Biochimica et Biophysica Acta, 636 (1981) 193–200
Elsevier/North-Holland Biomedical Press

BBA 48072

THE DETERGENT AND SALT EFFECT ON THE LIGHT-HARVESTING CHLOROPHYLL *a*/*b* COMPLEX FROM GREEN PLANTS

M.D. IL'INA, E.A. KOTOVA and A.Yu. BORISOV

A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Corpus 'A', Moscow 117234 (U.S.S.R.)

(Received February 10th, 1981)

Key words: Fluorescence; Pigment-protein complex; Detergent effect; Light-harvesting complex; Salt effect; (Pea chloroplasts)

The light-harvesting accessory pigment-protein complex (LHC) with a chlorophyll (Chl) *a*/*b* ratio of 1.2 was isolated by treating pea chloroplasts with Triton X-100. The LHC was used to investigate the action of ionic (sodium dodecyl sulfate) and non-ionic (Triton X-100) detergents. By optical methods (absorption and fluorescence spectra, measurements of fluorescence yield, φ, and lifetime, τ) two successive stages of the process were demonstrated, namely (1) interaction between detergent monomers and proteins and (2) solubilization of pigments into detergent micelles, which is facilitated by the presence of salts. The concentration ranges, characteristic of these stages, differ by 1.5–2 orders of magnitude for SDS, but slightly overlap for Triton X-100. At the second stage, certain changes occur in LHC absorption and fluorescence spectra. Several stable states of the LHC were established: (1) an aggregated state formed in the presence of 10 mM $MgSO_4$ with $\tau \approx 0.6$ ns; (2) the dialyzed LHC with $\tau \approx 0.9$ ns; (3) the states of the LHC in detergent solution with $\tau \approx 2.3$, 2.9, 3.4 ns; (4) a 30 kilodalton monomer obtained by SDS-polyacrylamide gel electrophoresis with $\tau \approx 4.1$ ns. The fluorescence parameters of the LHC states were compared with those of Chl *a* in detergent micelles (for the micelles $\tau = 5.6$–6.0 ns). The τ/φ ratio (the criterion for emission heterogeneity) for the LHC in the absence of a detergent was shown to be higher at least by a factor of 3.5 than that for Chl *a* in the presence of a detergent. Successive additions of the detergent to the LHC cause gradual decrease in the τ/φ ratio, and for the LHC monomer it reaches practically the same value as for Chl *a* in detergent micelles. The results are discussed on the basis of the data obtained previously. It is suggested that in vivo LHCs do not form such aggregates as in water solution without a detergent.

Introduction

A substantial advance in the investigation of the primary processes of plant photosynthesis during the recent years became possible owing to success in isolation of structural components of chloroplast membranes, i.e. three pigment-protein complexes: those of Photosystem I and Photosystem II and the light-harvesting accessory complex (LHC). Their chemical composition and functional properties are well described in a number of reviews [1,2]. However, the quantitative data on the fluorescence of these complexes are scarce. Measurement of the fluorescence parameters τ and φ with a system more homogeneous than chloroplasts seems to be fruitful. It will extend our knowledge of energy migration and primary photochemistry in green plants. However, the correlation between the state of pigments and proteins in isolated complexes and intact chloroplasts should be estimated.

It is known that detergents are usually employed for membrane disintegration in procedures of isolation of pigment-protein complexes. Proteins devoid of a hydrophobic membrane undergo certain structural changes in water solution, often aggregate and

Abbreviations: LHC, light-harvesting complex; Chl, chlorophyll; SDS, sodium dodecyl sulfate.

precipitate. This makes optical measurements more difficult. That is why surfactants are frequently added to solubilize proteins and reduce light scattering. But the presence of surfactants cannot but affect the activity and properties of an object under study. The most comprehensive elucidation of this problem was made by Helenius and Simons [3], but information concerning detergent interaction with plant pigment-protein complexes is lacking.

Detergents are known to exist as monomers in water solution at concentrations below the critical level, whereas above it they form micelles. According to the available data [3], only detergent monomers interact with proteins. They bind to hydrophobic sites of the latter by alkyl tails and often induce conformational changes or even disruption of the protein macrostructure, which leads to the binding of new detergent molecules (the cooperative effect). The higher the critical micellar concentration, the more detergent molecules are bound cooperatively and the greater is the probability of protein denaturation. Micelle formation diminishes the concentration of free detergent monomers in solution and prevents protein denaturation, although it facilitates its delipidation. As far as pigment-protein complexes are concenrned, their pigments can form mixed micelles with detergents because both detergents and pigments are amphiphiles.

