The 77 K fluorescence spectrum of the Photosystem I pigment–protein complex CPIa

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Photosystem I *Pigment–protein complex* *CPIa* *Fluorescence* *Light-harvesting complex*

1. INTRODUCTION

'Low-salt' thylakoids solubilized in SDS show a high F_{730}/F_{685} fluorescence ratio at 77 K; this ratio is drastically decreased upon cation addition (Mg^{2+}, H^+) [1,2]. This effect has been attributed to the cation-induced dissociation of the supramolecular structures of pigment–protein complexes [3,4] and in particular of complex CPIa, derived from PS I [1,2,4].

CPIa isolated by sucrose density gradient centrifugation [5] has 100 Chl per P700 (Chl *a*/Chl *b* = 4.5) and emits strongly at 732–735 nm, at 77 K; it is composed of (a) the reaction center complex CPI, with 30 Chl per P700, which emits at 720 nm, at 77 K; (b) the light-harvesting components of PS I (LHC-I) (24-, 23-, 21-, 20-kDa polypeptides, Chl *a*, Chl *b* [Chl *a*/Chl *b* = 3.8], xanthophylls) [5–7] and (c) low molecular mass polypeptides of 16, 14, 11, 10 and 9 kDa [5–7]. The polypeptide composition of CPIa is identical to that of Triton X-100-extracted PS I-110 particles [6].

LHC-I can be isolated from Triton X-100-extracted particles after mild SDS–PAGE; its electrophoretic mobility is identical to that of $LHCP^2$, the light-harvesting dimer form [6]. Thus, LHC-I is also present in the $LHCP^2$ band separated by SDS–PAGE of SDS-solubilized thylakoids, and in the $LHCP^{1+2}$ light-harvesting complex mixture separated from thylakoids by SDS-sucrose density gradient centrifugation [5,6]. Its presence in these bands is more pronounced whenever the thylakoid starting material is a fraction derived from stroma lamellae, since the latter are enriched in PS I particles.

The presence of LHC-I can be verified by its high long-wavelength fluorescence emission at 77 K. Indeed, LHC-I separated from PS I-110 particles by mild SDS–PAGE exhibits high 729 nm fluorescence [6]. It has been proposed that this fluorescence emanates from a chlorophyll-containing polypeptide of 21 kDa [6], since it was found that the LHCP oligomer mixture of stroma lamellae is rich in this polypeptide [6,8].

In an attempt to separate the 21-kDa polypeptide of LHC-I, and to correlate the low-temperature fluorescence characteristics of CPIa components with the polypeptide composition and organization of the complex, efforts have been made to isolate the LHC-I components from either the CPIa or $LHCP^2$ complex, separated after mild SDS–PAGE of chloroplast thylakoids, or from the $LHCP^{1+2}$ oligomer mixture, obtained after SDS–sucrose density gradient centrifugation of stroma lamellae.

The results show that an LHC-I preparation containing only the 21-kDa polypeptide can be successfully isolated by mild SDS–PAGE of the isolated complex CPIa, after its partial dissociation in the presence of Na^+. This preparation ex-

Abbreviations: Chl, chlorophyll; CPI, the Chl *a*-rich P700–pigment–protein complex originating in Photosystem I; CPIa, the pigment–protein complex containing CPI and the light-harvesting components of Photosystem I; LHCP, light-harvesting pigment–protein complex; LHC-I, the light-harvesting complex of Photosystem I; PAGE, polyacrylamide gel electrophoresis; PS, photosystem

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hibits high 717 nm emission at 77 K; upon cation addition the 717 nm emission is gradually blue-shifted. Similarly, the 720 nm emission of CPI is blue-shifted in the presence of cations. It is concluded that the emission spectrum of CPIa (and of PS I) depends on the organizational state of the pigment–protein complex components.

2. MATERIALS AND METHODS

Chloroplasts were isolated from etiolated pea or bean plants exposed to continuous light for some days. Thylakoids, isolated as in [5], were washed twice with 0.05 M Tricine–NaOH (pH 7.3).

