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Letter

Relevance of the chlorophyll phytyl chain on lamellar phase formation and organisation

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Abstract

A series of modified chlorophylls (chlorophyll *a*, pyrochlorophyll *a*, Zn-pheophytin *a* and Zn-pheophorbide *a*) have been inserted into lamellar phases of sodium bis-(2-ethylhexyl)-sulfosuccinate (AOT). The role played by the different functional groups in affecting the bilayer formation and organisation has been investigated by means of the NMR quadrupolar splitting technique. Evidence is reported for the first time on the capacity of the phytyl chain of the chlorophylls to anchor the tetrapyrroles into the bilayer, favouring at the same time the regular formation of the lamellae.

Keywords: Photosynthesis; Membrane organisation; Chlorophyll–protein

1. Introduction

Chlorophyll *a* (Chl *a*) is the main photosynthetic pigment in higher plants and many algae, playing a fundamental role in light harvesting and energy transduction. Electron and X-ray crystallography of different photosynthetic complexes [1–3] has allowed for the evaluation of the chlorophyll location within. More than 50% of the total chlorophyll involved in green plant photosynthesis are bound to the light-harvesting chlorophyll *a*/*b* protein complex associated with photosystem II (LHC-II) [4–6], an integral membrane protein whose structure has been solved by electron diffraction [3]. The native complex consists of three monomers related by crystallographic threefold symmetry. Each monomer con-

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tains three α-helical transmembrane domains, binding at least 12 molecules of Chls *a* and *b*. The tetrapyrrole macrocycles are arranged on two levels with respect to the depth of the membrane. Some of them have electron density protruding into the hydrophobic bilayer, which may correspond to parts of the hydrophobic C_{20}-phytyl chain. Several Chls seem to be suspended between the polypeptide loops on the membrane surface and the membrane-spanning α-helices, and it is still not clear if some of the Chls are only loosely bound and lost during isolation from the membrane. A well-defined lipid interaction has also been shown for the peridinin–chlorophyll–protein from dinoflagellates [3]. The possibility that chlorophylls can interact directly with the lipid matrix of the membrane, or be separated by interdispersed lipids, emphasises the relevance of studies on the molecular organisation and dynamics of chlorophylls in anisotropic amphiphilic systems.

Lamellar phases exhibiting a close similarity with biological membranes represent a model system to study the mutual influence exercised by a host on the lipid organisation, and vice versa. Previous studies on the insertion of Chl into sodium bis-(2-ethylhexyl)-sulfosuccinate (AOT) lamellar phases have already provided evidence for a catalytic function of the pigment on lamellae formation, and an active role in determining their organisation [7]. We have now extended these studies to a series of modified chlorophylls, in order to understand the role played by the different functional groups on pigment insertion and lamellar phase organisation. The ordering of the lamellar phases was studied by means of NMR quadrupolar splitting technique of the ^2H_2O (99.9% isotope enrichment) used as solvent. The surfactant was chosen on account of the large lamellar region present in the phase diagram of the AOT–water system [8–10].

In addition to Chl, the following pigments were used as host pigments: pyrochlorophyll *a* (Pyro-Chl), which lacks the 13^2-carbomethoxy-group, Zn-pheophytin *a* (Zn-Phe) in which the central Mg is replaced by Zn, and Zn-pheophorbide *a* (Zn-Pheid) which in addition lacks the 17^3-phytyl chain.

2. Experimental

Chl was isolated from fresh spinach leaves as previously described [11–13]. Purity and concentration were routinely checked using the criteria described elsewhere [14,15]. Pyro-Chl, Zn-Phe and Zn-Pheid were synthesised by standard procedures [16,17]. All pigments were stored in the dark at 243 K under N_2.

Lamellar phases were prepared by weighing appropriate amounts of AOT and ^2H_2O into glass tubes, which subsequently were sealed immediately. The contents of the tubes were mixed by repeated centrifugation, and kept at 298 K throughout. Lamellar phases containing the pigments were prepared by dissolving appropriate amounts of the chlorophylls in a 1% AOT solution, further addition of AOT and sealing. AOT from Sigma was used without further purification.

^{2}H-quadrupolar splittings were determined with a model XL 200 NMR spectrometer (Varian), equipped with a superconducting magnet of 4.7 T, using a quadrupolar echo sequence [18].

