FLUORESCENCE TRANSFER IN GLUTARALDEHYDE FIXED PARTICLES OF THE RED ALGA *PORPHYRIDIUM CRUENTUM* (N)

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1. Introduction

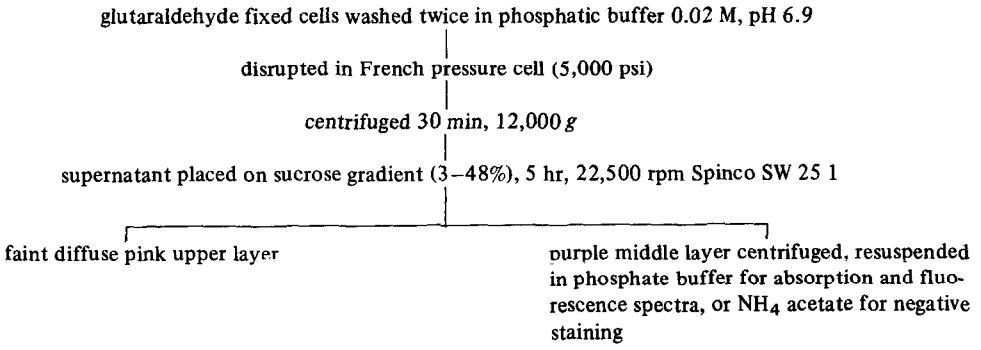
Phycobiliproteins are not physically integrated into the thylacoids of the photosynthetic apparatus of red algae. They are localized in compact macromolecular aggregates "phycobilisomes", arranged in a regular manner at the outer surface of the thylacoids [1]. As soon as the chloroplast membrane is destroyed there is a prompt and total dissociation of the phycobiliproteins from the lamellae. This is prevented by glutaraldehyde fixation [2], which changes the permeability of the cell wall [3] and cross-links the proteins to the aldehyde group with a strong covalent bond. This study was undertaken to investigate whether this treatment has any influence on the energy transfer between phycobilins and chlorophyll.

2. Materials and methods

Porphyridium cruentum Neageli (Indiana no. 161) was grown in 10 litre bottles in Jones et al. medium [4], with forced aeration (2.5% CO_2 in air), and mechanical agitation. The temperature of incubation was 20° and the light intensity 2,500–3,000 lux. The cells were harvested after 7 days, washed twice and resuspended in phosphate buffer 0.02 M, pH 6.9, at the concentration of 10^8 cells ml^{-1}. This suspension was divided in two parts: one part "whole intact cells", was used as reference, the other part, fixed with glutaraldehyde (2% w/v, 15 min, 4°), was treated as outlined in fig. 1 (see also [2]).

The absorption spectra were measured with a Cary 14 spectrophotometer. With intact cells scattering errors were minimized by the Shibata technique [5] using opal glass plates; residual apparent absorption at 720 nm was applied as a uniform scattering correction at all wavelengths.

