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The effects of Triton X-100 and n-octyl β-D-glucopyranoside on energy transfer in photosynthetic membranes

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The effects of the non-ionic detergents Triton X-100 and n-octyl β-D-glucopyranoside on energy transfer between pigment–protein complexes of *Pisum sativum* thylakoids were investigated. This was done by monitoring the 77K fluorescence-emission characteristics of stacked and unstacked thylakoids exposed to a range of detergent concentrations. At sub-critical micellar concentrations, the detergents had little effect, whereas above these concentrations they caused increases of up to 20-fold in short-wavelength fluorescence intensity and a shift in its maximum wavelength from 685 to 680nm. Fluorescence-emission intensities at 695 and 735nm were relatively unaffected by detergent treatments, although Triton X-100 caused a wavelength shift in the emission peak from 735 to 728nm. The results are discussed in terms of reversible dissociation of pigment–protein complexes induced by mild detergent solubilization and the consequent cessation of inter-complex energy transfer.

The photosynthetic membranes of higher plants contain three major membrane-spanning chlorophyll–protein complexes, i.e. Photosystem II (PSII), the light-harvesting chlorophyll–protein complex (LHCP-II) and Photosystem I (PSI) (Anderson, 1982). The LHCP-II complex is normally associated with PSII *in vivo*, although a mobile sub-population of LHCP-II may dissociate from PSII and re-associate with PSI upon phosphorylation during State I-to-State II transitions (Kyle *et al.*, 1983; Larsson *et al.*, 1983). The PSI complex also contains a separate LHCP-I, but the total amount of LHCP-I in the thylakoid membrane is far exceeded by that of LHCP-II (Mullet *et al.*, 1980; Haworth *et al.*, 1983). During steady-state photosynthesis, energy is transferred from LHCP-II to PSII reaction centres or between neighbouring PSII complexes, or it may 'spill over' to PSI. Energy is also transferred from LHCP-I to PSI reaction centres and possibly, under some circumstances, between PSI complexes (Haworth *et al.*, 1983). The regulation of energy transfer between these chlorophyll–protein complexes is of vital importance in the maintainance of optimal rates of photosynthetic electron flow during the fluctuating environmental conditions, and in particular the quality and intensity of incident radiation, that obtain in natural habitats.

Native thylakoid membranes exhibit three principal 77K fluorescence-emission maxima, at approximately 685nm (F_1), 695nm (F_2) and 735nm (F_3) (Fig. 1). The 685nm fluorescence is believed to arise from antenna chlorophylls associated with the reaction-centre polypeptides of PSII (Rijgersberg *et al.*, 1979; Breton, 1982). Most of this fluorescence arises from excitation energy originally passed on from LHCP complexes interacting with the reaction-centre polypeptides (Breton, 1982). LHCP-II itself fluoresces only very weakly in the native membrane, even at 77K, probably owing to effective quenching by antenna chlorophylls associated with PSII. The 695nm 'fluorescence' probably arises from a charge recombination in PSII reaction centres between phaeophytin$^-$ and P680$^+$ (Breton, 1982; Klimov *et al.*, 1980), and is therefore more correctly described as luminescence. The 735nm fluorescence emission is a composite peak generally attributed to PSI (Breton, 1982).

In the present paper we report the characterization of the effects of the mild non-ionic surfactants Triton X-100 and n-octyl β-D-glucopyranoside

Abbreviations used: LHCP, light-harvesting chlorophyll–protein complex; PSI, Photosystem I; PSII, Photosystem II.

on energy transfer between pigment–protein complexes, as monitored by 77K fluorescence-emission spectra. The aim of this work was to obtain intact but dissociated, i.e. non-energy-transferring, chlorophyll–protein complexes under the mildest possible solubilization conditions for use in subsequent reconstitution studies of interactions between these pigment–protein complexes, such as the investigation of the mechanism of inter-complex energy transfer.