3. Results and discussion

The quadrupolar splitting of the ^2H_2O NMR signals originates from the interaction of the ^{2}H quadrupolar moment with electric field gradients, such as those present on the surfactant aggregate surfaces. The technique is very sensitive to the anisotropy and orientation of the aggregate director axis, which undergoes motions that are slow compared to the nuclear relaxation. The quadrupolar interaction leads to a splitting of the NMR signal into $2I$ equidistant peaks. I is the spin quantum number of the quadrupolar nuclei, which is 1 for ^{2}H. The magnitude of the quadrupolar splitting, $\Delta\nu$, is described by the following equation [19]

$$\Delta\nu = \frac{3|\chi S|}{4I(2I-1)}$$

where χ is the nuclear quadrupolar coupling constant, and S is the order parameter defined as:

$$S = \frac{1}{2}\langle 3\cos^2\theta - 1\rangle$$

where θ is the angle between the principle axis of the quadrupolar tensor and the symmetry axis of the liquid crystal.

In addition to the quadrupole splitting signals, a central peak at the resonance Larmor frequency may be observed in the ^2H_2O powder-like spectrum of a liquid crystal, which is due to some extent of isotropic phase or phase inhomogenities. The latter reflect the coexistence of regular lamellae with defective phases, such as curved barriers, rippled structures or interlamellar bilayer disks [20–22].

Quadrupolar spectra of the lamellar samples containing pigments, as well as of the lamellar phase sample without chlorophylls, at 30% AOT, were recorded at different times after sample preparation (Fig. 1). The progressive disappearing of the central signal and the progressive increase of the split peaks, indicate the formation of the ordered lamellar phase. The time course of these changes is rather different for the different samples. In the sample without pigment, immediately after the preparation, no evidence of the ordered phase formation was observed (see Fig. 1a). The spectrum after 48 days is still given by a convolution of two components, indicative of the contemporary presence of significant amounts of isotropic and lamellar phases. The central peak relative to the isotropic phase becomes lower and lower in time, until it completely disappears after 198 days. In all samples containing pigments, the presence of the ordered phase is already evident immediately after their preparation. The time course is speeded up slightly with Pyro-Chl (trace c) increases further with Chl (trace b), and even more with Zn-Phe (trace d). Conversely, a rather different behaviour is evidenced by the samples containing Zn-Pheid (trace e), which show a sensitive slowing down of the lamellar phase formation compared to the sample without pigments. In fact, in the presence of Zn-Pheid a very high quantity of isotropic phase is observed even at our longest observation time.

To improve the assaying of the lamellar phase formation, it is convenient to introduce a parameter, R, defined as the ratio of the amplitudes of the quadrupolar splitting and the isotropic signal. The R-values of samples prepared at different AOT concentrations (15%, 20%, 30% w/w), as well as with different pigments at the same concentration (5×10^{-5} M) and at two different times (48 and 198 days) after sample preparation, are reported in Fig. 2A,B. The R values of samples that do not contain pigments are also shown. In all samples, lamellar phase formation increases with increasing AOT concentration. At low AOT concentration (15%), all solutions except that