This paper deals with fluorescence and absorption properties of the LHC in water solution. The effect of detergents and salts on these properties was investigated in detail. The data obtained were compared with those for the LHC in the chloroplast membrane and suggestions as to the structural organization of the LHC in vivo are given.

Materials and Methods

The LHC was isolated from pea chloroplasts treated with 3.3% Triton X-100 by separation of the mixture of complexes from solubilized lipids on DEAE-cellulose column and elution with 0.3 M NaCl followed by either of the two procedures [4]: (1) the salting-out of the LHC in the cold with a subsequent dialysis at 16°C against 5 mM Tris-HCl buffer (pH 8) with four changes during 8 h (LHC-I); (2) fractionation of the mixture of complexes on anion-exchange DEAE-Sephadex A-25, the LHC being eluted with a volume of 0.1 M NaCl/0.05% Triton X-100 constituting 0.35–0.55 column bed volume (LHC-II).

Chl *a* micelles in 0.1% Triton X-100 or 5% SDS were prepared by adding Chl *a* dissolved in acetone to the detergent solution, the acetone volume being about 2–3% of the overall volume.

Electrophoresis in 10% polyacrylamide gel was performed at room temperature in potassium-phosphate buffer (pH 7) containing 0.1% SDS, as described [5]. The dialyzed LHC-I was incubated at room temperature with 1% SDS for 2 h and then applied onto the gel (20 μg Chl per tube). Molecular weight markers were α and γ subunits of factor F_1 from bovine heart mitochondria (54 and 33 kdalton, respectively [6].

The absorption spectra were measured with SF-10 (LOMO, U.S.S.R) and Specord UV-VIS dual-beam recording spectrophotometers, the spectra of fluorescence emission excited by the 436 nm mercury line with an Aminco Bowman spectrofluorimeter. Light-induced absorbance changes in the 600–750 nm spectral range were determined as described [4] with a modified SF-10 instrument. The sensitivity of the differential scheme amounted to $5 \cdot 10^{-5}$ absorbance units.

Fluorescence τ and φ were measured with a phase-type fluorometer [7]: τ with an accuracy of about ± 0.02 ns, the relative values of φ in μA units (± 0.005). Fluorescence was excited by 404 and 436 nm lines from a high-pressure mercury lamp and detected through a glass filter transmitting the $\lambda > 620$ nm wavelength range. The intensity of the exciting light was 10^5 erg $\cdot$ cm^{-2} $\cdot$ s^{-1}.

Optical measurements were made at room temperature in 1.0 cm pathlength cells. For fluorescence measurements, samples with absorbances below 0.10 in the red peak were employed. The dependence of τ, φ and τ/φ on the detergent concentration was studied with the same sample by gradually increasing the detergent content in the medium. The measurements were carried out either immediately after the addition of a detergent, or 10 min later, as specified in the legends to figures. When the τ/φ ratio for the LHC was compared with that for CHl *a* in the detergent, the difference in the absorbances of these objects at the exciting light wavelengths was taken into account.

Both LHC-I and LHC-II have the Chl *a*/*b* ratio of

the order of 1.2. LHC-I contained one P-700 per 650–700 Chl molecules. LHC-II exhibited no light-induced absorbance changes around 700 nm in the presence of $5 \cdot 10^{-6}$ M *N,N,N',N'*-tetramethyl-*p* phenylenediamine. The protein content of LHC-II was estimated basing on the absorbance at 280 nm [8] corrected for the Triton X-100 contribution. The latter could be determined in each fraction of the elution profile by measuring the absorbance levels at 280 nm before and after the elution of the pigmented products. This estimate implied that colorless proteins were removed in the course of chromatography on DEAE-cellulose. Triton X-100 was determined by using the experimentally determined extinction coefficient, $E^{\%}_{280\text{nm},1\text{cm}} = 22$. The protein/detergent weight ratio for LHC-II was approx. 20 : 1. NaCl and Triton X-100 concentrations in the samples with non-dialyzed LHC-II were estimated according to the extent of dilution of the initial sample.

Chlorophyll *a* and Tris were purchased from Sigma Chemical Co. (St. Louis, MO), Triton X-100 from Ferak (Berlin), sodium dodecyl sulfate from Serva Feinbiochemica GmbH and Co. (Heidelberg).