The pigment–protein complexes were separated by mild SDS–PAGE at 4°C [9] (SDS/Chl = 10, Chl/ml = 600–700 μg). The CPIa or LHCP^2 zones were excised after separation and rerun on new polyacrylamide gels. The new gels contained 100 mM NaCl in their stacker for dissociation of CPIa and LHCP^2 complexes to their components [3].

Sucrose density gradient centrifugation was as in [5], but the gradient contained 0.1% SDS–0.2% Triton X-100–0.2% deoxycholate. The LHCP^{1+2} oligomer band was isolated and reapplied on a new gradient [5–22% sucrose containing 0.1% SDS in 0.05 M Tris–borate buffer (pH 9.4)]. Centrifugation was done at 10°C for 5–6 h in an SW 65 rotor and at 400000 × g.

Stroma lamellae (144000 × g fraction) were separated from French press-disrupted pea chloroplasts as in [10]; they were washed in Tricine–NaOH (pH 7.3).

Dissociating SDS–PAGE on slabs was done as in [11] and the polypeptides resolved were stained with Coomassie or $AgNO_3$ [12]. The samples used for polypeptide analysis were concentrated by lyophilization. The pigment–protein complexes were excised from the gels and eluted by grinding in a mortar in the presence of water. They were then recovered in the supernatant after centrifugation.

Fluorescence spectra were recorded as in [1], and chlorophyll was determined according to [13].

3. RESULTS AND DISCUSSION

Recent analysis by mild SDS–PAGE of the Triton X-100-extracted PS I-110 particles (a preparation containing 110 Chl per P700 [14]) has shown that these particles are composed of CPIa, CPI and LHC-I [6]. The latter complex had low temperature emission at 729 nm, and electrophoretic mobility after mild SDS–PAGE similar to that of the LHCP^2 dimer form of the light-harvesting complex; when analyzed further by dissociating SDS–PAGE it was found to contain two polypeptide doublets of 24–23 and 21–20 kDa [6].

The CPIa complex separated directly from SDS-solubilized thylakoids after SDS–sucrose density gradient centrifugation [5] has identical polypeptide composition with that of Triton X-100-extracted PS I-110 particles [6] (also shown in fig.1). CPIa therefore also contains LHC-I. Moreover, since LHC-I has identical electrophoretic mobility with that of the LHCP^2 dimer form of the light-harvesting complex, the LHCP^2 band separated by mild SDS–PAGE, as well as the LHCP^{1+2} oligomer mixture separated after sucrose density gradient centrifugation also contains the LHC-I component [6]. These complexes have been used here as starting material for further separation of LHC-I.

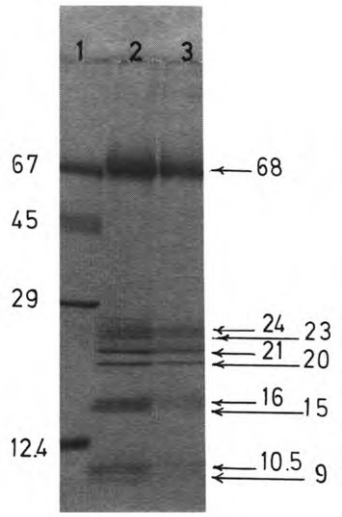

Fig.1. Polypeptide resolution by dissociating SDS–PAGE of Triton X-100-extracted PS I-110 particles (lane 2) and of the CPIa complex, separated from SDS-solubilized thylakoids after sucrose density gradient centrifugation (lane 3). Lane 1, standard protein markers.

3.1. *Isolation of LHC-I from CPIa and LHCP[2] complexes, separated after mild SDS–PAGE, or from LHCP[1+2] mixture, separated after SDS–sucrose density gradient centrifugation*

Fig.2 shows the electrophoretic separation of LHC-I from CPIa and LHCP[2] (fig.2C and B, respectively), after partial dissociation of the complexes to their components (in the presence of Na^+).