Experimental

Preparation of thylakoids

Dwarf pea plants (*Pisum sativum* L. var. Kelvedon Wonder) were grown in a greenhouse for up to 3 weeks before harvesting. The leaves were homogenized for two bursts of 1 s in medium containing 0.33 M-glucose, 50 mM-Na_2HPO_4/KH_2PO_4 buffer, pH 6.5, 5 mM-$MgCl_2$ and 25 mM-NaCl. Thylakoid membranes were sedimented by centrifugation at 3000*g* for 15 s. Chloroplast pellets were split into two batches, one for preparation of stacked and one for unstacked thylakoids. Stacked thylakoids were prepared by resuspension of chloroplasts in 5 mM-$MgCl_2$ and incubated on ice for 1 min before the addition of an equal volume of medium containing 0.66 M-sorbitol, 5 mM-$MgCl_2$ and 15 mM-NaCl. The mixture was incubated on ice for 1.5 h to allow thorough membrane stacking. Unstacked thylakoids were prepared by resuspending the chloroplast pellet in medium containing 7.5 mM-NaCl, 2.5 mM-sodium phosphate buffer, pH 7.05, and 0.1 M-sorbitol. Thylakoids were sedimented by centrifugation at 3000*g* for 5 min and resuspended in the same medium. Chlorophyll was determined as described by Bruinsma (1961). Absorption spectra were measured from 400 to 700 nm at 20°C in a Unicam SP. 1800 spectrophotometer. All procedures were carried out at 4°C unless otherwise indicated.

Detergent treatments

Detergent incubations were performed in the cylindrical glass sample tubes used for subsequent analysis of 77K fluorescence-emission spectra. Dark-adapted thylakoid suspensions at 50 μg of chlorophyll/ml were incubated on ice with the appropriate quantity of detergent in a total volume of 50 μl for 1 min. The tubes were then inserted into a storage vessel containing liquid N_2. The frozen samples were analysed as soon as possible after detergent incubations.

77K fluorescence-emission spectra

Fluorescence-emission spectra were measured at 77K and 50 μg of chlorophyll/ml and a total sample volume of 50 μl in the presence of the resuspension buffers used respectively for the preparation of stacked and unstacked thylakoids. Fluorescence emissions were collected at an angle of 30° from the broad-band blue actinic light via a fibre-optic light-guide. Actinic light-intensity was 13 $W \cdot m^{-2}$. Emission wavelengths ($\pm$ 1 nm) from 650 to 800 nm were detected by an E.M.I. 9558 photomultiplier tube and defined by a Corning 2–60 filter and an Applied Photophysics f3.4 grating monochromator. Scans were controlled and data recorded, averaged (of five scans), and normalized on a B.B.C. model 'B' computer. The sample vessel consisted of a brass-encased glass Dewar flask filled with liquid N_2, into which glass tubes containing samples were immersed.

Results and discussion

The addition of a surfactant such as Triton X-100 to thylakoid membranes results in the incorporation of surfactant molecules into the membranes as long as the surfactant concentration is appreciably below its critical micellar concentration (Helenius & Simons, 1975). Solubilization of the membrane components and the formation of complex lipid–protein–detergent micelles normally occurs at detergent concentrations in the region of or above the critical micellar concentration. In the case of thylakoid membranes, such a solubilization would be expected to disrupt greatly or even abolish inter-complex energy transfer, since this transfer depends on a close physical association of the pigment–protein complexes. In Fig. 1 it is shown that the incubation of thylakoids with increasing Triton X-100 concentrations results in a dramatic increase in F_1 fluorescence emission relative to F_2 and F_3 in the normalized spectra until, at the highest detergent concentration, no F_2 or F_3 peaks are discernible. In Fig. 2 it is shown that this is due to a 10–20-fold increase in the fluorescence yield of F_1, with little increase in F_2 yield and no increase at all in F_3 yield. Comparison of Triton X-100 titrations with stacked and unstacked thylakoids shows that the latter exhibited an onset in the increase in F_1 yield at 1 mg/ml, whereas in stacked thylakoids this occurred at 3 mg of Triton-100/ml. It is likely that the stacked membranes are less susceptible to detergent solubilization than are unstacked membranes as a result of the strong trans-thylakoid interactions between LHCP-II complexes, which occur in stacked thylakoids. n-Octyl β-D-glucopyranoside solubilization of thylakoids resulted in very similar changes in F_1 emission yield to those caused by Triton X-100 (Fig. 3). Once again, there was relatively little change in the F_2 or F_3 yields after detergent treatments (results not shown).

