

# Effect of $Mg^{2+}$ on excitation energy transfer between LHC II and LHC I in a chlorophyll-protein complex

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A chlorophyll-protein preparation has been isolated from the thylakoid membranes of *Pisum sativum* L. by the use of Triton X-100 in the presence of a Tris-HCl buffer. Analysis of this preparation by means of SDS-PAGE and freeze-fracture electron microscopy indicates that it contains the reaction centre complex of photosystem I (PS I) and its associated peripheral light-harvesting complex (LHC I), together with the peripheral light-harvesting complex of PS II (LHC II). The reaction centre complex of PS II is absent from this preparation. The polypeptide composition of the complex is similar to that of one previously isolated from barley [(1987) *Eur. J. Biochem.* 163, 221-230]. Fluorescence spectroscopy of the preparation at 77 K indicates that excitation of chlorophyll *b* at 472 nm (LHC II) gives fluorescence emission at 735 nm from LHC I, thereby indicating excitation energy transfer between the two complexes. The extent of excitation energy transfer from LHC II to LHC I is increased by the absence of  $Mg^{2+}$  from the medium, a phenomenon similar to effects of  $Mg^{2+}$  depletion promoting 'spillover' of excitation energy from PS II to PS I in chloroplast thylakoid membranes.

Photosystem I; Photosystem II; Light-harvesting complex; Chlorophyll-protein complex; Excitation energy transfer; Thylakoid membrane; Membrane stacking

## 1. INTRODUCTION

Distribution of absorbed excitation energy between photosystem (PS) I and PS II in chloroplast thylakoid membranes *in vitro* is known to be affected by the cation concentration of the suspending medium and is therefore correlated with cation-induced membrane ultrastructural changes [1,2]. Low cation concentrations cause thylakoid membranes to become unstacked and make excitation energy distribution appear to favour PS I at the expense of PS II, as judged by chlorophyll

fluorescence spectroscopy. Unstacking is thought to result from electrostatic repulsion between fixed negative charges at the thylakoid surface, with cations serving to maintain the stacked condition by providing electrostatic screening of these surface negative charges [3,4]. The increased 'spillover' of excitation energy from PS II to PS I is thought to arise as a result of this unstacking, since in unstacked membranes the lateral separation of PS II from PS I [5] will be destroyed. Although such cation-effects were originally put forward as a mechanism for physiological control of excitation energy distribution *in vivo* [3,4] it now seems that phosphorylation of light-harvesting complexes [6,7] is a more plausible mechanism for control of spatial organisation of

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membrane components [8]. The direct physiological relevance of control of stacking by cations is therefore unclear.

Here we describe a membrane-free chlorophyll-protein preparation from pea in which excitation energy transfer from a PS II light-harvesting complex (LHC II) to a PS I light-harvesting complex (LHC I) is favoured by the absence of cations from the medium. This result implies that unstacking of thylakoid membranes is not a necessary link between cation concentration and changes in excitation energy distribution in photosynthetic systems.

## 2. MATERIALS AND METHODS

In order to prepare the Tris-HCl material, mature leaves of 12-day-old peas grown under a 12-h photoperiod were homogenised in ice-cold sucrose isolation medium as described in [9]. The homogenate was filtered through 16 layers of muslin and the filtrate centrifuged at  $1000 \times g$  for 5 min to obtain a chloroplast pellet. The pellet was resuspended in 6.2 mM Tris, 48 mM glycine, pH 8.3 (TG buffer), in order to lyse the chloroplasts, and thylakoid membranes were recovered by centrifugation at  $10000 \times g$ . The thylakoid pellet was resuspended in TG buffer, and DNase and  $MgCl_2$  added to final concentrations of  $100 \mu g/ml$  and 2.5 mM, respectively. This solution was left to incubate for 40 min on ice after which time the thylakoids were recovered by centrifugation at  $20000 \times g$  for 10 min. The thylakoids were then washed in 50 mM sorbitol, 5 mM EDTA (pH 7.8) followed by two washes in TG buffer. The resulting pellet was resuspended in a small volume of 25 mM Tris-HCl (pH 8.3) and an aliquot removed for chlorophyll determination. The thylakoid suspension was then diluted with the Tris-HCl buffer and Triton X-100 was added from a 5% (w/v) stock solution such that the final concentrations of chlorophyll and detergent were 0.5 mg/ml and 0.5% (w/v), respectively. This suspension was incubated at room temperature for