CPIa was separated by mild SDS–PAGE of SDS-solubilized thylakoids; the CPIa zone was ex-

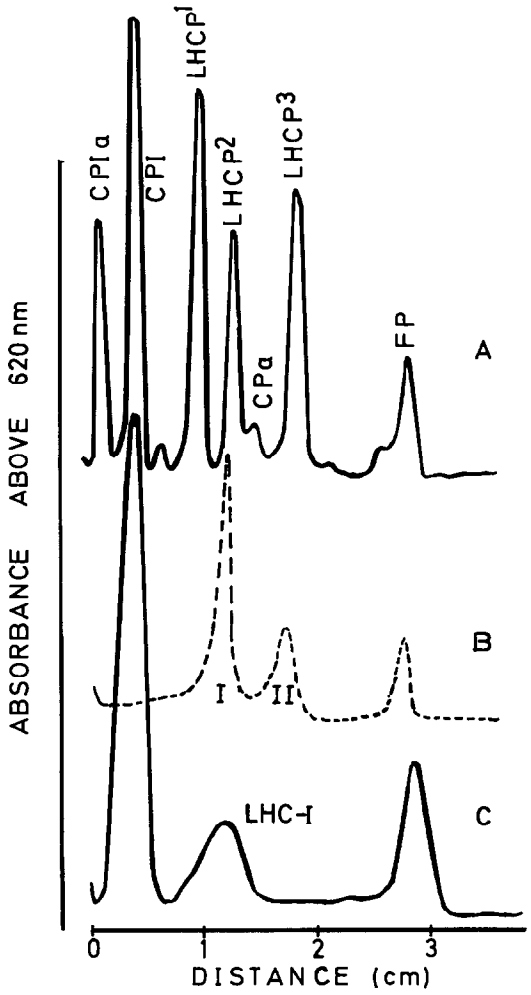

Fig.2. Electrophoretic profiles of (A) the pigment–protein complexes separated from SDS-solubilized thylakoids after mild SDS–PAGE; (B) LHCP[2] and (C) CPIa bands excised from the gel (A) and reelectrophoresed under partially dissociating conditions.

