Photosynthetic electron transport in thylakoid preparations from two marine red algae (*Rhodophyta*)

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Thylakoid membrane preparations active in photosynthetic electron transport have been obtained from two marine red algae, *Griffithsia monilis* and *Anotrichium tenue*. High concentrations (0.5–1.0 M) of salts such as phosphate, citrate, succinate and tartrate stabilized functional binding of phycobilisomes to the membrane and also stabilized Photosystem II-catalysed electron-transport activity. High concentrations (1.0 M) of chloride and nitrate, or 30 mM-Tricine/NaOH buffer (pH 7.2) in the absence of salts, detached phycobilisomes and inhibited electron transport through Photosystem II. The O₂-evolving system was identified as the electron-transport chain component that was inhibited under these conditions. Washing membranes with buffers containing 1.0–1.5 M-sorbitol and 5–50 mM concentrations of various salts removed the outer part of the phycobilisome but retained 30–70% of the allophycocyanin 'core' of the phycobilisome. These preparations were 30–70% active in O₂ evolution compared with unwashed membranes. In the sensitivity of their O₂-evolving apparatus to the composition of the medium *in vitro*, the red algae resembled blue–green algae and differed from other eukaryotic algae and higher plants. It is suggested that an environment of structured water may be essential for the functional integrity of Photosystem II in biliprotein-containing algae.

Red algae utilize water-soluble phycobiliproteins as their major light-harvesting pigments, and in this respect resemble the prokaryotic blue–green algae (*Cyanophyta*) rather than other eukaryotic algae or higher plants (Coombs & Greenwood, 1976). In red algae the three main types of phycobiliproteins, phycoerythrin, phycocyanin and allophycocyanin, are aggregated into granules called phycobilisomes attached to the external thylakoid surface, and are thought to transfer absorbed light energy mainly to the antenna chlorophyll of Photosystem II (for review, see Gantt, 1980; Glazer, 1981). The structure of red-algal phycobilisomes corresponds to the energy-transfer pathway: an array of rods with phycoerythrin at the outer ends, phycocyanin in the

middle and allophycocyanin at the core in direct contact with the chlorophyll-containing membrane (Gantt, 1980; Gantt & Lipschultz, 1980).

Blue–green algae, which also possess phycobilisomes, differ from higher plants in their thylakoid organization, and a number of studies have suggested that special conditions, for example high concentrations of polyols and/or 10–20 mM concentrations of bivalent cations (Fredericks & Jagendorf, 1964; McSwain *et al.*, 1976; Binder *et al.*, 1976; Stewart & Bendall, 1980) are required for retention of Photosystem II activity. No such precautions are essential in the preparation of Photosystem II-active thylakoids from higher plants.

Although eukaryotic, the red algae have a thylakoid organization closely resembling that of the blue–green algae, yet very little attention has been paid to electron transport in this biliprotein-containing group. It is pertinent to ask whether the electron-transport properties of red algae resemble more closely those of blue–green algae or those of higher plants.

A technique has recently been developed for retaining the phycobilisomes attached to the thylakoids of biliprotein-containing algae during mem-

Abbreviations used: Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; SPC, 0.5 M-sucrose/0.3 M-potassium citrate/0.5 M-potassium phosphate buffer, pH 7.5; DMBQ, 2,6-dimethyl-1,4-benzoquinone; DCIP, 2,6-dichloroindophenol (reduced form denoted by DCIPH₂); DBMIB, 2,5-dibromo-6-isopropyl-3-methyl-1,4-benzoquinone.

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brane isolation (Kato & Gantt, 1979; Dilworth & Gantt, 1981). So far this technique has been used for studies on energy transfer, but it also offers the potential for comparison of the electron-transport properties of thylakoids with and without attached phycobilisomes. The technique involves the use of isolation buffers containing high concentrations of sucrose (0.5M), phosphate (0.5M) and citrate (0.3M), and raises several interesting questions in the light of more recent work: it has now been shown (Stewart, 1982) that in spinach chloroplasts high concentrations of certain salts [those high on the Hofmeister or lyotropic series (e.g. phosphates and citrates)] were more effective than uncouplers in stimulating both Photosystem I- and Photosystem II-catalysed electron-transport reactions. The most marked stimulatory effect of these salts was on the O_2 -evolving system of Photosystem II, which was also protected by the salts against inactivation by heat and chaotropic agents. It was suggested that ions high on the Hofmeister series stabilized a highly active conformation of the O_2 -evolving complex, possibly by stabilizing regions of structured water around the complex.