Fig. 1



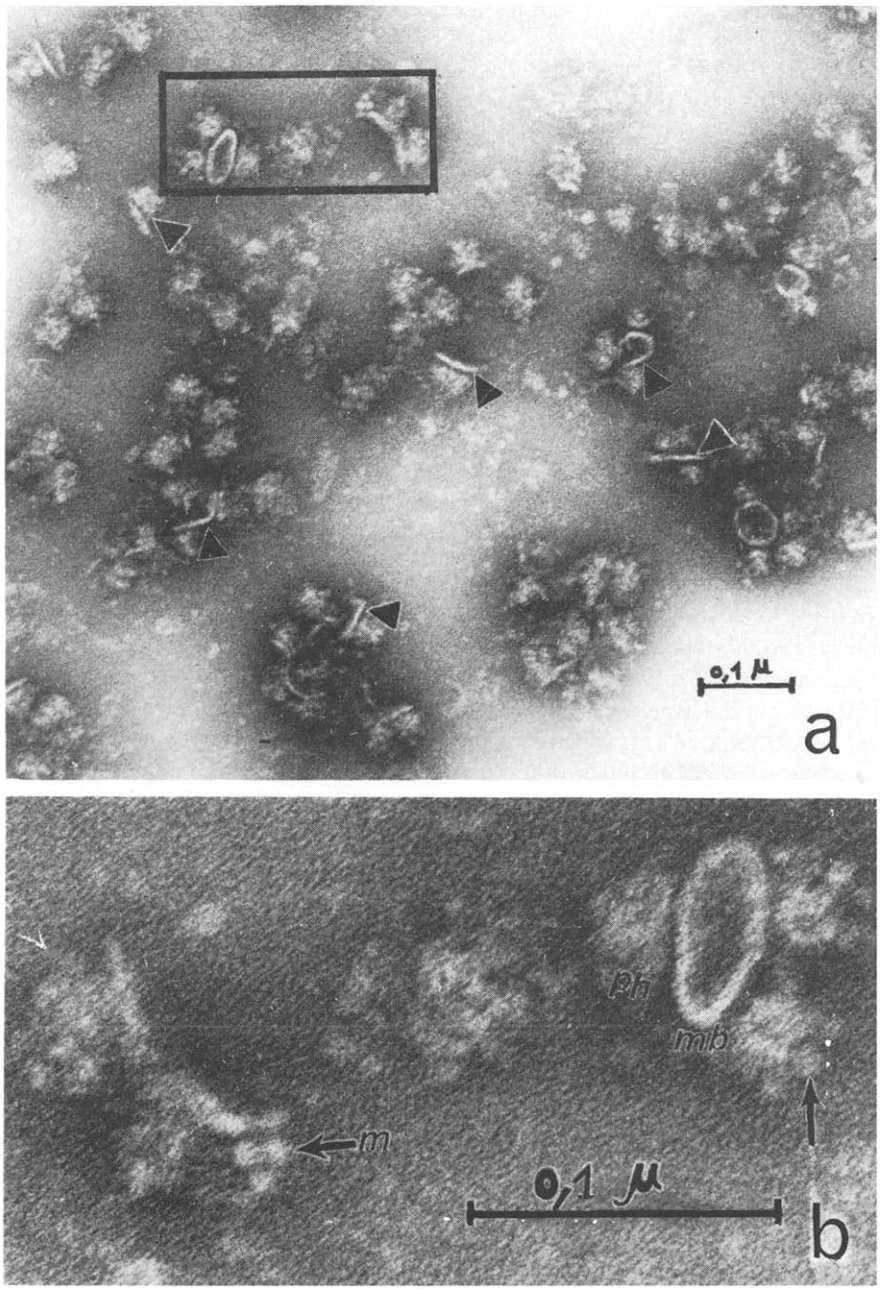


Fig. 2. (a) Phosphotungstic acid 1%. Fraction of prefixed cells. Large granules or phycobilisomes attached to lamellar fragments. Note the homogeneity of the fraction. X 125,5 50
(b) Enlargment of fig. 2 (a) showing the subunits (m) of phycobilisomes (ph) corresponding to hexamere of phycoerythrine. Note different view of contact between lamellae and phycobilisome. X 419,500.

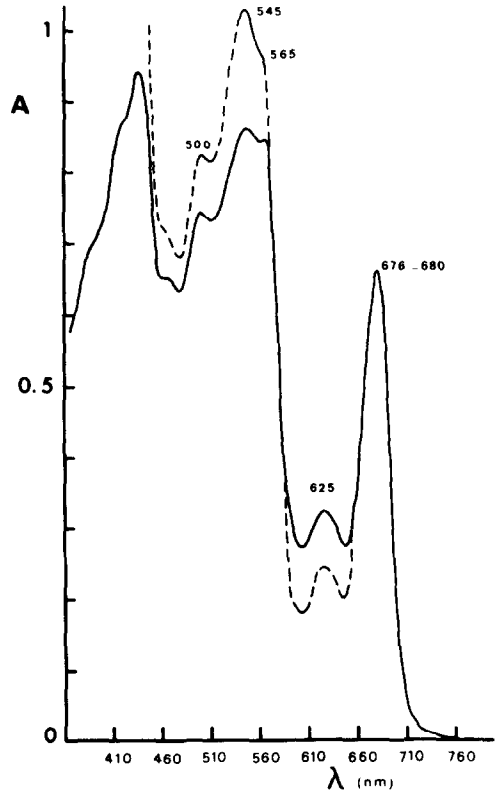


Fig. 3. Absorption spectra of whole intact cells (—) and glutaraldehyde fixed particles after disruption in the French pressure cell and sucrose gradient centrifugation (see text) (- - -).

