooling from the liquid crystalline phase  $L_{\alpha}$  [Tenchov et al., *Biophys. J.* 56 (1989) 757]. The  $P_{\beta}'(\text{mst})$  phase of DPPC clearly differs from the stable  $P_{\beta}'$  phase by increased ( $\approx 27\%$ ) ANS emission intensity, by enhanced ( $\approx 23\%$ ) average radiative rate constant, and by reduced ( $\approx 18\%$ ) non-radiative quenching rate constant. The fluorescence intensity peak at the  $P_{\beta}' \rightarrow L_{\alpha}$  transition temperature is replaced by a large, reversible stepwise intensity drop at the  $P_{\beta}'(\text{mst}) \rightarrow L_{\alpha}$  transition. No such effects have been found for dimyristoylphosphatidylcholine (DMPC) dispersions confirming previous results that DMPC does not form a  $P_{\beta}'(\text{mst})$  phase. Since ANS is known to predominantly reside in the interfacial region, the observed effects indicate differences between the stable and metastable rippled phases in the organization and dynamics of their lipid/water interfaces. The data demonstrate that the metastable rippled phase manifests its appearance also through interactions with small molecules (ANS size  $\sim 8$  Å). © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Phase transition; Phospholipid; Polymorphism; Lipid bilayer; ANS; Time-resolved emission spectroscopy

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**ASSESSMENT OF CHLOROPHYLL FLUORESCENCE  
AND PHOTOSYNTHETIC OXYGEN EVOLUTION  
PARAMETERS IN DEVELOPMENT OF BIOSENSORS  
FOR DETECTION OF Q<sub>B</sub> BINDING HERBICIDES**

**Radka Vladkova, Pavlina Ivanova, Vassilena Krasteva,  
Amarendra N. Misra\*, Emilia Apostolova**

*(Submitted by Academician K. Kumanov on December 23, 2008)*

**Abstract**

Thylakoid membranes isolated from pea leaves were used for comparing the sensitivity of the parameters of Pulse-Amplitude-Modulation (PAM) chlorophyll fluorescence and photosynthetic oxygen evolution to elaborate a biosensor for detection of Q<sub>B</sub> binding herbicide, atrazine. Non-photochemical quenching parameters (qN and NPQ) and flash oxygen evolution (estimated as the amplitude of the oxygen evolution after third flash, Y<sub>3</sub>) are the most suitable parameters for monitoring the effect of atrazine.

**Key words:** thylakoid membrane, atrazine, non-photochemical quenching, oxygen evolution, biosensor

4.

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ASSESSMENT OF SENSITIVITY OF PHOTOSYNTHETIC  
OXYGEN EVOLUTION AND CHLOROPHYLL  
FLUORESCENCE PARAMETERS TO COPPER  
FOR APPLICATION IN BIOSENSORS

Anelia Dobrikova, Radka Vladkova, Georgy Rashkov,  
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*(Submitted by Academician K. Kumanov on March 12, 2009)*

**Abstract**

The effects of copper ions on the parameters of the photosynthetic oxygen evolution measured by polarographic oxygen rate electrode and Pulse-Amplitude-Modulation (PAM) chlorophyll fluorescence of pea thylakoid membranes are compared. Data reveal that the non-photochemical quenching parameters and flash-induced oxygen evolution are suitable for detection of copper in solutions.

**Key words:** thylakoid membranes, photosystem II, copper, oxygen evolution, PAM fluorometry



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## Bilayer structural destabilization by low amounts of chlorophyll *a*

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### ABSTRACT

The present study shows that small admixtures of one chlorophyll *a* (Chl*a*) molecule per several hundred lipid molecules have strong destabilizing effect on lipid bilayers. This effect is clearly displayed in the properties of the L<sub>α</sub>–H<sub>II</sub> transformations and results from a Chl*a* preference for the H<sub>II</sub> relative to the L<sub>α</sub> phase. Chl*a* disfavors the lamellar liquid crystalline phase L<sub>α</sub> and induces its replacement with inverted hexagonal phase H<sub>II</sub>, as is consistently demonstrated by DSC and X-ray diffraction measurements on phosphatidylethanolamine (PE) dispersions. Chl*a* lowers the L<sub>α</sub>–H<sub>II</sub> transition temperature (42 °C) of the fully hydrated dipalmitoleoyl PE (DPOPE) by ~8 °C and ~17 °C at Chl*a*/DPOPE molar ratios of 1:500 and 1:100, respectively. Similar Chl*a* effect was recorded also for dielaidoyl PE dispersions. The lowering of the transition temperature and the accompanying significant loss of transition cooperativity reflect the Chl*a* repartitioning and preference for the H<sub>II</sub> phase. The reduction of the H<sub>II</sub> phase lattice constant in the presence of Chl*a* is an indication that Chl*a* favors H<sub>II</sub> phase formation by decreasing the radius of spontaneous monolayer curvature, and not by filling up the interstitial spaces between the H<sub>II</sub> phase cylinders. The observed Chl*a* preference for H<sub>II</sub> phase and the substantial bilayer destabilization in the vicinity of a bilayer-to-nonbilayer phase transformation caused by low Chl*a* concentrations can be of interest as a potential regulatory or membrane-damaging factor.

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Nitric Oxide

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## Photoelectron transport ability of chloroplast thylakoid membranes treated with NO donor SNP: Changes in flash oxygen evolution and chlorophyll fluorescence

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### ABSTRACT

The nitric oxide (NO) donor sodium nitroprusside (SNP) is frequently used in plant science *in vivo*. The present *in vitro* study reveals its effects on the photosynthetic oxygen evolution and the chlorophyll fluorescence directly on isolated pea thylakoid membranes. It was found that even at very low amounts of SNP (chlorophyll/SNP molar ratio ~ 67:1), the SNP-donated NO stimulates with more than 50% the overall photosystem II electron transport rate and diminishes the evolution of molecular oxygen. It was also found that the target site for SNP-donated NO is the donor side of photosystem II. Compared with other NO-donors used in plant science, SNP seems to be the only one exhibiting stimulation of electron transport through photosystem II.