30 min with constant stirring, followed by centrifugation at  $30000 \times g$  for 30 min at  $4^\circ C$ . This centrifugation step results in the production of a large green pellet and a green supernatant. The pellet, representing the Tris-HCl preparation, was resuspended in TG buffer and stored at  $-20^\circ C$  or used for further analysis.

PS I particles were prepared from mature leaves of *Pisum sativum* by the method of Mullet et al. [10], with the modifications described in [9].

Polyacrylamide gel electrophoresis was carried out using the buffer system of Laemmli [11]. Chlorophyll determinations were performed using the equations described in [12].

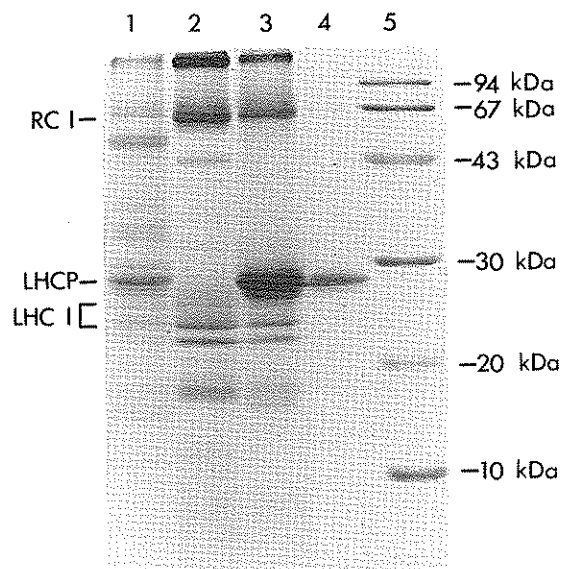
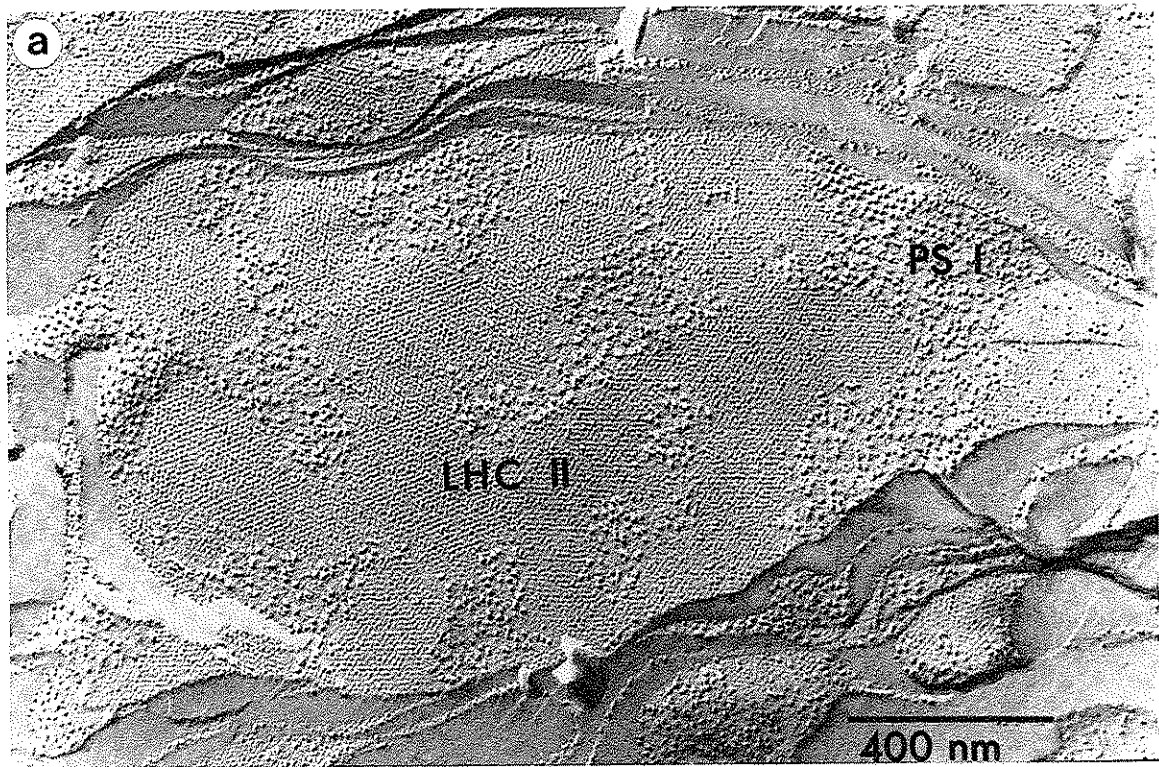