Results and Discussion

Preliminary measurements of the fluorescence lifetime for pigment-protein complexes obtained by ion-exchange chromatography with 0.05% Triton X-100 [4] showed that τ may have an arbitrary value over the range of 0.5–5.5 ns, dependig on the extent of dilution of the initial sample. This fact prompted us to conclude that one should be careful in the evaluation of fluorescence data with samples obtained in the presence of detergents. Therefore, we started to examine thoroughly the action of nonionic detergent Triton X-100 and of anionic detergent SDS on the plant light-harvesting complex. These surfactants were chosen because of their being widely employed in preparation of pigment-protein complexes.

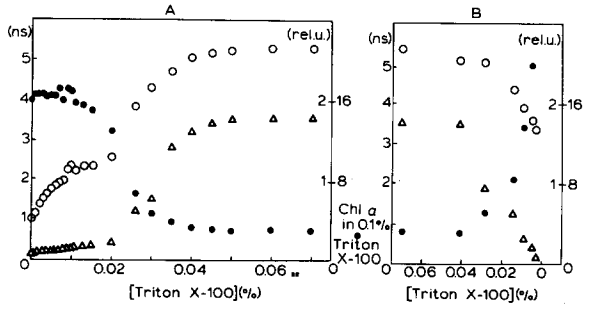

Fig. 1. The dependence of fluorescence lifetime (τ) (○), relative quantum yield (φ) (△) and τ/φ (●) ratio on Triton X-100 concentration for the dialyzed LHC-I in 5 mM Tris-HCl buffer, pH 8: (A) with gradual increase in detergent concentration in the medium; (B) upon dilution of LHC-I containing 0.07% Triton X-100 with 5 mM Tris-HCl buffer, pH 8. $A_{676\text{nm}} = 0.1$.

Triton X-100 effect. Fig. 1A demonstrates changes in the fluorescence parameters τ, φ and τ/φ of the dialyzed LHC-I occurring upon gradual increase of the Triton X-100 concentration in the incubation mixture from 0.001 to 0.07%. Two stages are discernable since there is some delay in the τ increase, when the detergent concentration changes from 0.009 to 0.015%. At the first stage, τ rises from 1.0 to 2.3 ns and at the second from 2.3 to 5.3 ns, the latter value being constant at concentrations above 0.045%. The next day measurement of τ showed that it reached 5.8 ns and became identical with τ measured for Chl *a* in 0.1% Triton X-100 micelles. When the sample of the nondialyzed LHC-II containing 10–15 mM NaCl was titrated with Triton X-100, the second state of the fluorescence increase began at concentrations below 0.01% and the first stage could hardly be distinguished in the titration curve.

As seen in Fig. 1A, the increase in τ is accompanied by a similar change in φ, with φ rising faster than τ over the concentration range of 0.013–0.04%. Thereby, the τ/φ ratio remains constant, until the Triton X-100 concentration exceeds 0.013%. Then τ/φ gradually decreases until at concentrations above 0.04% it becomes 5.9-times lower than the initial level. Finally, τ/φ reaches the value similar to that for Chl *a* in 0.1% Triton X-100.

A combined study of LHC-I absorption and fluorescence emission spectra showed no change in the peak positions in the LHC-I spectra upon addition of Triton X-100 at concentrations lower than 0.015%. Further rise in the concentration caused a gradual shift of the red maximum in the absorption spectrum from 676 to 668 nm (Fig. 2A and B) and of the fluorescence maximum from 682 to 677 nm. Finally, these spectral characteristics of the complex became similar to those of Chl *a* solubilized in 0.1% Triton X-100. Besides, the maximum in the Soret band of Chl *b* absorption spectrum gradually shifted from 472

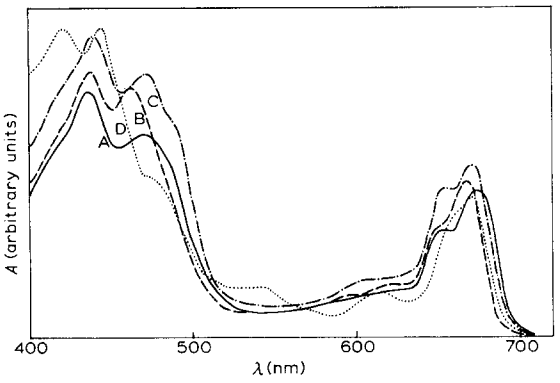

Fig. 2. Absorption spectra of the dialyzed LHC-I. (A) Without additions; (B) in the presence of 0.07% Triton X-100; (C) in the presence of 5% SDS; (D) the sample similar to (C) after overnight storage at 0°C.

to 462 nm, and the carotenoid shoulder disappeared at 490 nm (see Fig. 2B).

