The effects of oxygen solubility and high concentrations of salts on photosynthetic electron transport in chloroplast membranes

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(Received 25 July 1983/Accepted 9 November 1983)

Chloroplast membranes were isolated in different media containing Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] and high concentrations of sorbitol (0.33 M), potassium citrate (0.75 M) or Na₂SO₄ (1.0 M). Due to the complexity of the media, the oxygen solubility is strongly modified by high concentrations of salts (oxygen solubility for 0.33 M-sorbitol, 0.21 mmol/litre; for 0.75 M-potassium citrate, 0.121 mmol/litre; and for 1.0 M-Na₂SO₄, 0.112 mmol/litre). The knowledge of these values is necessary to interpret the rate of O₂ evolution. For thylakoids isolated in 'sorbitol buffer' and then tested in high concentrations of potassium citrate, a slight stimulation of O₂ evolution is observed (143–173 µmol of O₂/h per mg of chlorophyll *a*) with potassium ferricyanide as electron acceptor. When we monitor the potassium ferricyanide reduction, no stimulation of electron transport is obtained even if the observed phenomenon is identical with the Photosystem-II oxygen evolution. In the same experiments no stimulation of the photophosphorylation was recorded, but when thylakoids are directly isolated in 0.75 M-potassium citrate, O₂ evolution, ferricyanide reduction and photophosphorylation are inhibited by high concentrations of salts. The behaviour of thylakoids seems to be influenced by their initial treatment.

The use of photosynthetic systems in energy-conversion processes has been studied by numerous authors (Hall, 1976; Benemann *et al.*, 1980; San Pietro, 1980). Isolated chloroplast membranes (thylakoids) are able to perform the photolysis of water, but the limiting step for their use in continuous reactors is the stability of PSI and PSII activities over a long period of time. The stabilization of the photochemical activities constitutes an important problem and various approaches can be considered.

Photosynthetic electron transport and the ultra-structure of chloroplast membranes (Izawa & Good, 1966; Barber, 1980) can be affected and improved by the addition of some ions. A functional stabilization of the photosystem's activities has also been obtained by several methods of immobilization (Cocquempot *et al.*, 1979, 1981) or

by glutaraldehyde fixation (Hardt & Kok, 1976; Papageorgiou, 1980).

The organization and the structural integrity of organelles are largely responsible for the efficiency of the light-energy transfer. Changes in the concentrations of uni- or bi-valent cations, especially, modify the stacking of thylakoids and photosynthetic activity (Thomasset *et al.*, 1982). Other authors have shown that chaotropic ions [NO₃⁻, ClO₄⁻, CCl₃CO₂⁻ (trichloroacetate)] destabilize membrane enzyme complexes and macromolecules (Hatefi & Hanstein, 1974). Anti-chaotropic ions [SO₄²⁻, HPO₄²⁻, C₆H₅O₇³⁻ (citrate)] can be used to perform the reconstitution of a water-insoluble multi-protein-lipid complex (Complex II, succinate:ubiquinone oxidoreductase; Davis & Hatefi, 1972), to stabilize native protein structure and to protect protein against denaturation (Jencks, 1969). Zilinskas & Glick (1981) have also studied the effects of various environmental perturbations (high concentrations of sodium salts, SCN⁻, NO₃⁻, C₆H₅O₇³⁻, SO₄²⁻, PO₄³⁻) on phycobilisome integrity to explain the different forces that maintain the phycobilisome structure.

Abbreviations used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; pBq, *p*-benzoquinone; DMBQ, 2,6-dimethyl-1,4-benzoquinone; DCDB, 3,6-dichloro-2,5-dimethoxy-1,4-benzoquinone; PSI and PSII, Photosystems I and II; PD_{ox.}, oxidized phenylene-1,4-diamine.

Stewart (1982) has studied the effect of high concentrations of salts (especially potassium citrate) on the activity of the chloroplast electron-transport chain. A considerable stimulation of oxygen evolution has been observed in untreated Tris-washed and trypsin-treated chloroplasts.

The purpose of the present paper is to discuss the effects of high concentrations of salts on the activity of electron transport in thylakoids measured with an oxygen electrode. In such a study we report the effects of high concentrations of citrate and sulphate on the oxygen-solubility values, on electron transport from water to an electron acceptor (ferricyanide, pBq, Methyl Viologen) and on photophosphorylation of the thylakoids.