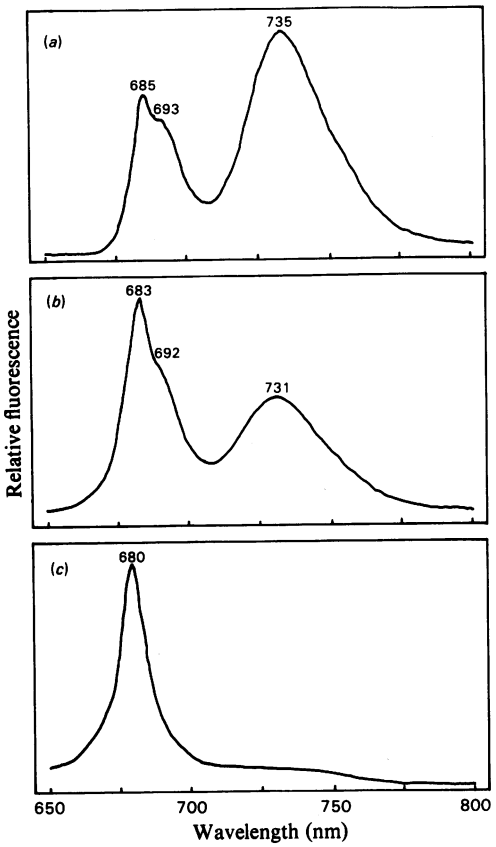

Fig. 1. *77K fluorescence-emission spectra of (a) intact unstacked thylakoids, (b) thylakoids incubated with 1mg of Triton X-100/ml and (c) thylakoids incubated with 0.3g of Triton X-100/ml*

All incubations were for 1min. Scans were performed as described in the Experimental section.

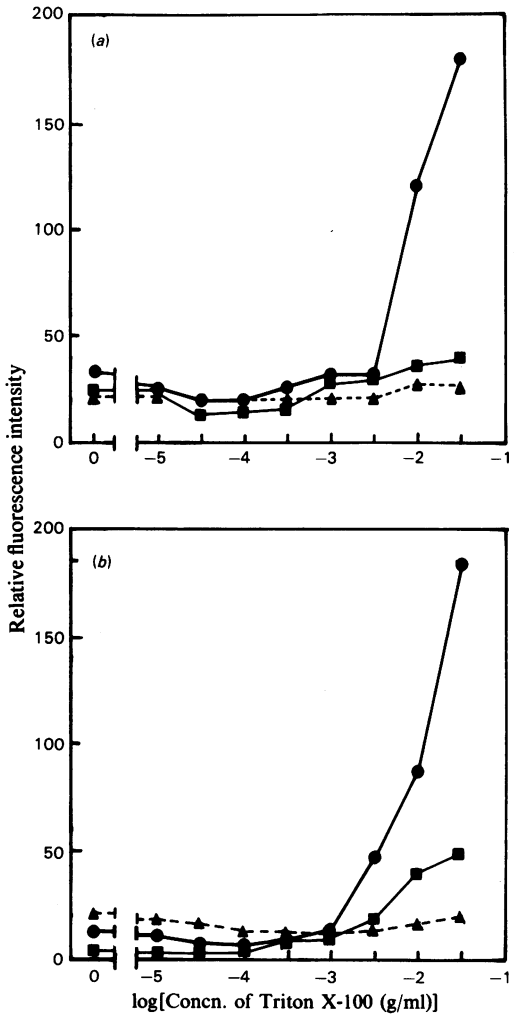

Fig. 2. *Effect of Triton X-100 concentration on the 77K fluorescence yield of (a) stacked thylakoids and (b) unstacked thylakoids*

●, F_1 fluorescence yields (680–685nm); ■, F_2 fluorescence yield (692–694nm); ▲, F_3 fluorescence yield (728–735nm). Thylakoids were incubated for 1min with Triton X-100 as described in the Experimental section.