Successive dilution of the sample containing 0.07% Triton X-100 by 5 mM Tris-HCl buffer led to the reverse process of τ and φ quenching (Fig. 1B). Since not only the amount of the detergent but also the amount of the complex changed in the course of this process, the value of φ was regarded as fluorescence intensity multiplied by the dilution of the sample. Comparison of the data presented in Fig. 1B with those in Fig. 1A revealed a difference in the concentration dependence of τ. When the Triton concentration reached 0.003%, τ dropped to 3.35 ns, but not to 1.5 ns, as in Fig. 1A. The question concerning the stages of the quenching remains open, for its solution requires more points on the τ and φ curves at concentrations of 0.005–0.015%. We failed to detect spectral changes in this experiment. The accuracy of estimation of the peak position did not exceed ±2 nm because of the low absorbance of the samples obtained by dilution (final absorbance 0.0044).

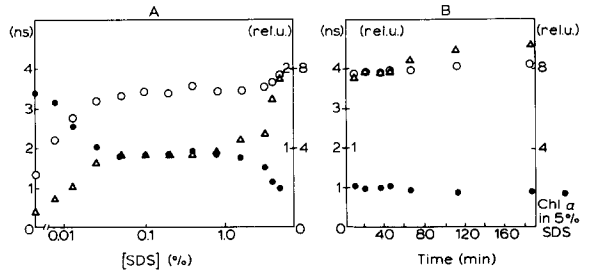

Fig. 3. (A) The dependence of fluorescence lifetime (τ) (○), relative quantum yield (φ) (△) and τ/φ ratio (●) on SDS concentration (up to 5%) for LHC-II in 5 mM Tris-HCl buffer, pH 8. The interval between each detergent addition and measurement was about 10 min. (B) Time courses of τ, φ and τ/φ for LHC-II with 5% SDS. A_{674nm} = 0.05.

SDS effect. Figs. 3–5 demonstrate results of the fluorescence studies with SDS. In the experiment with LHC-II containing approx. 10–15 mM NaCl and approx. 0.022% Triton X-100 (Fig. 3A), two stages are distinctly seen in the process of fluorescence enhancement. It is remarkable that the SDS concentration at which the second stage starts differs by two orders of magnitude from the concentration at which the first stage terminates. When the detergent content in the sample increase from 0.0075 to 0.05%, τ shows a rise from 1.3 to 3.4 ns. Over the concentration range of 0.05–1.5%, the values of τ and φ remain constant. The second stage of fluorescence enhancement develops slower than that in the case of Triton X-100. 3-h incubation of the LHC with 5% SDS (Fig. 3B) caused increase in τ only from 3.85 to 4.1 ns. During both stages, φ grew faster than τ, which resulted in a 4-fold decrease in the τ/φ ratio, the latter becoming equal to that for Chl *a* in 5% SDS or in 0.1% Triton X-100. It should be noted that τ measured for Chl *a* in SDS micelles amounted to 5.8 ns.

The time of incubation of the object with SDS being as short as possible (Fig. 4), we observed: (a) τ on the steady level lower than in Fig. 3A (2.86 ns); (b) slower fluorescence increase at the first stage, with the τ/φ ratio being constant and τ being stabilized to some extent in the region of 2.2 ns. (Similar results were obtained with the dialyzed LHC-I. Com-

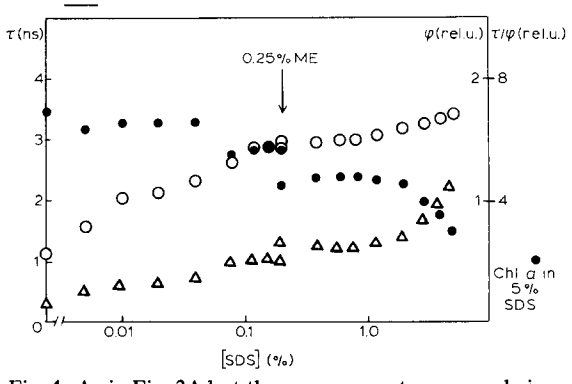

Fig. 4. As in Fig. 3A but the measurements were made immediately after addition of the detergent. 0.25% β-mercaptoethanol (ME) was added against 0.2% SDS background. A_{674nm} = 0.04.