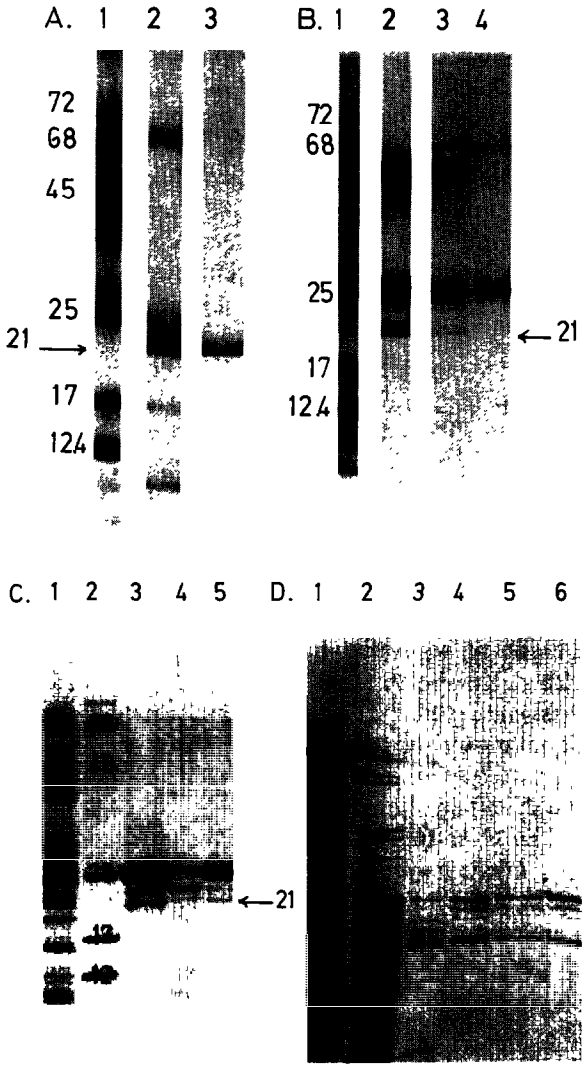

Fig.3. Polypeptide analysis by dissociating SDS–PAGE of the CPIa band excised from the gel, eluted in H_2O and lyophilized (A-2) and LHC-I separated from CPIa (A-3); the LHCP[2] band excised from the gel, eluted in H_2O and lyophilized (B-2) and bands I and II separated from LHCP[2] (B-3, B-4, respectively); the LHCP[1+2] oligomer mixture, separated by sucrose density gradient centrifugation of thylakoids (C-3) and the upper and lower band separated from LHCP[1+2] by further centrifugation (C-4, C-5, respectively); the stroma lamellae fraction (D-2), the CPIa complex (D-3) and the LHCP[1+2] (D-4) separated from stroma lamellae by sucrose density gradient centrifugation, and the upper and lower band separated from the latter complex by sucrose density gradient centrifugation (D-5, D-6, respectively). A-1, B-1, C-2, D-1, standard protein markers. C-1, total thylakoids. (A, B, D) silver nitrate staining; (C) Coomassie staining.

cised from the gel and reapplied on a new gel containing 100 mM Na^+ in the stacking gel. As shown in fig.2C, CPIa is resolved into CPI, LHC-I and free pigment. The polypeptide composition of each complex is shown in fig.3. It is clear that CPIa, separated directly from SDS-solubilized thylakoids after mild SDS–PAGE, contains identical polypeptides to those of CPIa separated after sucrose density gradient centrifugation (cf. fig.1, lane 3; fig.3A, lane 2). The CPI component of CPIa contains only the 68-kDa polypeptide, while LHC-I is composed of the 21-kDa polypeptide (fig.3A, lane 3). This result is at variance with the previous finding that LHC-I separated from PS I-110 particles contains the two polypeptide doublets of 24–23 and 21–20 kDa [6]. This suggests that probably the presence of Triton X-100 in the PS I-110 preparation, as well as the absence of Na^+ from the gel, keeps the 4 polypeptide components of CPIa organized in such a way that they cannot be separated by mild SDS–PAGE. It is clear, therefore, that when Na^+ is used to dissociate the CPIa complex isolated from thylakoids after mild SDS–PAGE, the LHC-I separated after further SDS–PAGE contains only the 21-kDa polypeptide.

The 77 K fluorescence spectral characteristics of the pigment–protein complexes, derived from CPIa, are shown in fig.4 and table 1. The spectra were recorded in situ in the gel slices as soon as the electrophoretic separation was over. The peak emission positions of CPIa, separated electrophoretically, are at 728 and 685 nm; those of the CPI component at 681 and 722 nm, while those of LHC-I vary with different preparations (e.g., at 691 and 717 nm, or 681 and 717 nm). This suggests that the pigment–protein complex LHC-I is quite unstable after isolation, and depending on the state of its organization various peak emissions may be found. This is further supported by the earlier finding that the peak emission of LHC-I isolated from Triton X-100-extracted PS I-110 particles is located at 729 nm.

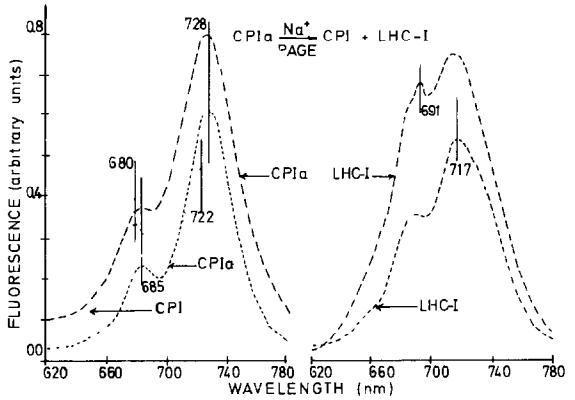

Fig.4. Fluorescence spectra at 77 K (in situ) of CPIa excised from a gel after mild SDS–PAGE of thylakoids, and of its components CPI and LHC-I resolved after partial dissociation of CPIa.