The reason for the increase in the yield of F_1 fluorescence emission is a physical dissociation of LHCP-II complexes from PSII complexes. This has been demonstrated by the centrifugation at 190000*g* of the solubilized membranes to yield fractions highly enriched respectively in LHCP-II and the photosystem reaction centres, as judged by polyacrylamide-gel electrophoresis (Murphy, 1984). The dissociated LHCP-II complexes are unable to transfer excitation energy to the antenna chlorophylls of PSII, and the result is a strong F_1 fluorescence emission. The loss of ability to transfer excitation energy may be substantially reversed on subsequent re-incorporation of the solubilized pigment–protein complexes into proteoliposomes (Murphy, 1984). This reconstitution, which was achieved by detergent-dialysis of solubilized thylakoids in the presence of various acyl lipids, resulted in the restoration of a 77K fluorescence-emission spectrum typical of native thylakoids rather than of solubilized thylakoids. The F_1 fluorescence peak in native thylakoids is normally at 685nm and is believed to be due to antenna chlorophyll emission (Rijgersberg *et al.*, 1979; Breton, 1982). In Fig. 4 it is shown that Triton X-100 solubilization of thylakoid membranes was accompanied by a shift in the F_1 peak from 685 to 680nm. This corresponds to the fluorescence-emission maximum of free LHCP-II (Anderson *et al.*, 1978), and further indicates that

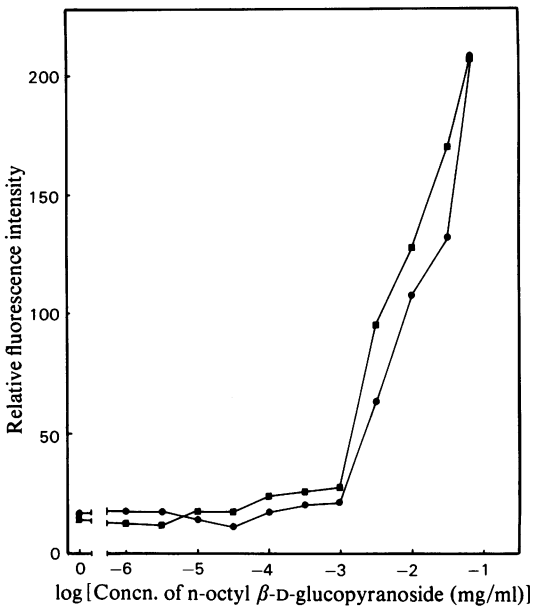

Fig. 3. *Effect of n-octyl β-D-glucopyranoside concentration on the 77 K fluorescence yield at 680–685 nm (F_1) of stacked (●) and unstacked (■) thylakoids*

Thylakoids were incubated with n-octyl β-D-glucopyranoside for 1 min as described in the Experimental section.

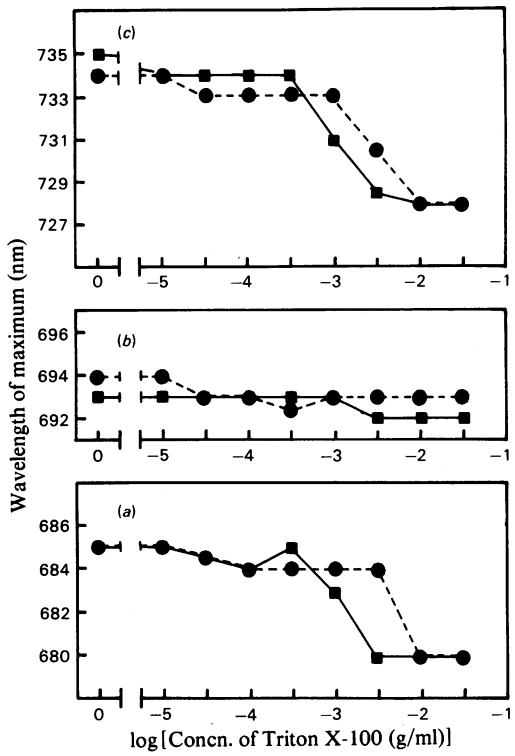

Fig. 4. *Effect of Triton X-100 concentration on the wavelengths of F_1 (a), F_2 (b) and F_3 (c) 77 K fluorescence-emission maxima*

●, Stacked thylakoids; ■, unstacked thylakoids. Thylakoids were incubated with Triton X-100 for 1 min as described in the Experimental section.