Fig.1. SDS-polyacrylamide gel analysis of polypeptide constituents of the Tris-HCl pellet. Lanes: (1) unfractionated thylakoid membranes; (2) PS I prepared by the method of Mullet et al. [10]; (3) Tris-HCl pellet; (4) purified LHC II; (5) molecular mass markers. RC I, the PS I reaction centre polypeptides; LHCP, the major apoprotein of the chlorophyll *a/b* light-harvesting complex of PS II; LHC I, the chlorophyll *a/b* light-harvesting complex of PS I. The gel is stained with Coomassie brilliant blue.

Fig.2. Freeze-fracture electron micrographs of pellets resulting from the solubilisation of thylakoids with Triton X-100 in the presence of 25 mM Tris-HCl (pH 8.3). (a) Detailed structure in the absence of  $MgCl_2$ ; the two component chlorophyll-protein complexes of this material, PS I and LHC II, are labelled. (b) Gross structure in the absence of  $MgCl_2$ . (c) Gross structure in the presence of  $MgCl_2$  showing a region of close sheet appression (arrow).



Fluorescence spectroscopy at 77 K was carried out using a Perkin-Elmer LS-5 luminescence spectrometer. Samples were diluted to a final chlorophyll concentration of 5  $\mu\text{g}/\text{ml}$  prior to rapid freezing in liquid nitrogen. For freeze-fracture electron microscopy, samples were thermally quenched from 22°C using a liquid nitrogen jet freezer, then fractured and replicated at -135°C in a Polaron freeze-fracture unit. Replicas were cleaned with sodium hypochlorite, washed, placed on 400 mesh uncoated grids and examined in a Philips EM301G transmission electron microscope.

### 3. RESULTS AND DISCUSSION

Fig.1 shows an SDS-polyacrylamide gel analysis of the pellet obtained by the Tris-HCl method. For comparison, polypeptide profiles of unfractionated thylakoids, a Mullet-type PS I preparation [10], and LHC II purified by the method of Burke et al. [13] are also shown. Comparison of tracks 2 and 3 of fig.1 indicates that the Tris-HCl material contains the reaction centre polypeptides of PS I together with the peripheral PS I light-harvesting complex (LHC I), and a number of low molecular mass proteins which are also found in the Mullet-type PS I preparation. In addition to these PS I-associated polypeptides, the Tris-HCl preparation also contains heavily stained bands representing the LHC II apoproteins with molecular masses in the 24–26 kDa range. Experiments involving the *in vitro* incorporation of  $^{32}\text{P}$ -labelled orthophosphate into thylakoid phosphoproteins show that none of the phosphoproteins of the PS II reaction centre complex are present in the Tris-HCl preparation (not shown). A similar LHC II-PS I pigment-protein complex has recently been isolated from barley by Bassi and Simpson [14].