Table 1

77 K fluorescence characteristics of the pigment–protein complexes CPIa and $LHCP^2$, separated by mild SDS–PAGE, and of their components, obtained after partial dissociation of the complexes

Sample		F_{max} (77 K) (nm)	Ratio F_{730}/F_{685}
(A)	CPIa	685, 728	2.12
	CPI	681, 721	1.40
	LHC-I	691, 717	1.10
		681, 717	1.50
	$LHCP^2$	685, 735	0.20
	Band I	685, 735	0.18
	Band II	685, 735	0.07
(B)	CPIa	685, 727	2.88
	$LHCP^2$	686, 727	0.88

CPIa and $LHCP^2$ were isolated from SDS-solubilized bean thylakoids (A) or from pea stroma lamellae (B). Separation of their components was achieved by mild SDS–PAGE (see section 2)

Fig.2 also shows the electrophoretic profile of $LHCP^2$ (fig.2B) which was separated by mild SDS–PAGE from SDS-solubilized thylakoids. As shown, the $LHCP^2$ band is further resolved into 3 pigment zones: bands I and II and the free pigment zone. The two bands I and II contain variable amounts of LHC-II and LHC-I components, as judged from their polypeptide composition (fig.3B, lanes 3,4) and their low-temperature fluorescence spectra (fig.5 and table 1). Band I is enriched in LHC-I components [i.e., long-wavelength-emitting components (fig.5) and polypeptides of 24–20 kDa (fig.3B, lane 3)] as compared to band II, which is enriched in the 25-kDa polypeptide component of LHC-II (fig.3B, lane 4) and is depleted in long-wavelength-emitting

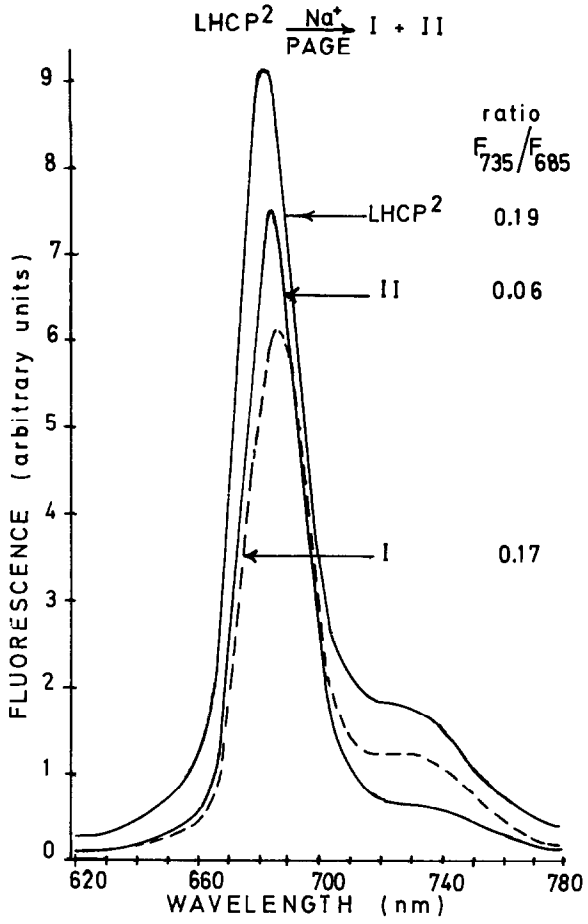

Fig.5. Fluorescence spectra at 77 K (in situ) of $LHCP^2$ excised from a gel after mild SDS–PAGE of thylakoids, and of its components I and II, resolved after partial dissociation of $LHCP^2$.

components (fig.5). No clear separation of the 21-kDa polypeptide can be achieved in this way. This suggests that the association of LHC-I with LHC-II components is very strong. Indeed, separation of the 21-kDa polypeptide of LHC-I from the $LHCP^{1+2}$ oligomer mixture obtained after SDS–sucrose density gradient centrifugation was also unsuccessful. The $LHCP^{1+2}$ oligomer mixture obtained from SDS-solubilized thylakoids or stroma lamellae was removed from the gradient; it was then reapplied on a new gradient and centrifuged for 5 h at 400000 × *g*. Fig.6 is a schematic presentation of the result. As shown, two bands can be further separated from the $LHCP^{1+2}$ mixture. Their Chl *a*/Chl *b* ratio and polypeptide