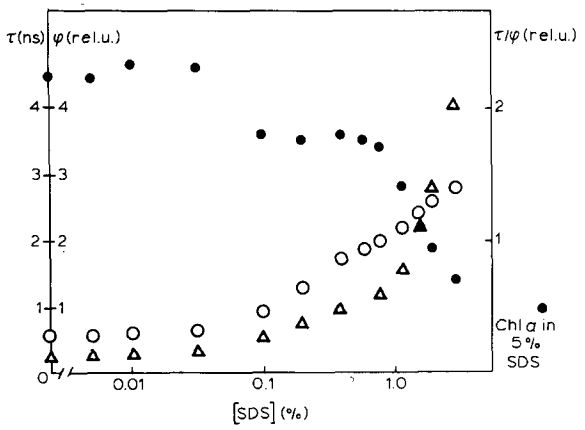

Fig. 5. As in Fig. 3A, but for the nondialyzed LHC-I. A_{676nm} = 0.06. NaCl concentration in the sample is higher than 50 mM.

pared to LHC-II, it showed a lower initial value of τ, namely about 0.7 ns.) Addition of 0.25% β-mercaptoethanol to this sample against the 0.2% SDS background induced a slight fluorescence increase (φ became 25% higher, τ changed from 2.86 to 2.95 ns), but did not alter the character of the concentration dependence.

The second stage of SDS effect on the LHC (at a concentration above 1.5–2%) was accompanied by blue shifts of the absorption (676 → 671 nm, Fig. 2A and C) and fluorescence (682 → 678 nm) maxima. The storage of the sample with SDS at 0°C led to: (a) increase in τ from 4.1 to 5.8 ns; (b) shift of the carotenoid shoulder from 490 to 480 nm; (c) complete pheophytinization of chlorophylls which manifested in shifts of the Soret bands (from 438 to 422 nm for Chl *a* and from 472 to 444 nm for Chl *b*) and in the emergence of new peaks near 515 and 545 nm (see Fig. 2D). It is noteworthy that pheophytinization of Chl *a* also takes place upon solubilization of the pure pigment in 5% SDS.

A study of the dilution effect with the sample stored with 3% SDS at 0°C showed that when the SDS concentration was reduced by two orders of magnitude (from 3 to 0.03%), τ dropped from 5.8 to 5.0 ns, with φ decreasing proportionally.

Fig. 5 illustrates the dependence, similar to those in Figs. 3 and 4, for the LHC-I which was not subjected to dialysis after the salting-out of the solution. The NaCl concentration exceeds 50 mM in this case. This object is characterized by: (a) a low initial value of τ (0.55 ns); (b) the development of the first stage of fluorescence enhancement at detergent concentrations higher than 0.1%; (c) continuous transition from the first stage to the second. The latter begins somewhat earlier than that in Figs. 3 and 4, i.e. at 0.9–1.0% SDS, as evidenced by the sharp rise in φ.

Two stages of interaction between detergents and LHC. The data presented in Figs. 1–5 indicate that interaction between detergents and the LHC involves two successive stages, each occurring over a specific concentration range (Table I). It is difficult to distinguish these stages for Triton X-100 since the concentration specific for the second stage is only about twice as high as that corresponding to the first stage. However, in the case of SDS, these concentration ranges differ by 1.5–2 orders of magnitude. The first stage develops at detergent concentrations far below the critical one, with no change occurring in the

TABLE I

INTERACTION BETWEEN DETERGENTS AND LHC

Stage	Object	[NaCl] (mM)	[Triton X-100] [a] (%)	[SDS] (%)
I. Binding to proteins	LHC-I	–	<0.009 [b]	–
	LHC-II	≈10–15	<0.003	<0.01 [c]
	LHC-I	>50		0.1–0.9 [d]
II. Solubilization of pigments into micelles	LHC-I	–	⩾0.02 [b]	–
	LHC-II	≈10–15	⩾0.008	⩾1.5–2 [e]
	LHC-I	>50	–	⩾0.9–1.0 [d]

[a] The critical micellar concentration for Triton X-100 is $3.2 \cdot 10^{-4}$ M (approx. 0.02%); for SDS $2.5 \cdot 10^{-3}$ M (approx. 0.07%) [9], [b] data taken from Fig. 1A, [c] data from Fig. 4, [d] data from Fig. 5, [e] data from Figs. 3A and 4.

visible region of the LHC absorption spectrum. The second stage is characterized by the following: (a) it develops at detergent concentrations above the critical level; (b) it is accompanied by blue shifts of Chl *a* absorption and fluorescence maxima, which eventually makes the spectra similar to those of Chl *a* in detergent micelles; (c) the τ/φ ratio drops abruptly and reaches the same value as that for Chl *a* dissolved in the detergent; (d) the value of τ after prolonged incubation of the LHC with the detergent becomes similar to that for Chl *a* in the detergent micelles. All this makes it possible to attribute the first stage to the interaction between detergent monomers and proteins of the LHC and the second to the extraction of pigments from the LHC and the formation of mixed micelles.