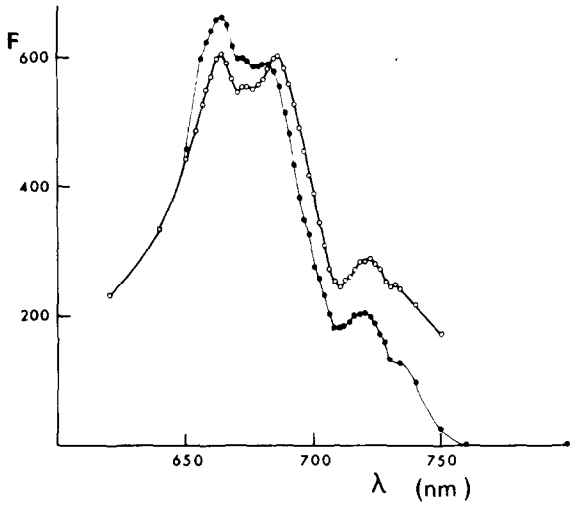


Fig. 4. Fluorescence emission spectra of whole cells (o—o) and glutaraldehyde fixed particles (•—•) (same as in fig. 3). Intensity of fluorescence in arbitrary units.

The instrument used to measure fluorescence has been described elsewhere [6]. In order to eliminate reabsorption of fluorescence rather dilute samples were used, with absorbance around 0.3 at 680 nm (1 cm path length). The emission spectra were evaluated by correcting each curve for the wavelength dependence of the photomultiplier sensitivity and the spectral efficiency of the monochromator, as well as for the variation in photon energy. These fluorescence emission spectra were measured in steady state conditions, eliminating the influence of fluorescence induction.

For electron microscopic examination (negative staining) the material to be examined was dialysed overnight in ammonium acetate 0.01 M, pH 7. After centrifugation one drop was colored with phosphotungstic acid (1% w/v) as previously described [2], before examination in a Hitachi electron microscope H U 11 A operating at 75 kV and using the high resolution device.

3. Results

After sucrose gradient centrifugation, the tube containing the extract from prefixed cells shows a faint pink band at the top of the gradient and a lower purple band. The upper diffuse band contains a small amount of soluble phycoerythrin which has not been fixed; all the chlorophyll is in the middle layer. This fraction, after centrifugation and resuspension in buffer, was studied for structure, absorption and fluorescence emission spectra.

The structure of the membrane fragments is shown in fig. 2. The fragments, rather homogeneous in size, consist of membrane pieces always closely associated with one or more small granules.

Fig. 3 shows the absorption spectra of whole intact cells and glutaraldehyde fixed particles adjusted to give equal absorbance at 676 nm. The particles have the same pigment composition as whole algae. We must notice that there is no shift in the absorption peaks of

phycoerythrin (500–545–565 nm), phycocyanin (625 nm) or chlorophyll *a* (676 nm). The ratio of phycoerythrin to chlorophyll is constant in whole cells and equals 1.3. In the particles it is 1.6. This augmentation could not be due only to the release of the geometrical flattening effect [7], resulting from an inhomogeneous distribution of pigments in intact cells, as this should be equally true for chlorophyll as well as for phycobilins. It seems more likely to be an effect of the complete accessibility of the phycoerythrin granules (see fig. 1) to the solvent.

Fig. 4 represents the fluorescence emission spectra, measured in steady-state conditions. When excited at 544 nm, glutaraldehyde particles are able to transfer energy to phycocyanin (660 nm) and chlorophyll (685 and 720 nm) as well as intact cells.

4. Discussion

The very labile association between chlorophyll bearing thylacoids and phycobiliproteins is stabilized by glutaraldehyde. The phycobilisomes remain associated to the membrane fragments of the chloroplast of *Porphyridium cruentum*, after disruption and purification on a sucrose gradient. These homogeneous particles have the same pigment composition as whole cells, and the glutaraldehyde treatment does not alter the efficiency of transfer of energy between phycoerythrin, phycocyanin and chlorophyll.

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