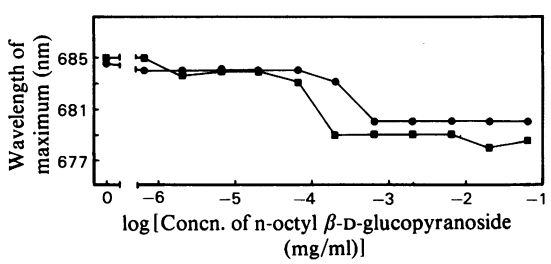

Fig. 5. *Effect of n-octyl β-D-glucopyranoside concentration on the wavelength of the F_1 77 K fluorescence-emission maximum*

●, Stacked thylakoids; ■, unstacked thylakoids. Thylakoids were incubated with n-octyl β-D-glucopyranoside for 1 min as described in the Experimental section.

LHCP-II has become dissociated from the PSII antenna chlorophylls, which normally quench its fluorescence. n-Octyl β-D-glucopyranoside also caused a shift in the F_1 peak from 685 to 679 nm (Fig. 5). In the case of both Triton X-100 and n-octyl β-D-glucopyranoside treatments, the detergent concentration at which the effects on the wavelength of the F_1 maximum are manifest were significantly lower than those giving rise to increases in F_1 yield. This may indicate that the membrane environment of the pigment–protein complexes is subtly altered at sub-solubilizing detergent concentrations. In contrast, the wavelength of the F_2 maximum showed no change on solubilization. This indicates that the environment of the chlorophylls adjacent to the reaction centre of PSII was not disrupted by Triton X-100 or n-octyl β-D-glucopyranoside solubilization.

The only change in F_2 fluorescence that followed Triton X-100 sobulization was a 3–4-fold increase in intensity (Fig. 2). This was possibly due to the solubilization of most of the mobile plastoquinone pool, leading to an over-reduction of Q and a consequent increase in the phaeophytin$^-$-to-P680$^+$ back-reaction, which gives rise to the F_2 emission. F_3 fluorescence, which arises from PSI complexes, also exhibited a blue-shifting of its maximum wavelength in the presence of Triton X-100 (Fig. 4), but not with n-octyl β-D-glucopyranoside (results not shown). This was probably due to an alteration in the environment of the chlorophylls in the PSI complex on solubilization, since it was substantially reversible by detergent removal and reconstitution of the PSI complexes into proteoliposomes (Murphy, 1984).

The differential susceptibility of stacked and

unstacked thylakoids to Triton X-100 solubilization, as demonstrated in Figs. 2 and 3, was also reflected in the delayed blue-shifting of the F_1 and F_3 peaks shown in stacked membranes (Figs. 4 and 5). Once again, the onset of this effect occurred at a 3-fold higher detergent concentration in stacked compared with unstacked thylakoids. It has previously been reported that Mg^{2+} dramatically diminishes the ability of Triton X-100 to disrupt thylakoid membranes (Arntzen *et al.*, 1977). The data in the present paper indicate that this may be due to the increase in thylakoid stacking induced in the presence of Mg^{2+}. The lowest Triton X-100 concentration that showed a significant effect on thylakoid 77K fluorescence emission was 0.3mg/ml. In a similar study on wheat thylakoids, it was found that the fluorescence yield of Triton-treated membranes began to increase dramatically at a detergent concentration of 0.36mg/ml (Bartzatt *et al.*, 1983). This range of concentrations (0.45–0.60mM) is of the same order as the experimentally determined critical micellar concentration of 0.24mM (Kushner & Hubbard, 1954). In the present study the lowest detergent/chlorophyll ratio resulting in a significant wavelength shift in the F_1 and F_3 peaks was 6:1. The large increases in F_1 intensity began at detergent/chlorophyll ratios of 20:1 in unstacked thylakoids and 60:1 in stacked thylakoids.