The aims of the present study were to obtain data on the electron-transport activities of thylakoid preparations from two species of marine red algae, to compare the properties of Photosystem II-catalysed electron transport with published information for other marine algae, higher plants and blue-green algae and to study the relationship between phycobilisome binding and stability of Photosystem II in red-algal thylakoids.

The two red algae chosen, *Griffithsia monilis* (Harvey) and *Anotrichium tenue* (C. Agardh) Naegeli, are both multinucleate-celled algae of the order Ceramiales. They offer the advantage that the cells are easily broken by gentle homogenization (Larkum & Weyrauch, 1977; Lilley & Larkum, 1981) and do not release the large amounts of mucus that make the leathery, thalloid types of red algae difficult to work with.

Materials and methods

Plants of *Griffithsia* and *Anotrichium* were collected from intertidal rocks at Little Sirius Point, Sydney Harbour, N.S.W., Australia. Strands of healthy red cells were washed in clean seawater and stored overnight in the dark at 18°C in aerated seawater before preparation of thylakoids.

Thylakoids with attached phycobilisomes were prepared by homogenizing the cells for 15 s at setting 5 in a Sorvall Omnimixer (Ivan Sorvall Inc., Newtown, CT, U.S.A.) in ice-cold SPC buffer (Dilworth & Gantt, 1981). The brei was squeezed through one layer of Miracloth (Chicopee Mills, Milltown, NJ, U.S.A.) and filtered through a further

two layers. Thylakoids pelleted from the filtrate by centrifuging at 12000g for 10 min were resuspended in SPC buffer.

Chlorophyll *a* was assayed by the method of Arnon *et al.* (1974).

Electron transport was assayed as O_2 exchange by using a Rank oxygen electrode (Rank, Botolphsham, Cambridge, U.K.), or as reduction of DCIP by using a dual-wavelength difference spectrophotometer (Johnson Foundation, University of Pennsylvania, Philadelphia, PA, U.S.A.). The methods used have been described in detail previously (Stewart, 1982).

For the assay of phycobiliproteins, phycobilisomes were released from the membranes and dissociated by diluting membrane samples 100-fold in 10 mM-potassium phosphate buffer, pH 7.0 (Gantt *et al.*, 1976) and incubating at 20°C for 4 h. The membranes were pelleted by centrifuging at 15000g for 1 h, and the absorption spectrum of the supernatant was recorded. Amounts of phycoerythrin were estimated from the absorbance at 545 nm, and amounts of phycocyanin and allophycocyanin were calculated from the absorbance values at 620 nm and 650 nm by using the equations derived by Gantt & Lipschultz (1974).

Results

In agreement with the work of Dilworth & Gantt (1981) it was found that when *Griffithsia* or

Fig. 1. Absorption spectra of thylakoid membrane preparations from *G. monilis* ----, Membranes in SPC buffer, pH 7.5; —, membranes washed and resuspended in a buffer containing 1.0M-sorbitol, 50mM-KCl, 5mM-MgCl₂ and 30mM-Tricine/NaOH, pH 7.5. Chlorophyll *a* concentration was 6.8 µg/ml.

Anotrichium cells were homogenized and centrifuged in SPC buffer, the phycobiliproteins pelleted with the thylakoids. The thylakoids absorbed strongly in the green region of the spectrum (Fig. 1) and were able to perform electron transport efficiently in low-intensity green light (Fig. 2), indicating that the functional connection between the phycobilisomes and the membrane was preserved. As in spinach chloroplasts (Stewart, 1982) the rates of Photosystem I- and Photosystem II-catalysed partial reactions were very high when assayed in saturating light in SPC buffer, and were not stimulated by the uncoupler NH_4Cl (Table 1). There was good preservation of electron transport through the complete chain from water to Methyl Viologen.

Red thylakoids suspended in SPC buffer were remarkably stable, like spinach chloroplasts suspended in 1.0M-potassium citrate, pH 7.5 (Stewart, 1982). No appreciable change in Photosystem II activity occurred over storage for 6 h on ice, and the thylakoids were resistant to inactivation by mild heating (70% activity remaining after 3 min at 47°C).