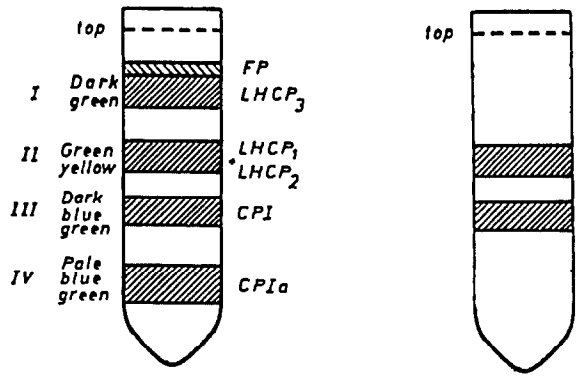

Fig.6. Separation of pigment–protein complexes by SDS–sucrose density gradient centrifugation (left). The $LHCP^{1+2}$ oligomer mixture was removed from the gradient, reapplied on a new gradient and centrifuged for an additional 5 h at 400000 × *g* (right).

composition are shown in fig.3C,D and table 2. The upper band has a high Chl *a*/Chl *b* ratio and is enriched in the 21-kDa polypeptide component of LHC-I; in contrast, the lower band has a low Chl *a*/Chl *b* ratio and is enriched in the 25-kDa polypeptide component of LHC-II. In this case again the separation of the LHC-I from the LHC-II components is only partial.

Table 2

Chl *a*/Chl *b* ratio of the $LHCP^{1+2}$ light-harvesting complex mixture, separated after sucrose density gradient centrifugation, and of the components separated by a new centrifugation on sucrose density gradients (see section 2)

Sample	Chl *a*/Chl *b*
(A)	
Pea thylakoids	3.28
$LHCP^{1+2}$	2.14
Upper band	4.30
Lower band	1.70
(B)	
Stroma lamellae	5.9
$LHCP^{1+2}$	2.4
Upper band	8.4
Lower band	2.6

The $LHCP^{1+2}$ light-harvesting complex mixture was isolated from pea thylakoids (A) or from pea stroma lamellae (B)

3.2. *Cation-induced changes in the low-temperature emission spectra of the CPIa complex and its components CPI and LHC-I*

As shown earlier, the isolated CPIa has a high 77 K emission at about 730 nm [1] (isolation by SDS–sucrose density gradient centrifugation [5]); the long-wavelength emission is blue-shifted to about 680 nm, and the F_{730}/F_{685} ratio is drastically diminished when cations are added [1]. Fig.7 shows the gradual changes induced by increasing concentrations of Mg^{2+} added to the CPIa complex isolated from SDS-solubilized thylakoids after sucrose density gradient centrifugation. Since under similar cation concentrations the oligomeric structures of the pigment–protein complexes are dissociated into their components [3], it has been proposed that the changes observed in the low-temperature fluorescence spectra of CPIa reflect the dissociation of the CPIa complex to its components. Thus the organized CPIa emits at about 735 nm, its CPI component at 720 nm and LHC-I at 730 nm. However, depending on the state of organization of each pigment–protein complex separately, one may find different peak position maxima. At full dissociation of each complex the emission maximum will shift to 680 nm as shown