Reduction of the detergent concentration and, consequently, of the micelle content in the sample (Fig. 1B) entails decrease in τ and φ. This is, apparently, due to the accumulation of pigments in the remaining micelles, as well as to their release into the water solution and the reverse adsorption on proteins. Further investigation of this phenomenon is important for understanding the mechanisms of the photosynthetic membrane self-assembly.

Our attention was drawn to the fact in the case of SDS the pigments were solubilized at concentrations much higher than the critical level known for this detergent. This might be accounted for by an increase in the critical micellar concentration caused by strong SDS binding to LHC proteins or by electrostatic repulsion between negatively charged groups on the surface of SDS micelles and LHC proteins. It is noteworthy that electrophoretic separation of pigment-protein complexes in the presence of SDS is successfully carried out due to the above-mentioned peculiarity. Otherwise, complexes would easily lose all the pigments and would, consequently, be unidentifiable. A certain part of the pigment still appears to be lost, which thus hinders determination of the real molecular weight and composition of complexes.

As follows from Table I, the presence of a salt diminishes the detergent concentration at which pigment solubilization starts by a factor of 1.5–2.5. The effect of salts seems to be evidenced in neutralization of the negative charges of micelles in the case of SDS and in the lowering of the extent of detergent hydration in the case of Triton X-100. This should be borne in mind when salt gradients are used in ion-exchange chromatography for separation of complexes in the presence of Triton X-100. The binding of SDS to proteins is affected by salts in the opposite manner. The concentration at which the first stage begins increases at least by one order of magnitude when the salt content in the sample exceeds 50 mM. This is, probably, due to change in the protein conformation and decrease in accessibility of the detergent to protein binding sites.

Polyacrylamide gel electrophoresis with SDS. LHC-I was subjected to electrophoresis in the presence of 0.1% SDS to separate the products obtained by prolonged incubation of the complex with 1% SDS. The electrophoresis yielded two green bands. They were cut out of the gel, crushed with a scalpel and homogenized in 5 mM Tris-HCl buffer (pH 8) with 0.3% SDS. The absorption and fluorescence spectra were recorded; τ and φ were measured for the eluates obtained. A fraction of molecular weight of about 29–31 kdalton had the spectral properties of the original complex and $\tau \approx 4.1$ ns, which did not change when the SDS concentration increased to 0.6%. The second (more mobile) band localized in the region of 12–15 kdalton contained pheophytins *a* and *b* and carotenoids with a spectrum similar to that in Fig. 2D. It was a fraction of the so-called 'free' pigments solubilized by detergent micelles. Its fluorescence lifetime amounts to 5.4 ns. The difference in the τ/φ ratios for these two fractions did not exceed 20%. The extract from the first band being kept at 0°C, the pigments from the LHC were completely solubilized as its τ increased to 5.5 ns and the absorption spectrum became similar to that of the second fraction.

Salt effect. The data shown in Fig. 5 point out that the presence of salts causes shortening of the initial τ value of the LHC. In this connection, we investigated τ variations depending on the Mg^{2+} content in the medium. The increase in Mg^{2+} concentration from 2.5 to 10 mM gave rise to visible aggregation of the complex accompanied by proportional quenching of τ and φ. As follows from Fig. 6, τ of the dialyzed LHC-I dropped from 1.07 to 0.77 ns in the presence of 10 mM $MgSO_4$. 5 min exposure of this sample to the exciting light brought about further decrease in τ to 0.65 ns, the latter value remaining unaltered for several months.

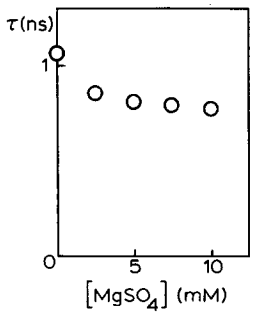

Fig. 6. The effect of $MgSO_4$ on fluorescence lifetime (τ) of the dialyzed LHC-I in 5 mM Tris-HCl buffer, pH 8. A_{676nm} = 0.055.