The effects of high concentrations of other salts were investigated. It was found that a number of salts preserved high Photosystem II activity (assayed as electron transport from water to the lipophilic acceptor 2,6-dimethyl-1,4-benzoquinone) and these same salts also stabilized functional binding of phycobilisomes (Table 2). In membrane

preparations from *Griffithsia*, high concentrations of phosphate, citrate, succinate and tartrate acted as stabilizers. However, molar concentrations of chloride and nitrate were inhibitory. Electron-transport activity appeared to have a stronger dependence on the stabilizing salts than did phycobilisome binding, since at low citrate concentrations the former was less effectively preserved than the latter.

As Table 2 shows, Photosystem II activity was inhibited and phycoerythrin removed if the thylakoids were washed with dilute Tricine buffer without added salts. The O_2 -evolving mechanism proved to be the most sensitive component of Photosystem II to the composition of the medium: thylakoids completely inhibited in Hill activity by incubation in 30mM-Tricine/NaOH buffer, pH 7.2, could be reactivated by addition of *sym*-1,5-diphenylcarbonohydrazide, an artificial donor to Photosystem II bypassing the water-oxidation site (Fig. 3). In contrast, Photosystem I activity in the partial reaction $\text{DCIPH}_2 \rightarrow \text{Methyl Viologen}$ was relatively insensitive to hypo-osmotic shock, with a high rate of electron transport (570 μmol of O_2/h per mg of chlorophyll *a* in the presence of 3mM- NH_4Cl) retained in the hypo-osmotic Tricine buffer.

Fig. 2. Rates of O_2 evolution in low-intensity red light and green light in *G. monilis* thylakoids with attached phycobilisomes

Red light (●) was filtered through a Corning 2-62 filter and green light (▲) through a Schott 545nm interference filter. Intensities were varied by using Balzers neutral density filters. The light-saturated rate of O_2 evolution ($\text{H}_2\text{O} \rightarrow \text{DMBQ}$ reaction) was 611 μmol of O_2/h per mg of chlorophyll.

Table 1. Electron transport in *G. monilis* and *A. tenue* thylakoids with attached phycobilisomes

Thylakoids were isolated in SPC buffer as described in the Materials and methods section. Electron transport was assayed in saturating red light (Corning 2-62 filter, 600 μmol of photons/ m^2 per s) in 3.0ml of SPC buffer containing thylakoids equivalent to 20 μg of chlorophyll *a*. Values listed are ranges for three to five preparations. Concentrations of reagents were: 0.5mM-DMBQ, 1.0mM- $\text{K}_3\text{Fe}(\text{CN})_6$, 3mM- NH_4Cl , 1.0 μM -DBMIB, 0.1mM-Methyl Viologen, 0.5mM- NaN_3 , 75 μM -DCIP (plus 2.5mM-sodium D-isoascorbate).

Reaction	Rate of O_2 exchange (μmol of O_2/h per mg of chlorophyll <i>a</i>)	
	<i>Griffithsia</i>	<i>Anotrichium</i>
$\text{H}_2\text{O} \rightarrow \text{DMBQ} + \text{K}_3\text{Fe}(\text{CN})_6$	510–650	650–790
$\text{H}_2\text{O} \rightarrow \text{K}_3\text{Fe}(\text{CN})_6$	200–320	350–580
$\text{H}_2\text{O} \rightarrow \text{K}_3\text{Fe}(\text{CN})_6$ + NH_4Cl	200–320	350–580
$\text{H}_2\text{O} \rightarrow \text{K}_3\text{Fe}(\text{CN})_6$ + DBMIB	120–160	210–300
$\text{H}_2\text{O} \rightarrow \text{Methyl Viologen}$ + NaN_3 + NH_4Cl	80–100	310–550
$\text{DCIPH}_2 \rightarrow \text{Methyl Viologen}$	490–580	730–860
$\text{DCIPH}_2 \rightarrow \text{Methyl Viologen}$ + NH_4Cl	490–580	730–860

Table 2. *Effect of high concentrations of salts on retention of phycobilisomes and stability of Photosystem II activity*
 Samples (0.2 ml) of *Griffithsia* thylakoids in SPC buffer were diluted to 20 ml with 30 mM-Tricine/NaOH buffer, pH 7.5, containing the additions shown. Thylakoids were incubated for 15 min at 5°C, pelleted by centrifuging (12000 g, 20 min) and resuspended in 0.5 ml of the same buffers. O₂ evolution (H₂O → DMBQ reaction) was assayed at 25°C in SPC buffer in low-intensity green light (Schott 545 nm interference filter, 45 μmol of photons/m² per s) or in saturating red light (Corning 2-62 filter, 600 μmol of photons/m² per s). Phycoerythrin content was estimated from the absorbance at 545 nm as described in the Materials and methods section. Control rates of O₂ evolution for unwashed membranes (μmol of O₂/h per mg of chlorophyll *a*) were 516 (red light) and 254 (green light).