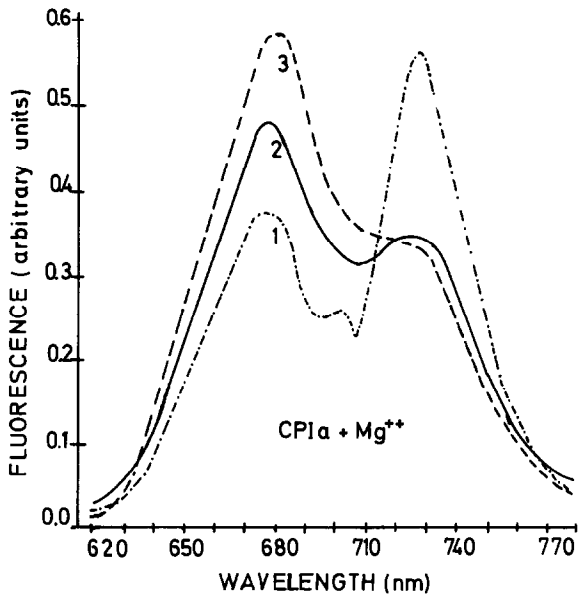

Fig.7. The effect of $MgCl_2$ on the 77 K fluorescence spectra of CPIa, separated by sucrose density gradient centrifugation from SDS-solubilized thylakoids. (1) 0.09 mM Mg^{2+}, (2) 1 mM Mg^{2+}, (3) 1.6 mM Mg^{2+}.

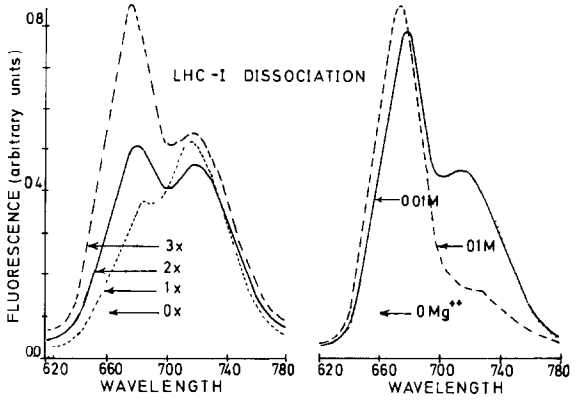

Fig.8. Fluorescence spectra of LHC-I at 77 K (in situ) as affected by freezing-thawing (left) or $MgCl_2$ (right). Left: the gel slice containing LHC-I was frozen in liquid N_2 and the spectra recorded (0×), then thawed and refrozen successively 1–3 more times (1×–3×). Right: the gel slice was immersed for 1 min at 25°C in solutions containing 0, 0.01 or 0.1 M $MgCl_2$, and then the spectra were recorded.

for the isolated CPI [1,3]. Fig.8 shows the effect of freeze-thawing and of Mg^{2+} addition on the spectra of LHC-I, isolated after mild SDS–PAGE of CPIa. It is clear that in both cases the long-wavelength emission is blue-shifted gradually to 680 nm, while the F_{730}/F_{685} ratio is drastically decreased. These spectra were recorded in situ in the gel slice containing the LHC-I, after excision of the band and immediate freezing in liquid nitrogen. The same band was allowed to thaw, then dipped again in liquid nitrogen and its spectrum rerecorded. The effect of Mg^{2+} on the fluorescence spectrum of the LHC-I band was studied after excising the LHC-I band from a number of gels; the gel slices were immersed in various concentrations of $MgCl_2$ for 1 min at 25°C; then the spectra of the gel slices were recorded. It is clear from these experiments that the low-temperature fluorescence changes observed with CPIa, during its dissociation, are analogous to those observed with its components CPI [1], and LHC-I (fig.8), after their dissociation.

We can conclude, therefore, that the organizational state of CPIa, and of its separated components, is reflected by the 77 K emission of the complex.

This conclusion is further supported by earlier work on the organization of CPIa occurring in

vivo, during chloroplast development. In greening etiolated plant tissue (whole leaves or isolated thylakoids) it has been found that a good correlation exists between the appearance of CPIa and the appearance and increase of the long-wavelength fluorescence emission at 77 K [15]. Thus in intermittent light plants where the 21–24-kDa polypeptides of LHC-I are missing [16] and no organized CPIa can be detected, but only CPI is present [17,18], only a faint emission at about 714 nm (and a high at 690 nm) can be observed. The 714 nm peak is shifted gradually to 720 nm as exposure to intermittent light is prolonged; after transfer of the plants to continuous light the 720 nm emission peak is further shifted to 730 nm, and finally to 735 nm [15]. Parallel to this shift the synthesis of the LHC-I components and their assembly into the organized CPIa complex takes place [15].

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