The lowest detergent/chlorophyll ratios at which there were significant increases in F_1 intensity were independent of chlorophyll concentration over the range 10–50μg of chlorophyll/ml (results not shown). In a related study it was found that Triton X-100-induced stimulation of PSI activity in pea thylakoids occurred at a detergent/chlorophyll ratio of 40:1 down to chlorophyll concentrations as low as 5μg/ml, and that the most efficient detergent/chlorophyll ratio for PSII solubilization was 25:1 at chlorophyll concentrations as high as 2mg/ml (D. J. Murphy & R. T. Prinsley, unpublished work). These results suggest that thylakoids may be effectively solubilized in the presence of 20:1 to 40:1 detergent/chlorophyll ratios over a large range of chlorophyll concentrations.

In all cases these membrane preparations were incubated with Triton X-100 or n-octyl β-D-glucopyranoside for only 1min in order to observe the above changes. Detergent incubations for up to 15min resulted in a greater extent of solubilization, as reflected in the continuing increase in F_1 intensity, particularly at the lower 'active' detergent concentration, i.e. 0.3–10mg/ml. At higher detergent concentrations, i.e. above 10mg/ml, solubilization was complete within 20s–2min. Incubations for longer than 15min rarely increased the extent of solubilization of chlorophyll–protein even at very low detergent concentrations.

We have found that a 10min incubation at 0°C with a detergent/chlorophyll ratio of 20:1 is adequate for the complete dissociation of the LHCP-II, PSII, LHCP-I, and PSI complexes. This was tested at chlorophyll concentrations over the range 25–500μg/ml. The dissociated pigment–protein complexes were still capable of intra-complex energy and electron transfer, but were unable to effect such transfers between one another (Murphy, 1984). These mild solubilization conditions resulted in the preparation of intact pigment–protein complexes that could be further separated by density-gradient centrifugation or column chromatography. No free chlorophyll was present in the preparations. The solubilized complexes could be reconstituted into proteoliposomes of various lipid compositions, whereupon inter-complex energy and electron transfer was restored (Murphy, 1984). This relatively simple system may be readily utilized for studying the mechanisms governing the interaction of both pigment-containing and non-pigment-containing protein complexes in photosynthetic membranes.

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References

Anderson, J. M. (1982) *Trends Biochem. Sci.* **7**, 288–292
Anderson, J. M., Waldron, J. C. & Thorne, S. W. (1978) *FEBS Lett.* **92**, 227–233
Arntzen, C. J., Armond, P. A., Briantais, J.-M., Burke, J. J. & Novitzky, W. P. (1977) *Brookhaven Symp. Biol.* **28**, 316–337
Bartzatt, R. A., Yang, C. M. & Markwell, J. P. (1983) *Biochim. Biophys. Acta* **725**, 341–348
Breton, J. (1982) *FEBS Lett.* **147**, 16–20
Bruinsma, J. (1961) *Biochim. Biophys. Acta* **52**, 576–578
Haworth, P., Watson, J. L. Arntzen, C. J. (1983) *Biochim. Biophys. Acta* **724**, 151–158
Helenius, A. & Simons, K. (1975) *Biochim. Biophys. Acta* **415**, 29–79
Klimov, V. V., Ke, B. & Dolan, E. (1980) *FEBS Lett.* **118**, 123–126
Kushner, L. M. & Hubbard, W. D. (1954) *J. Phys. Chem.* **58**, 1163–1167
Kyle, D. J., Staehelin, L. A. & Arntzen, C. J. (1983) *Arch. Biochem. Biophys.* **222**, 527–541
Larsson, U., Jergil, B. & Andersson, B. (1983) *Eur. J. Biochem.* **136**, 25–29
Mullet, J. E., Burke, J. J. & Arntzen, C. J. (1980) *Plant Physiol.* **65**, 814–822
Murphy, D. J. (1984) in *Structure, Function and Metabolism of Plant Lipids* (Siegenthaler, P. A., ed.), Elsevier/North-Holland, Amsterdam, in the press
Rijgersberg, C. P., Amsez, J., Thielen, A. P. G. M. & Swager, J. A. (1979) *Biochim. Biophys. Acta* **545**, 473–482