Fig.2a represents a freeze-fracture electron micrograph showing the structure of the Tris-HCl material. The fracture planes expose extensive sheet-like structures in which two distinct size-classes of particle can be clearly distinguished. The larger particles range in diameter from 13 to 17 nm, while the smaller particles display a more uniform diameter of 8 nm. Comparison of this material with that shown in micrographs published

by other authors [16,17] indicates that the small particles represent individual LHC II complexes while the larger particles represent PS I reaction centre complexes with associated LHC I. The LHC II complexes are arranged in tightly packed arrays which are strikingly similar in form to the crystalline sheets of purified LHC II described by Kuhlbrandt et al. [17]. Interspersed among these arrays, and occupying the same fracture surfaces, are groups of less tightly packed PS I particles.

Fig.2b and c shows the gross organisation of the Tris-HCl material in the absence and presence of  $\text{MgCl}_2$ . No consistent differences between these two treatments could be discerned, although some samples showed isolated regions of increased appression of the sheet-like structures in the presence of cations (fig.2c). In addition, the presence of  $\text{MgCl}_2$  induced no apparent changes in the distribution of the two component complexes relative to each other within the sheets.

Fig.3a shows 77 K fluorescence emission spectra of the Tris-HCl material in the presence and absence of  $\text{MgCl}_2$ . Both spectra exhibit a fluorescence maximum at 682 nm arising from the LHC II component, together with a larger fluorescence maximum at 735 nm arising from the light-harvesting component of PS I (LHC I) [18–20]. In the presence of 5 mM  $\text{MgCl}_2$ , the material has an intensity of PS I fluorescence emission ( $F_{735}$ ) approximately twice that due to LHC II ( $F_{682}$ ). The  $F_{735}/F_{682}$  rises to 5.3 in the absence of  $\text{MgCl}_2$  (fig.3a). It has been shown by Argyroudi-Akoyunoglou [21] that addition of  $\text{Mg}^{2+}$  to SDS-solubilised PS I and LHC I preparations causes a blue-shift in their long-wavelength fluorescence maxima at 77 K. In order to test whether a similar phenomenon is responsible for the  $\text{Mg}^{2+}$ -dependent changes shown in fig.3a, 77 K fluorescence emission spectra were obtained for Mullet-type PS I preparations in the presence and absence of added cations. Fig.3b shows that although some 682 nm fluorescence is observed in these preparations (possibly due to small amounts of contaminating LHC II), the addition of 5 mM  $\text{MgCl}_2$  causes no change in the size of the peak at 682 nm relative to that at 735 nm.

Since excitation at 472 nm will occur primarily through chlorophyll *b*, most of which is associated with the LHC II component of the Tris-HCl material, the relative change in the spectrum