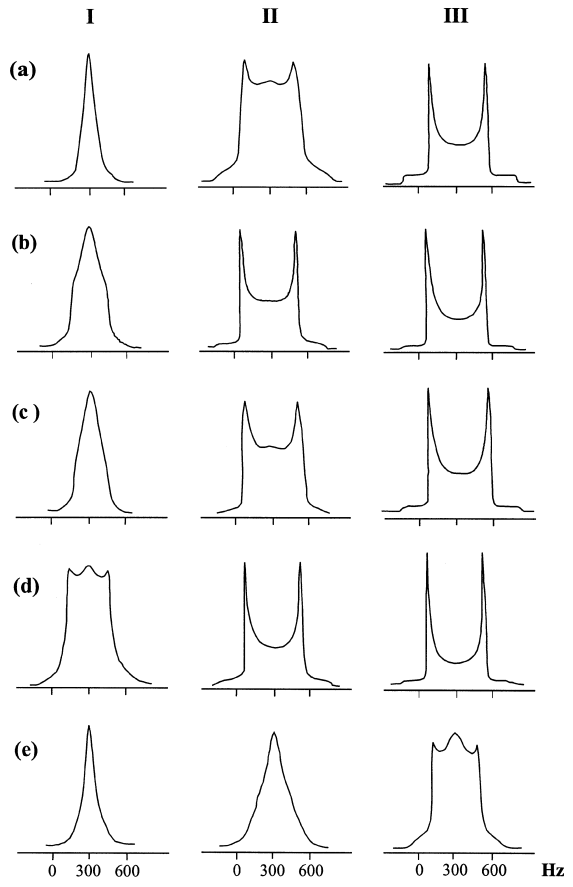

Fig. 1. Deuterium NMR spectra recorded just after sample preparation (I), after 48 days (II) and 198 days (III) from sample preparation of 30% (w/w) AOT solutions: (a) in the absence of pigments; (b) in the presence of chlorophyll *a*; (c) in the presence of pyrochlorophyll *a*; (d) in the presence of Zn-phephytin *a*; and (e) in the presence of Zn-pheophorbide *a*.

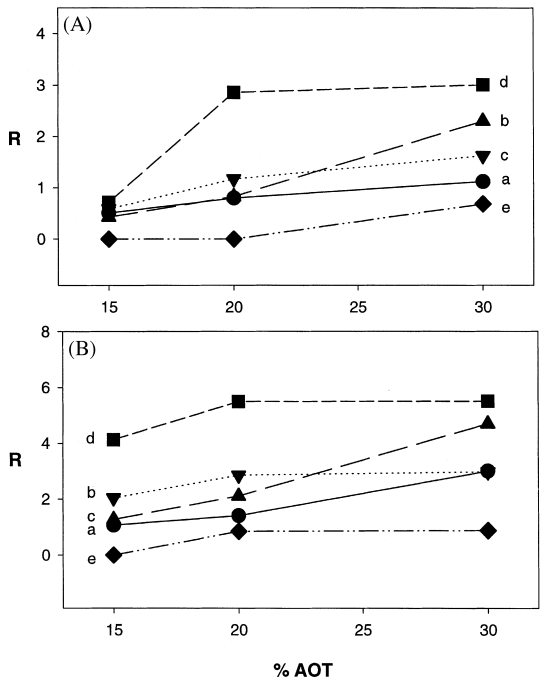

Fig. 2. *R*-Values vs. % (w/w) of AOT in lamellar phases (A) after 48 days and (B) 198 days from sample preparation: (a) in the absence of pigments; (b) in the presence of chlorophyll *a*; (c) in the presence of pyrochlorophyll *a*; (d) in the presence of Zn-pheophytin *a*; and (e) in the presence of Zn-pheophorbide *a*.

containing the Zn-Phe, show only a very slow lamellar phase formation, if judged from the absence of well-defined splitting signals up to 198 days from the sample preparation.

It is also evident that the presence of the different pigments influences the anisotropic phase formation, speeding up the process with respect to the corresponding sample of AOT alone, and, in the case of Zn-Phe and Chl, also promoting the formation of more ordered lamellae. The last mentioned effect is shown in the spectra recorded after 198 days, by the higher value of *R* relative to the two pigments at all the AOT concentrations. The Zn-Pheid represents again the unique exception with respect to the general behaviour. The addition of this phorphyrin not only fails to accelerate the ordered phase formation, but seems to even hinder the formation of the lamellae. This experimental evidence is emphasised in the data presented in Fig. 3A, where the *R* values for samples containing 30% AOT are reported as a function of the time elapsed after preparation.

Despite the different rate of lamellar phase formation, the size of the splitting, $\Delta\nu$, reaches the same equilibrium value for all samples, irrespective of the particular pigment present, and irrespective of its presence. This information is inferred by the analysis of the data reported in Fig. 3B, where the value of the ratio, f, between the quadrupolar splitting relative to the relaxed ($\Delta\nu^{r}$) and unrelaxed ($\Delta\nu^{u}$) samples, is reported as a function of time for all the samples examined, taking as relaxed value that recorded after 198 days. This ratio is defined as [20]