Washing and resuspension buffer (additions to 30 mM-Tricine/NaOH, pH 7.5)	Rate of O ₂ evolution (% of control)		Phycoerythrin retained in thylakoid pellet (% of control)
	Low-intensity green light	Saturating red light	
No addition	0	3.9	0.8
1.0 M-Potassium phosphate	94	98	100
1.0 M-Potassium succinate	115	109	96
1.0 M-Sodium tartrate	101	124	99
0.75 M-Potassium citrate	96	122	100
0.5 M-Potassium citrate	90	95	96
0.3 M-Potassium citrate	57	54	78
0.2 M-Potassium citrate	21	15	47
1.0 M-KCl	0	0	2.8
1.0 M-NaNO ₃	0	0	1.7

Table 3. *Effect of medium composition on retention of phycobiliproteins and stability of O₂ evolution in washed thylakoids of G. monilis and A. tenue*

Samples (0.3 ml) of thylakoids were diluted to 30 ml and incubated for 30 min in the buffers shown. Each incubation mixture was divided between two tubes (20 ml plus 10 ml) and centrifuged at 15000 g for 10 min. The smaller pellets were resuspended in 2.0 ml of 10 mM-potassium phosphate buffer, pH 7.0, for measurement of phycobiliproteins as described in the Materials and methods section. Control amounts of phycobiliproteins in unwashed membranes (mg/mg of chlorophyll *a*) were, for *Griffithsia* and *Anotrichium* respectively: phycoerythrin, 12.1 and 12.4; phycocyanin, 0.9 and 0.7; allophycocyanin, 0.3 and 0.7. The larger pellets were resuspended in 0.6 ml of the same buffers used for washing, plus 10 mM-KCl. When the washing buffer was hypo-osmotic (lines 1–3 of each table), 0.3 M-sorbitol was added to the resuspending buffer. O₂ evolution was assayed in SPC buffer within 1 h, with 0.5 mM-DMBQ plus 1 mM-K₃Fe(CN)₆ as acceptor. Control rates for unwashed membranes (μmol of O₂/h per mg of chlorophyll *a*) were 431 (*Griffithsia*) and 686 (*Anotrichium*).

Washing buffer (additions to 30 mM-Tricine/NaOH, pH 7.5)	Phycobiliprotein retained (% of control)							
	Rate of O ₂ evolution (% of control)							
	<i>monilis</i>	<i>tenue</i>	Phycoerythrin		Phycocyanin		Allophycocyanin	
No additions	3.9	0	<i>monilis</i>	<i>tenue</i>	<i>monilis</i>	<i>tenue</i>	<i>monilis</i>	<i>tenue</i>
10 mM-MgCl ₂	23.4	0	0.8	0.8	0.4	0.8	1.4	3.7
50 mM-KCl	6.0	0	1.2	1.4	2.1	3.5	9.7	10.6
1.0 M-sorbitol	23.4	4.2	2.0	1.9	3.8	0.5	7.9	4.3
1.0 M-sorbitol, 10 mM-MgCl ₂	37.0	48.8	1.8	3.1	1.9	4.8	7.1	18.7
1.0 M-sorbitol, 10 mM-CaCl ₂	36.9	52.8	5.4	11.5	7.8	13.9	33.2	49.2
1.0 M-sorbitol, 10 mM-MgSO ₄	84.5	88.3	5.4	12.0	8.4	17.9	34.7	53.1
1.0 M-sorbitol, 10 mM-Mg(NO ₃) ₂	26.9	26.1	6.2	17.3	15.5	53.7	79.0	86.7
1.5 M-sorbitol, 5 mM-MgCl ₂	57.7	67.5	4.7	8.2	6.8	10.8	24.3	24.1
1.0 M-sorbitol, 50 mM-KCl	71.0	65.0	7.6	17.5	12.4	42.3	67.5	59.8
1.0 M-sorbitol, 50 mM-KCl, 5 mM-MgCl ₂	73.2	68.1	2.4	3.3	2.5	14.2	69.8	55.8
			3.6	9.0	5.7	22.9	70.2	64.4