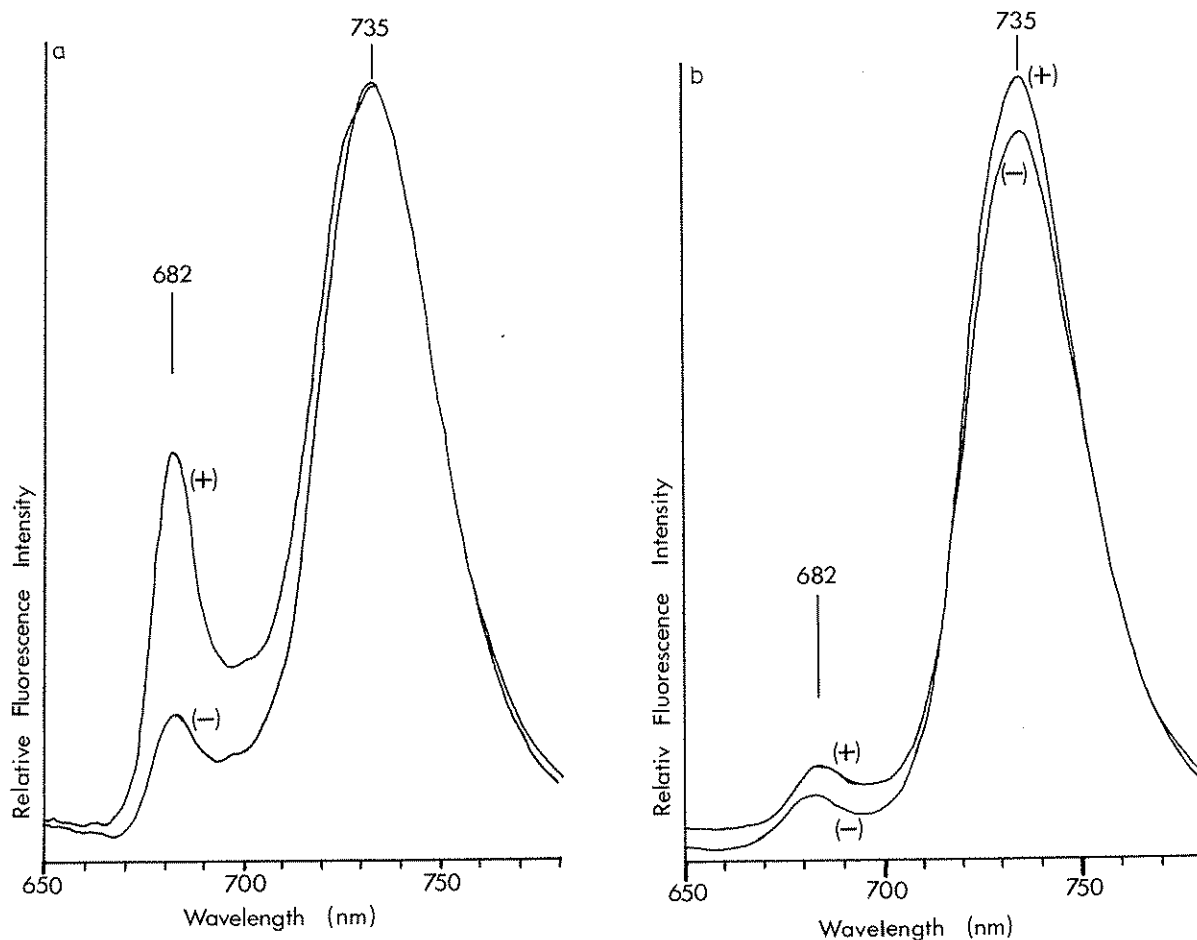


Fig.3. Low temperature (77 K) fluorescence emission spectra of (a) the Tris-HCl pellet in the presence (+) and absence (-) of 5 mM  $MgCl_2$ , and (b) PS I prepared by the method of Mullet et al. [10] in the presence (+) and absence (-) of 5 mM  $MgCl_2$ . Spectra were obtained using 472 nm excitation with a slit width of 2.5 nm, and an emission slit width of 5 nm. Spectra in (a) were normalised to the maximum at 735 nm.

observed in fig.3a could indicate either increased excitation energy transfer from LHC II to LHC I, or increased non-radiative decay of energy absorbed by LHC II. Relative fluorescence intensity is expressed in arbitrary units, and to facilitate comparison the two spectra in fig.3a were normalised to  $F_{735}$ . There is therefore no way of distinguishing between these two possibilities from the data in fig.3a alone.