Materials and methods

Thylakoid preparations

Intact chloroplasts were isolated from lettuce (*Lactuca sativa*) leaves by the method of Epel & Neuman (1973). The blending medium was 0.33 M-sorbitol / 10 mM- $\text{Na}_4\text{P}_2\text{O}_7$ / 20 mM- NaCl / 1 mM- MgCl_2 / 1 mM- MnCl_2 / 2 mM-EDTA / 0.15 mM- K_2HPO_4 / KH_2PO_4 adjusted to pH 6.8. Thylakoids were obtained by osmotic shock, centrifuged at 3000g for 30 s at 4°C and resuspended in 0.33 M-sorbitol / 50 mM-Hepes / 1 mM- MgCl_2 / 0.1 mM- MnCl_2 / 0.5 mM-EDTA / 0.15 mM- KH_2PO_4 / K_2HPO_4 adjusted to pH 7.4 ('sorbitol buffer') or in 20 mM-Hepes containing either 0.75 M-potassium citrate ('potassium citrate buffer') or 1.0 M- Na_2SO_4 (' Na_2SO_4 buffer'), both adjusted to pH 7.4. Chlorophyll was assayed by the method of Arnon (1949).

Electron-transport assays

Reaction media contained sorbitol buffer, potassium citrate buffer, or Na_2SO_4 buffer. NH_4Cl at 5 mM was used to uncouple phosphorylation.

Oxygen production. Electron transport was assayed at 22°C with a Clark oxygen electrode calibrated with air-saturated water. Reaction media were illuminated at a saturating intensity by a 100 W quartz/iodine lamp and filtered with a red filter ($600\text{ nm} < \lambda < 900\text{ nm}$; 500 W/m^2). pBq (1 mM), ferricyanide (5 mM) or Methyl Viologen (0.85 mM) (and 4 mM- NaN_3) were used as the electron acceptors.

Ferricyanide reduction. To perform these experiments, a closed circulating system was used. Reaction media were continuously moved from a batch reactor (15 ml) through a cuvette by means of a peristaltic pump. The rate of potassium ferricyanide (0.8 mM) reduction was continuously recorded by a spectrophotometer at 420 nm (Legoy *et*

al., 1982). The reaction medium was illuminated in the reactor under continuous saturating light.

Photophosphorylation assays. ATP produced during non-cyclic photophosphorylation was measured by using ferricyanide as electron acceptor by the luciferin/luciferase method of Pradet (1967).

Measurements of oxygen solubility

The solubility of the oxygen of the air in the buffers (sorbitol, potassium citrate and Na_2SO_4) was determined by a modified Winkler (1889) titrimetric procedure. This method is based on the transfer of the buffer's dissolved oxygen at saturation by a nitrogen flux, which is then 'trapped' in the reaction media containing $\text{Mn}(\text{OH})_2$ at a high pH, in the presence of excess KIO_4 . The solution is then made acidic and the resulting iodine is titrated with thiosulphate.

Fluorescence-emission spectra

These were recorded at 77 K with a laboratory-built apparatus that was described by Sironval *et al.* (1968).

Results

Effects of high salt concentrations on the solubility of the oxygen in the different reaction media

It is well known that the solubility of gases changes with salt concentrations, temperature and pressure (Wilhelm *et al.*, 1977). Numerous authors have studied the variation of this parameter in some aqueous solution of HCl , NH_4Cl , BaCl_2 , CaCl_2 or in numerous nitrate or sulphate salts. They have explained the decrease of solubility caused by increasing salt concentrations by hydration theories. All of these studies have been well summarized by Battino & Clever (1966). Müller (1912), MacArthur (1916) and Joslyn & Supplee (1949) have also shown the occurrence of a progressive and rapid decrease in oxygen solubility with an increase in the concentration of sucrose, dextrose or glycerol.

In our study, reaction media are complex and it seems necessary to determine the quantity of dissolved oxygen at saturation for the temperature and the pressure generally used in photoreduction experiments. The solubility of oxygen in our buffers was determined by a modified Winkler (1889) titrimetric procedure (see the Materials and methods section). Table 1 indicates that the oxygen solubility can be affected by a factor of 2 for different reaction media. In the case of a high concentration of Na_2SO_4 and potassium citrate, this parameter is 0.112 and 0.121 mmol/litre respectively. These values are very different from those obtained in water or sorbitol buffer (0.259 and 0.21 mmol/litre respectively). The errors in

oxygen-solubility measurements can be estimated at about 2.5–4%, depending on the method of determination.