$$f = \frac{\Delta\nu^{u}}{\Delta\nu^{r}} = \frac{1}{1+\eta}$$

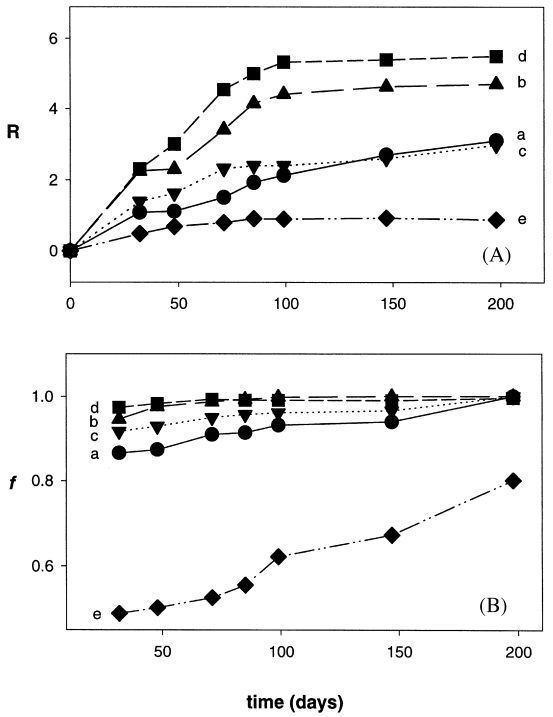

Fig. 3. (A) Time dependence of the *R* parameter and (B) time dependence of the *f* parameter relative to 30% (w/w) AOT lamellar phases: (a) in the absence of pigments; (b) in the presence of chlorophyll *a*; (c) in the presence of pyrochlorophyll *a*; (d) in the presence of Zn-pheophytin *a*; and (e) in the presence of Zn-pheophorbide *a*.

and can be immediately related to the asymmetry parameter η, which is zero for highly ordered lamellae. The ratio f is therefore indicative of the amount of defects present in the lamellar phase. The experimental evidence that the size of the splitting, $\Delta\nu$, reaches the same equilibrium value for all samples, indicates, in addition, that the presence of the pigments does not modify the structure of the mesophase. Once again, it is evident that the Zn-Pheid negatively influences the formation of a perfect bilayer structure.

Taken together, the data clearly indicate an influence of the pigment structure on the kinetics of lamellar phase formation, with the most relevant effect exerted by the phytyl 'tail' at C-17[3]. The role of the phytyl chain in increasing the order parameter of nematic uniaxial liquid crystals has been already reported in the literature [23]. The NMR data presented here, underline the capacity of the chlorophyll aliphatic chain of anchoring the cyclic tetrapyrroles into the bilayer, favouring at the same time the formation of regular lamellae. By contrast, the absence of the tail has a disordering effect. The significance of this information becomes clear if we consider the importance of the non-random orientation of chlorophyll for the efficiency of the energy transfer mechanisms by Förster exciton mechanism. The observation that the pigment chain is able to influence the organisation of the matrix in which is embedded, supports the hypothesis that the binding of chlorophylls is coupled to the folding of the apoproteins and affects the polypeptide incorporation into the lipid membrane [24,25].

Last but not least, additional information can be inferred on the pigment organisation from the UV-Vis spectra (not shown). Immediately after the preparation, those samples characterised by the absence of the NMR splitting, showed a strong absorption at 730–740 nm, indicative of pigment aggregate formation. The formation of the lamellae was always accompanied by the disaggregation of the pigments, evidenced by the appearance of an absorption in the 670 nm region, which is characteristic of monomeric chlorophylls. After 48 days, this monomerisation is complete. The initial amount of aggregated pigment form was particularly low with Zn-Phe. The greatest influence of this pigment in accelerating the formation of the lamellae can, therefore, be ascribed to its lower capacity to form aggregated species. The coordination number of the Zn is lower as compared to that of the Mg, but it is not clear if and how the central metal is involved in aggregation in aqueous detergent phase [26,27].

It has been previously reported [7] that the presence of a negative charge on the polar head of the surfactant, in micellar systems, always results in the Chl aggregation. The experimental evidence reported above, therefore, suggests a preferential insertion of the pigments into the intralamellar space, the hydrophobic core of the anisotropic phase. The presence of the phytyl chain is, however, the factor determining the ordered insertion of the pigment, as evidenced by the data obtained with Zn-Pheid, that, although always present in the monomeric form, does not promote the formation of ordered lamellae.

Further experiments are in progress to study energy transfer and/or dissipation among the chlorophylls in these systems, and compare them to the light-harvesting complexes.

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