As mentioned previously, for a number of blue-green algae media were found that stabilized O₂ evolution even though most of the phycobiliprotein was lost. Similar conditions were effective for the red algae (Table 3). High concentrations of sorbitol and 5–50 mM concentrations of various salts removed substantial proportions of phycoerythrin and phycocyanin, which together con-

tributed to the red color of the algae. High concentrations of sorbitol and 5–50 mM concentrations of various salts removed substantial proportions of phycoerythrin and phycocyanin, which together con-

Fig. 3. Effect of hypo-osmotic shock on Photosystem II activity in *G. monilis* thylakoids

A sample of membranes (0.015 ml) was diluted to 1.5 ml with buffer containing 30 mM-Tricine/NaOH, pH 7.2. The rate of DCIP reduction in the light was measured immediately, after incubation for 6 min in the cuvette, and then after addition of 1 mM-sym-1,5-diphenylcarbonohydrazide (DPC). The concentration of DCIP was 17 μ M, and the measuring and reference wavelengths were 570 nm and 520 nm respectively. The numbers beside the traces are the rates of DCIP reduction (μ mol of DCIP/h per mg of chlorophyll *a*).

stitute approx. 90% of the phycobiliprotein in the algae (Gantt & Lipschultz, 1980), but preserved approx. 40–80% of the O_2 -evolving capacity of the unwashed membranes assayed in saturating red light. The absorption spectrum for active *Griffithsia* membranes washed in a buffer containing 1.0 M-sorbitol, 50 mM-KCl, 5 mM-MgCl₂ and 30 mM-Tricine/NaOH buffer, pH 7.5, is shown in Fig. 1 (note the substantial decrease in absorbance in the green region of the spectrum).

However, careful examination of the phycobiliprotein content revealed that the most active washed preparations retained substantial proportions of the 'core' component of the phycobilisome, allophycocyanin (Table 3). (Allophycocyanin is obscured by

Fig. 4. Relationship between retention of allophycocyanin and stability of O_2 evolution in washed thylakoids of *G. monilis* and *A. tenue*

Samples of thylakoids were washed and resuspended in the buffers listed in Table 3. Rates of O_2 evolution ($H_2O \rightarrow DMBQ$ reaction) were assayed in SPC buffer, and amounts of allophycocyanin measured as described in the Materials and methods section. Activities and allophycocyanin content are expressed as percentages of the values for unwashed membranes. ●, *G. monilis*; ○, *A. tenue*.

chlorophyll in the spectrum for washed membranes, and does not significantly affect total absorbance in the red region of the spectrum.) For both *Griffithsia* and *Anotrichium* there was an approximate correlation between retention of activity and retention of allophycocyanin (Fig. 4), though *Griffithsia* differed from *Anotrichium* in being somewhat more stable at low allophycocyanin concentrations, especially in the presence of either 1.0 M-sorbitol or 10 mM-MgCl₂.

Washed membranes of both algae, when assayed in the washing buffer (1.0 M-sorbitol/50 mM-KCl/5 mM-MgCl₂/30 mM-Tricine/NaOH buffer, pH 7.5) (Table 4) were less stable and active than membranes with attached phycobilisomes (Table 1), but showed electron-transport rates for the partial reactions of Photosystem I and Photosystem II comparable with rates that have been reported for other marine algae (Ben-Amotz & Avron, 1972; Schönfeld *et al.*, 1973) and for blue-green algae (Arnon *et al.*, 1974; Binder *et al.*, 1976; Ono & Murata, 1978; Stewart & Bendall, 1980). However, in washed *Griffithsia* thylakoids the rate of electron transport through the complete chain was low ($H_2O \rightarrow$ Methyl Viologen reaction), possibly indicating that a soluble component between the

Table 4. *Electron transport in active washed thylakoids of G. monilis and A. tenue*

Samples (0.4 ml) of thylakoids prepared in SPC buffer were diluted to 40 ml with buffer containing 1.0 M-sorbitol, 50 mM-KCl, 5 mM-MgCl₂ and 30 mM-Tricine/NaOH buffer, pH 7.5, incubated for 30 min at 5°C, pelleted by centrifuging (12 000 g, 10 min) and resuspended in the washing buffer. Electron transport was assayed at 25°C in the same sorbitol/MgCl₂/KCl/Tricine buffer. Concentrations of reagents were as listed in the legend to Table 1.