Table 1. *Oxygen solubility in reaction media*

The oxygen solubility is determined by a modified Winkler (1889) titrimetric procedure described in the Materials and methods section. Experiments and titration were performed at 22°C and at 1.026 MPa (770 mmHg), which are the conditions used to achieve the biophotolysis of water. The composition of the various buffers is given under 'Thylakoid preparations' in the Materials and methods section.

Reaction medium	Oxygen solubility (mmol/litre)
Water	0.259 ± 0.0025
Sorbitol buffer	0.21 ± 0.0025
Potassium citrate buffer	0.121 ± 0.004
Na ₂ SO ₄ buffer	0.112 ± 0.0025

Effects of high salt concentrations on the photo-reductive activity

PSII reactions. As noted by Stewart (1982), oxygen evolution seems to be stimulated by high concentrations of potassium citrate. This author has noted that electron transport from water to DMBQ is stimulated 2–3-fold in the presence of potassium citrate at a concentration of 0.7 M and above. She has also observed a stimulation of oxygen evolution when other acceptors are used (ferricyanide, PD_{ox}, DCDB, Methyl Viologen).

The solubility of oxygen in water at 22°C and at atmospheric pressure is generally used to express the activities of the Hill reaction. In this hypothesis, the rate of electron transport from water to potassium ferricyanide (or pBq; results not shown) of thylakoids isolated in sorbitol buffer is stimulated by a factor of 2 by high concentrations of potassium citrate or Na₂SO₄ (Fig. 1a). For

Fig. 1. *Effects of high concentrations of citrate or sulphate on the rate of electron transport from water to potassium ferricyanide* Thylakoids were isolated in sorbitol, potassium citrate or Na₂SO₄ buffer as described in the Materials and methods section. Electron transport was assayed at 22°C by using a Clark-type electrode in 6.0 ml of a buffer containing thylakoids (corresponding to 100 μg of chlorophyll a), 5 mM-potassium ferricyanide and 5 mM-NH₄Cl. (a) Shows oxygen evolution of thylakoids isolated in sorbitol buffer and tested in potassium citrate buffer (○), in Na₂SO₄ buffer (□) and in sorbitol buffer (△). (b) Shows oxygen evolution of thylakoids isolated in potassium citrate buffer and tested in sorbitol buffer (●) or in potassium citrate buffer (○) or of thylakoids isolated in Na₂SO₄ buffer and tested in sorbitol buffer (■) or in Na₂SO₄ buffer (□).

thylakoids isolated in potassium citrate buffer (or Na_2SO_4 buffer) the stimulation of oxygen evolution was also observed when they were tested in the same buffer (Fig. 1b).

The initial PSII activity is greater for thylakoids isolated in sorbitol buffer ($371 \mu\text{mol}$ of oxygen/h per mg of chlorophyll *a*) than for thylakoids isolated in potassium citrate buffer ($304 \mu\text{mol}$ of oxygen/h per mg of chlorophyll *a*) when they are tested in a high concentration of potassium citrate (Table 2, apparent values). The same results are also obtained for Na_2SO_4 buffer (Table 2, apparent values).

According to the oxygen-solubility measurements (Table 1), we can determine the true values of the oxygen evolution. A slight increase of 20% in the PSII activity is only observed for thylakoids isolated in sorbitol buffer ($143 \mu\text{mol}$ of oxygen/h per mg of chlorophyll *a*) and tested in potassium citrate buffer ($173 \mu\text{mol}$ of oxygen/h per mg of chlorophyll *a*; Table 2, true values). In contrast, for thylakoids isolated in high salt concentrations (potassium citrate: $142 \mu\text{mol}$ of oxygen/h per mg of chlorophyll, or sodium sulphate: $107 \mu\text{mol}$ of oxygen/h per mg of chlorophyll) and tested in each of these buffers, no stimulation is shown (Table 2, true values).

The presence of NH_4Cl stimulates the rate of electron transport by uncoupling photophosphorylation (Table 3). We have also noticed that photophosphorylation is directly uncoupled at high concentrations of potassium citrate (Table 3; Good *et al.*, 1966).

PSI and PSII reactions. The electron transport through the complete chain (from water to Methyl Viologen) also seems to be stimulated by high concentrations of citrate or sulphate (Table 4, apparent values). But when we use the correct

solubility values to express the oxygen uptake, a slight activation is also observed for thylakoids isolated in sorbitol buffer (Table 4, true values).

From this study we have noticed that the behaviour of thylakoids seems to be influenced by their initial physiological state. Stewart (1982) has observed that, for high concentrations of citrate, the degree of stimulation in untreated chloroplasts is much greater than in Tris-washed or trypsin-treated chloroplasts.