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## Sensors and Actuators B: Chemical

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## Prolonged sensitivity of immobilized thylakoid membranes in cross-linked matrix to atrazine

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## ABSTRACT

Freshly prepared pea thylakoid membranes were immobilized in bovine serum albumin–glutaraldehyde cross-linked matrix (BSA–GA matrix) and their stability under long term storage was analyzed by Pulse-Amplitude-Modulated (PAM) chlorophyll fluorescence and photosynthetic oxygen evolution measured by oxygen rate electrode. The thylakoid membranes stored at 4 °C showed prolonged stability in BSA–GA matrix and additional adsorption on nitrocellulose membrane filters gave them more stability. The sensitivity of the parameters of the oxygen evolution of thylakoid membranes to atrazine increased with immobilization. The half-inhibition time for oxygen evolution and quantum efficiency of photosynthesis could be prolonged to more than 15 days. These results suggest that the immobilized thylakoid membranes in BSA–GA matrix can be used as biological receptor in biosensors for a long period of time (up to 25 days) applying the proposed new method for atrazine detection by using polarographic oxygen rate electrode. This method is more sensitive, faster and easier to use than other methods for detection of herbicides based on determination of the photochemical activity of photosystem II.

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

Anelia Dobrikova, Radka Vladkova, Daniela Stanoeva,  
Antoaneta Popova, Maya Velitchkova

*(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





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## Plant Physiology and Biochemistry

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## Research article

## Effects of exogenous 24-epibrassinolide on the photosynthetic membranes under non-stress conditions



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## ABSTRACT

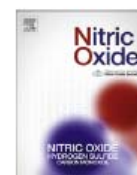
In the present work the effects of exogenous 24-epibrassinolide (EBR) on functional and structural characteristics of the thylakoid membranes under non-stress conditions were evaluated 48 h after spraying of pea plants with different concentrations of EBR (0.01, 0.1 and 1.0 mg.L<sup>-1</sup>). The results show that the application of 0.1 mg.L<sup>-1</sup> EBR has the most pronounced effect on the studied characteristics of the photosynthetic membranes. The observed changes in 540 nm light scattering and in the calorimetric transitions suggest alterations in the structural organization of the thylakoid membranes after EBR treatment, which in turn influence the kinetics of oxygen evolution, accelerate the electron transport rate, increase the effective quantum yield of photosystem II and the photochemical quenching. The EBR-induced changes in the photosynthetic membranes are most probably involved in the stress tolerance of plants.

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## Review

## Action and target sites of nitric oxide in chloroplasts



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## ABSTRACT

Nitric oxide (NO) is an important signalling molecule in plants under physiological and stress conditions. Here we review the influence of NO on chloroplasts which can be directly induced by interaction with the photosynthetic apparatus by influencing photophosphorylation, electron transport activity and oxidation state of the Mn clusters of the oxygen-evolving complex or by changes in gene expression. The influence of NO-induced changes in the photosynthetic apparatus on its functions and sensitivity to stress factors are discussed.

## SPECIFIC SOLVATION OF CHLOROPHYLL *b*: A SITE-SELECTION STUDY AT 5 K

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**Keywords.** Chlorophyll *b*, specific solvation, site-selection spectroscopy, vibrational structure, liquid helium temperature, photosynthesis.

### Summary

The site-selection fluorescence excitation spectra of chlorophyll *b* were recorded in three different types of polar solvent host at 5 K. The influence of specific solvation of chlorophyll *b* on the S<sub>1</sub> state vibrational modes active in these spectra was studied in the high frequency vibrational region above 1000 cm<sup>-1</sup>. The nucleophilic solvation of chlorophylls (*i.e.* one or two axial extra ligands at the central magnesium atom), unlike electrophilic interactions (hydrogen bonding), can be detected very successfully by the method used. The maximum down-shift of 20 - 30 cm<sup>-1</sup> of the high characteristic vibrational frequencies in the C=C stretching region estimated under electronic excitation of chlorophyll *b* is comparable with the effect of magnesium biligation and is explained in terms of an expansion of the tetrapyrrolic macrocycle core of the molecule. The broad-band fluorescence spectra of chlorophyll *b* in the same frozen solutions recorded at different laser excitation wavelengths are also presented and discussed.

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**Zero-phonon transitions of chlorophyll *a* in mature plant leaves  
revealed by spectral hole-burning method at 5 K**

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Key words: Low temperature fluorescence spectroscopy; Spectral hole burning; Zero phonon transition;  
Chlorophyll *a*; (*Zea mays*)

After monochromatic exposition in the 680–700 nm region persistent holes are formed in the fluorescence excitation spectra of green maize leaves. These narrow (up to  $1\text{ cm}^{-1}$ ) holes correspond to zero-phonon lines whose intensity may reach 45% of the total fluorescence. Thus the fluorescence bands  $F_{685}$  and  $F_{695}$ , originating mainly from Photosystem II (Rijgersberg, C.P., Ames, J., Thielen, A.P.G.M. and Swager, J.A. (1979) *Biochim. Biophys. Acta* 545, 473–482), are essentially of purely electronic nature. Unlike chlorophyll *a* in glassy matrices, the pigment in greening leaves cannot be completely phototransformed under high-dose  $\text{Kr}^+$  laser irradiation at 676.4 nm.