Stable states of the LHC. It is remarkable that at the first stage of interaction with the detergent, the LHC goes through a number of (at least four) quasi-steady states. They are characterized by certain values of τ: 2.3, 2.9, 3.4 and 4.1 ns (Table II). The higher the τ value, the greater the stability of the LHC state. Basing on the value of protein mobility in the electrical field, one may conclude that the state with $\tau \approx 4.1$ ns corresponds to the smallest structural unit (monomer) of the LHC. This follows from the fact that the subsequent treatment of the complex with SDS leads to its denaturation and complete delipidation, resulting in isolation of nonpigmented polypeptides with molecular weights of about 23–25 kdalton [10]. Another two states of the LHC may be added to those already mentioned above. One of them, with $\tau \approx 0.9$ ns, is observed in water solution without a detergent, the other ($\tau \approx 0.6$ ns) under higher ionic strength conditions.

TABLE II

FLUORESCENCE PARAMETERS (τ AND THE τ/φ RATIO) FOR DIFFERENT FORMS OF LHC IN WATER SOLUTION

	τ(ns)	(τ/φ of LHC)/(τ/φ of Chl *a* in detergent micelles)	
		LHC-I	LHC-II
LHC + salt	≈0.6	≈5.5	–
LHC	0.7–1.1	≈5.5	3.5
LHC + detergent	2.3	5.0	3.2
LCH + detergent	2.9	4.5	3.0
LCH + detergent	3.4	–	2.0
LHC + detergent subjected to electrophoresis	4.1	1.0	–

We determined the τ/φ ratio for all the states observed in our experiments. In the case of homogeneous emission, this ratio is known to be equal to τ_o, the radiative lifetime, or $1/K_{fl}$, where K_{fl} is the constant of intramolecular radiative deactivation. Changes in the τ/φ ratio determined by the phase-shift method allow us to estimate the heterogeneity of emission. According to the formulae described in Ref. 11, the appearance of highly quenched (i.e., short-lived) fluorescence components causes much greater decrease in the measured value of φ than in τ, with the τ/φ ratio higher than that in the case of homogeneous emission. The constancy of the τ/φ ratio with concomitant changes in both parameters indicates that the total rate of excitation decay changes for all the Chl molecules, e.g., energy migration to the quenching centers occurs.

The τ/φ ratio for LHC-I without a detergent is higher by a factor of 5–6 and for LHC-II by at least a factor of 3.5 than that for Chl *a* in the detergent solution and in the LHC monomer. We explain this fact by the heterogeneity of the emission and, in particular, by the presence of weakly fluorescent pigment forms. In the case of LHC-I, this may be in part due to contamination by Photosystem I. The addition of a salt to the LHC induces a proportional quenching of τ and φ, probably due to energy migration towards new quenching centers. If the incubation mixture contains a detergent, the LHC displays gradual fluorescence enhancement with a higher increase in φ than in τ: φ rises 20–25-fold; τ from 0.9 to 4.1 ns. It should be observed that the disproportion in φ and τ quenching is lowered most appreciably after the LHC reaches the state with $\tau \approx 3$ ns. The emission of the LHC monomer with $\tau = 4.1$ ns is homogeneous.

Thus, the adsorption of Chl *a* on proteins in the course of LHC formation manifests in the decrease of τ from 5.8 to 4.1 ns, with τ/φ ratio remaining constant. This means that the K_{fl} of Chl *a* does not change, while both τ and φ decrease as a result of specific interaction between Chl *a* and the proteins and/or energy migration to the quenching centers. Assuming the τ_o for Chl *a* in solution to be 15.2 ns [12], one can estimate the fluorescence yield to be 27% for the LHC monomer.

According to our observations, both the removal of the detergent and the addition of salts induce visible aggregation of the LHC. This is consistent with the data in Refs. 10 and 13, where a regular arrange-

ment of LHC aggregates was demonstrated by the freeze-fracture micrograph technique. The detergent dissociates these aggregates into more and more minute fragments, down to monomers. It breaks hydrophobic interactions which maintain the protein macrostructure. The experiment with β-mercaptoethanol shows that S-S bonds play a negligible role in interaction between the LHC proteins. The above-mentioned forms of the LHC (Table II) with different levels of protein aggregation have identical spectral maxima but differ in τ and in the τ/φ ratio. Hence the protein-protein interaction increases the number of excitation quenching centers and weakly fluorescent Chl *a* forms in hydrophobic sites of the proteins, rather than alters appreciably the ratio of different spectral forms of the complex.