Potassium ferricyanide reduction. Another way to measure the rate of electron transport from water to an electron acceptor is to monitor directly its reduction by spectrophotometry. In our experiments we chose to record the reduction of potassium ferricyanide at 420 nm in a batch reactor under continuous saturating light (Legoy *et al.*, 1982). We used the same working conditions as for the amperometric method.

For thylakoids isolated in sorbitol buffer and then tested in sorbitol buffer, potassium citrate buffer or Na_2SO_4 buffer, the reduction of ferricyanide seems to be of the same order. Although the same part of the photosynthetic electron chain is involved, no stimulation of the reduction of ferricyanide is observed, but a slight increase of 20% in oxygen evolution is noticed (Tables 5 and 2 respectively).

For thylakoids isolated in potassium citrate buffer (or in Na_2SO_4 buffer) and tested in this medium, only 70% of the Hill activity is recorded (compare with activity in sorbitol buffer; Table 5).

From the experiments we have also shown that the initial physiological state of the organelles influences their future catalytic behaviour.

Photophosphorylation. The photochemical reactions consist essentially of pigment excitations by photons. These reactions lead to NADP reduction

Table 2. Effects of high salt concentrations on electron transport from water to potassium ferricyanide (5 mM)

Thylakoids are isolated in sorbitol or potassium citrate or Na_2SO_4 buffer as described in the Materials and methods section. Electron transport is assayed in saturating red light (500 W/m^2) at 22°C with a Clark-type electrode in 6.0 ml of sorbitol or potassium citrate or Na_2SO_4 buffer containing thylakoids (corresponding to $100 \mu\text{g}$ of chlorophyll *a*). Uncoupling agent (NH_4Cl at 5 mM) was present in all experiments. The corrections used to convert 'apparent values' (AV) into 'true values' (TV) are as follows: sorbitol buffer: $\text{TV} = 2.1 \times \text{AV}/2.59$; potassium citrate buffer: $\text{TV} = 1.21 \times \text{AV}/2.59$; Na_2SO_4 buffer: $\text{TV} = 1.12 \times \text{AV}/2.59$.

Thylakoids isolated in ...	Oxygen evolution ($\mu\text{mol/h}$ per mg of chlorophyll <i>a</i>) in:					
	Sorbitol buffer		Potassium citrate buffer		Na_2SO_4 buffer	
	AV	TV	AV	TV	AV	TV
Reaction medium						
Sorbitol buffer	177	143				
Potassium citrate buffer	371	173	304	142		
Na_2SO_4 buffer	346	150			248	107

Table 3. *Effects of NH₄Cl on the rate of electron transport for thylakoid suspensions*

Thylakoids (corresponding to 100 µg of chlorophyll *a*) isolated in sorbitol, potassium citrate or Na₂SO₄ buffer were tested by using a Clark-type electrode in saturating red light (Good *et al.*, 1966). Evolution was tested in the absence or presence of uncoupling agent (NH₄Cl).

Thylakoids isolated and tested in:	Oxygen evolution (µmol of oxygen/h per mg of chlorophyll <i>a</i>)	
	Without NH ₄ Cl	With NH ₄ Cl
Sorbitol buffer	121	143
Potassium citrate buffer	134	142
Na ₂ SO ₄ buffer	57.5	107

Table 4. *Effects of high salt concentrations on electron transport from water to Methyl Viologen*

Thylakoids isolated in sorbitol, potassium citrate or Na₂SO₄ buffer were tested as described in the Materials and methods section. The concentrations of reagents were as follows: 0.85 mM-Methyl Viologen, 4 mM-NaN₃ and 5 mM-NH₄Cl.

Thylakoids isolated in ...	Oxygen uptake (µmol of oxygen/h per mg of chlorophyll <i>a</i>)					
	Sorbitol buffer		Potassium citrate buffer		Na ₂ SO ₄ buffer	
	Apparent values	True values	Apparent values	True values	Apparent values	True values
Reaction medium						
Sorbitol buffer	171	139				
Potassium citrate buffer	359	168	271	127		
Na ₂ SO ₄ buffer	348	149			251	108

Table 5. *Effects of high salt concentrations on potassium ferricyanide reduction*

Thylakoids were isolated in sorbitol, potassium citrate or Na₂SO₄ buffer and tested in these different reaction media. The experiments were performed in a batch reactor under saturating illumination. The rate of potassium ferricyanide reduction is recorded spectrophotometrically at 420 nm as described in the Materials and methods section. Concentrations of reagents were as follows: 5 mM-NH₄Cl and 0.8 mM-potassium ferricyanide (this excess of substrate is sufficient to test initial activity; Legoy *et al.*, 1982).