Reaction	Rate of O ₂ exchange (μ mol of O ₂ /h per mg of chlorophyll <i>a</i>)	
	<i>Griffithsia</i>	<i>Anotrichium</i>
H ₂ O → DMBQ + K ₃ Fe(CN) ₆	280–370	100–150
H ₂ O → K ₃ Fe(CN) ₆	170–240	80–90
H ₂ O → K ₃ Fe(CN) ₆ + NH ₄ Cl	170–240	110–130
H ₂ O → K ₃ Fe(CN) ₆ + DBMIB	100–150	50–70
H ₂ O → Methyl Viologen + NaN ₃	0–20	60–90
H ₂ O → Methyl Viologen + NaN ₃ + NH ₄ Cl	30–60	150–200
DCIPH ₂ → Methyl Viologen	260–340	200–290
DCIPH ₂ → Methyl Viologen + NH ₄ Cl	450–660	640–700

photosystems, such as plastocyanin or a soluble cytochrome, had been lost.

Discussion

The data in Tables 1 and 4 provide the first information on the Photosystem I- and Photosystem II-catalysed electron-transport activities of thylakoid preparations in red algae. In the sensitivity of their O₂-evolving apparatus to the composition of the medium the red-algal thylakoids were clearly similar to preparations from a number of blue-green algae, where the O₂-evolving complex has also been identified as the component of Photosystem II that is irreversibly inactivated when the thylakoids are rinsed with dilute buffers (Biggins, 1967; Fujita & Suzuki, 1971; Stewart & Bendall, 1980). This finding is in agreement with the close evolutionary relationship that has been suggested for the two algal groups on the basis of immunological cross reactivity between their corresponding phycobiliproteins (Glazer, 1981).

In higher-plant chloroplasts, on the other hand, although high concentrations of phosphate and citrate stimulate O₂ evolution, washing in dilute buffers does not lead to irreversible inactivation of the O₂-evolving system. Nor is such sensitivity characteristic of other groups of marine algae. For

example, studies on intact chloroplasts of the marine green algae *Codium*, *Bryopsis* and *Caulerpa* (Chlorophyta) have shown that an osmotic strength comparable with that of seawater was required for maximum rates of CO₂ fixation (Grant *et al.*, 1976; Schönfeld *et al.*, 1973; Yamagishi *et al.*, 1981), but in broken chloroplasts from these algae and from the brine alga *Dunaliella salina* (Ben Amotz & Avron, 1972) electron transport from water to artificial acceptors was much less sensitive to the osmotic strength of the medium.

The nature of the correspondence between conditions preserving functional binding of phycobiliprotein and conditions preserving O₂ evolution in the red algae is worthy of comment. It is significant that the most important phycobilisome component in this respect was allophycocyanin, the pigment that is thought to constitute the 'core' of the phycobilisome in most intimate contact with the membrane (Gantt, 1980). The result suggests that there may be a close structural association between allophycocyanin and the electron-transport chain components of Photosystem II, so that when allophycocyanin is removed the O₂-evolving system is more accessible from the external medium and more strongly affected by its composition. *Anotrichium* appeared to be more fragile than *Griffithsia* in this respect, since in *Anotrichium* no conditions were found that preserved O₂ evolution when all the allophycocyanin was sheared away from the membranes. *Griffithsia* more closely resembled many blue-green algae, in that either a high concentration of sorbitol alone or MgCl₂ alone could preserve activity with retention of very little phycobiliprotein (Arnon *et al.*, 1974; McSwain *et al.*, 1976).

It is difficult to find a molecular explanation for the sensitivity *in vitro* of Photosystem II in red and blue-green algae. The remarkable stabilizing effect of ions high on the Hofmeister series, both on the phycobilisome and, as has now been shown, on O₂ evolution, suggests that an environment of structured water (Franks, 1977) may be important in preserving the functional integrity of the whole Photosystem II complex in these algae. It is unlikely to be coincidental that salts high on the Hofmeister series have also been shown to stimulate and stabilize O₂ evolution in higher-plant chloroplasts (Stewart, 1982). However, as mentioned previously, in higher plants O₂ evolution is not inactivated *in vitro* when the membranes are washed in dilute buffers. A possible explanation for this difference may be that, although the O₂-evolving complex itself may have similar properties in all oxygenic organisms, in higher-plant thylakoids the presence of the large membrane-spanning light-harvesting complex may help to preserve a favourable microenvironment around the complex *in vitro*. It will be of interest to study electron transport in other algal

groups, particularly the chlorophyll *c*-containing algae (the 'Chromophyta'), to discover whether the properties of Photosystem II show affinities with the higher plants (and green algae) or with the red and blue-green algae.

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