Relation to the in vivo state. It is interesting to compare different forms of the LHC in solution with its in vivo state. When the Photosystem II reaction centres are closed and there is no energy migration to Photosystem I (no spillover), $\tau = 1.5–1.6$ ns for spinach chloroplasts [14,15] and 1.6–1.8 ns for pea chloroplasts [7]. Lower values of τ may be attributed to the processes of chloroplast 'ageing' [7,16]. Taking into account that the closed reaction centres of Photosystem II are still excitation quenchers [17], one should expect the value of τ for the LHC in the thylakoid membrane to be higher than 1.6–1.7 ns. LHC aggregates with $\tau \leqslant 1$ ns fail to meet this requirement. The latter fact is in agreement with the results of the circular dichroism study of the LHC [18].

As shown in Ref. 19, the τ/φ ratio for Chl *a* in chloroplasts increases about 3.5-fold compared to that for Chl *a* in solution. However, this increase, due to heterogeneity of the emission, should not be ascribed exclusively to the LHC, but also to Chl *a* forms belonging to Photosystem I. Therefore, we believe the LHC in thylakoids to have $\tau \geqslant 3$ ns. Interaction between LHCs and other pigment-protein complexes results in decrease in τ. (The τ/φ ratio seems to be unchanged.) For instance, τ drops to approx. 1.7 ns in the absence of the spillover and to approx. 0.9 ns when the spillover takes place [7].

Note added in proof (Received May 8, 1981)

According to recent reports, the addition of the detergent to the LHC results in the disappearance of the circular dichroic signal of Chl *a* [18] and in the reduction of low-temperature fluorescence at 695 nm [13,20]. Together with our data on the decrease in emission heterogeneity (the drop in the τ/φ ratio) these findings provide evidence of the detergent-induced weakening of pigment-pigment interactions.

Acknowledgements

We are grateful to Dr. I.S. Tsybovsky for his assistance in performing electrophoresis and to Mrs. M. Krieger for correction of the English version of the manuscript.

References

1 Wessels, J.S.C. (1977) in Photosynthesis I: Photosynthetic Electron Transport and Photophosphorylation (Trebst, A. and Avron, M., eds.), pp. 563–573, Springer-Verlag, Berlin
2 Thornber, J.P., Markwell, J.P. and Reinmann, S. (1979) Photochem. Photobiol. 29, 1205–1216
3 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79
4 Il'ina, M.D. and Borisov, A.Yu. (1980) Biochim. Biophys. Acta 590, 345–352
5 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412
6 Knowles, A.F. and Penefsky, H.S. (1972) J. Biol. Chem. 247, 6624–6630
7 Il'ina, M.D. and Borisov, A.Yu. (1979) Biokhimiya 44, 40–49
8 Warburg, O. and Christian, W. (1942) Biochem. Z. 310, 384
9 Massini, P. and Voorn, G. (1968) Biochim. Biophys. Acta 153, 589–601
10 McDonnel, A. and Staehelin, L.A. (1980) J. Cell. Biol. 84, 40–56
11 Tumerman, L.A. and Sorokin, E.M. (1967) Mol. Biol. 1, 628–638
12 Brody, S.S. and Rabinowitch, E. (1957) Science 125, 555–557
13 Mullet, J.E. and Arntzen, C.J. (1980) Biochim. Biophys. Acta 589, 100–117
14 Briantais, J.M., Vernotte, C. and Moya, I. (1973) Biochim. Biophys. Acta 325, 530–538
15 Barber, J., Searle, G.F.W. and Tredwell, C.J. (1978) Biochim. Acta 501, 174–182
16 Sorokin, E.M., Fetisova, Z.G. and Tumerman, L.A. (1970) J. Prikl. Spektroskop. 13, 439–443
17 Duysens, L.N.M. (1979) Ciba Found. Symp. 61, 323–340
18 Gregory, R.P.F., Demeter, S. and Faludi-Daniel, A. (1980) Biochim. Biophys. Acta 591, 356–360
19 Borisov, A.Yu. and Il'ina, M.D. (1969) Mol. Biol. 3, 391–405
20 Ryrie, I.J., Anderson, J.M. and Goodchild, D.J. (1980) Eur. J. Biochem. 107, 345–354