Fig.4a shows 77 K fluorescence excitation spectra of the LHC II-PS I complex in the presence of  $Mg^{2+}$  as  $MgCl_2$  at 5 mM. For  $F_{683}$  fluorescence emission from LHC II (broken line), chlorophyll *a* excitation at 435 nm and chlorophyll *b* excitation

at 473 nm contributed almost equally, as expected for a chlorophyll *a/b* ratio of almost unity. For  $F_{735}$  emission from LHC I of PS I (continuous line), chlorophyll *b* excitation (473 nm) was markedly less effective than chlorophyll *a* (435 nm), as expected from the higher chlorophyll *a/b* ratio of LHC I [22,23] than that of LHC II. However, fig.4b shows that the difference between PS I and LHC II fluorescence emission with respect to the relative contributions of chlorophyll *a* and chlorophyll *b* is abolished when  $Mg^{2+}$  is absent from the medium. This result strongly suggests that excitation energy transfer from LHC II to LHC I occurs and is increased in the absence of

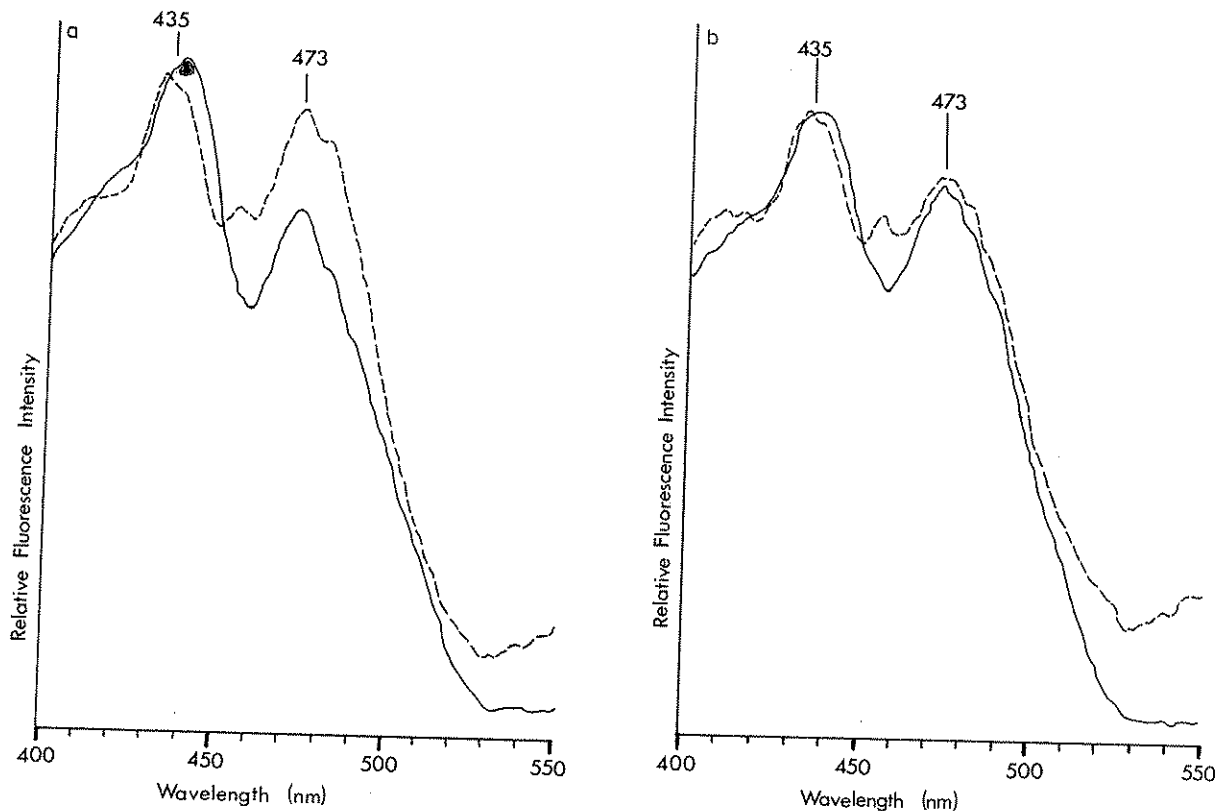


Fig.4. Low temperature (77 K) fluorescence excitation spectra of the Tris-HCl pellet in (a) the presence and (b) the absence of 5 mM  $MgCl_2$ . Fluorescence emission from the LHC II component was measured at 683 nm (broken line), while fluorescence emission from the PS I (LHC I) component was measured at 735 nm (solid line), as described in fig.3. For emission at 683 nm additional, minor maxima at 455 nm are seen and are likely to be second-order interference artefacts arising from the use of unblocked grating monochromators.

$Mg^{2+}$ . The results in fig.4 supporting this conclusion are summarised in table 1.

The results presented above lead us to conclude that transfer of excitation energy from LHC II to LHC I can be demonstrated to occur and to be under the control of cation concentration even in a chlorophyll-protein preparation containing only LHC II and PS I complexes. The N-terminal surface-exposed segment of the LHC II has been shown to be required for cation-induced thylakoid membrane stacking [8] and is also required for the aggregation of proteoliposomes containing isolated LHC II complexes [16]. It is possible that in the Tris-HCl material  $Mg^{2+}$  can screen fixed negative charges in the hydrophilic domain of the LHC II component, thereby facilitating LHC II  $\rightarrow$  LHC I excitation energy transfer between

hydrophobic domains and parallel to the sheet of LHC II particles. Cation-depletion would then be expected to allow electrostatic repulsion to predominate and drive neighbouring LHC II complexes apart, decreasing excitation energy transfer between them, and increasing LHC II  $\rightarrow$  LHC I excitation energy transfer as the less negatively charged PS I units become more homogeneously distributed amongst the LHC II components.

Such an explanation of the cation-mediated fluorescence changes reported in this paper requires that addition of  $MgCl_2$  to the Tris-HCl material should result in a spatial redistribution of the two component chlorophyll-protein complexes. This redistribution could take the form of an increased lateral separation between the LHC II and PS I components within any given sheet, or an

Table 1  
Fluorescence emission ratios of the Tris-HCl preparation

Emission $\lambda$ (nm)	$F_{\text{ex}=435}/F_{\text{ex}=473}$	
	+ $\text{Mg}^{2+}$	- $\text{Mg}^{2+}$
683	1.05	1.11
735	1.29	1.13

Summary of data obtained from the results in fig.4.  $F_{\text{ex}=435}/F_{\text{ex}=473}$  is the ratio of fluorescence emission with 435 nm excitation to fluorescence emission with 473 nm excitation

increase in the number of closely appressed sheets. These two possibilities are not mutually exclusive, but the latter possibility would be expected to result in a gross morphology similar to that of stacked thylakoid membranes. No evidence of a cation-induced lateral redistribution of chlorophyll-protein complexes could be found in any of the freeze-fracture replicas examined. Similarly, only slight evidence of increased sheet appression could be found upon addition of cations, and this was not found to be consistent between replicas.

Although our observations do not demonstrate categorically that membrane stacking is unnecessary for control of excitation energy distribution between PS I and PS II in thylakoids, they do raise the possibility that changes in stacking are secondary effects of changes in cation concentration, and that more localised electrostatic forces between membrane proteins play an important part in regulation of excitation energy transfer in photosynthesis. Protein phosphorylation is able to produce effects similar to cation depletion even in unstacked membrane systems [24], and the present finding suggests that localised electrostatic forces, possibly acting at the level of protein conformational changes, underlie the physiological control of membrane protein-protein interactions within and between photosynthetic units [24].

A further question arising from this work is whether excitation energy transfer from LHC II to PS I contributes to an increased absorption cross-section of PS I either in vitro or in vivo. For thylakoid membranes there is disagreement in the literature concerning the effects of cation depletion [25-27] and of protein phosphorylation [28,29] on

the rate of PS I photochemistry. It is possible that the kind of chlorophyll-protein preparation represented by the Tris-HCl material described here may serve as a useful model for determining the fate of excitation energy diverted away from PS II when its absorption cross-section is regulated in response to environmental change [5-8].

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