Thylakoids isolated in ...	Ferricyanide reduction (µmol of ferrocyanoide/h per mg of chlorophyll <i>a</i>)		
	Sorbitol buffer	Potassium citrate buffer	Na ₂ SO ₄ buffer
Reaction medium			
Sorbitol buffer	151		
Potassium citrate buffer	153	109	
Na ₂ SO ₄ buffer	148		108

Table 6. *Effects of high concentrations of citrate (or sulphate) on non-cyclic photophosphorylation*

Thylakoids isolated in sorbitol, potassium citrate or Na₂SO₄ buffer were tested in these different reaction media. Non-cyclic phosphorylation was studied with thylakoids (corresponding to 100 µg of chlorophyll *a*) in the different media containing 5 mM-potassium ferricyanide and 2 mM-ADP, under saturating illumination. ATP was measured with the luciferin/luciferase method as described by Pradet (1967).

Thylakoids isolated in ...	Ferricyanide reduction (µmol of ferrocyanoide/h per mg of chlorophyll <i>a</i>)		
	Sorbitol buffer	Potassium citrate buffer	Na ₂ SO ₄ buffer
Reaction medium			
Sorbitol buffer	180		
Potassium citrate buffer	186	108	
Na ₂ SO ₄ buffer	156		104

and ATP formation. Non-cyclic phosphorylation accompanies the electron transport from water to NADP. ATP production can also indicate the efficiency of electron transport through the two photosystems.

The results of ATP production are summarized on Table 6. For thylakoids isolated in sorbitol buffer, no significant stimulation is recorded for high concentrations of citrate. A high concentration of Na_2SO_4 seems to inhibit electron transport partially.

When thylakoids are directly isolated in high concentrations of potassium citrate (or Na_2SO_4), the complete activity is subsequently not restored (Table 6).

The results are similar to those obtained for ferricyanide reduction.

Fluorescence emission at 77 K

Fluorescence emission by chlorophyll *a* *in vivo* depends on the state of the photosynthetic membranes (Kautsky, 1931). The ratio of 77 K fluorescence emitted at 735 nm (F_{735}) to that at 695 nm (F_{695}) is used for evaluating the energy transfer from PSII to PSI (Murata, 1969; Mohanty *et al.*, 1971).

Fluorescence emission spectra are recorded at 77 K for thylakoids isolated in sorbitol buffer, potassium citrate buffer or Na_2SO_4 buffer. In all cases, three peaks are observed at 685 nm, 695 nm and 735 nm. The peaks are strongly associated either with the PSII pigment protein complex (685 nm, 695 nm) or with PSII. The fluorescence ratio (F_{735}/F_{695}) is almost the same for thylakoids isolated in sorbitol buffer (2.98) or Na_2SO_4 buffer (2.19). In this last case, the efficiency of energy transfer from PSI to PSII seems to be slightly affected.

Discussion

In this work, we have tried to improve photosynthetic activity by using high concentrations of potassium citrate or Na_2SO_4 . Stewart (1982) found that oxygen evolution rates were stimulated by high concentrations of certain anions. However, due to the complexity of the reaction media (Hepes and high concentrations of sorbitol, citrate or sulphate) we must still observe great care when explaining the experimental data. The oxygen solubility is strongly modified by high concentrations of salts (Wilhelm *et al.*, 1977) or sucrose (Joslyn & Supplee, 1949). Indeed, an oxygen electrode must give the same reading for any medium in equilibrium with atmospheric oxygen irrespective of the solubility of oxygen in this medium. The rates measured with an oxygen electrode can only be converted into μmol of

O_2 /unit time if the solubility of oxygen in the experimental medium is known. Stewart (1982) noticed that several salts containing anions that are high in the Hofmeister series can greatly stimulate oxygen evolution. But it appears that she failed to correct her results for differing oxygen solubilities. Indeed, in her paper no mention is made of the oxygen solubility in the different media used.

Thylakoids isolated in sorbitol buffer

When they are tested in potassium citrate buffer, a 20% stimulation of oxygen evolution is observed (Table 2, true values), whereas ferricyanide reduction remains unchanged (Table 5). The errors in oxygen-solubility measurements are estimated at about 2.5–4%, depending on the method of determination (Table 1), but they are not sufficient to explain such a slight stimulation. In one case oxygen evolution is observed, whereas in the other case the reduction of ferricyanide is recorded. These two molecules are quite different, and their diffusion through the reaction media certainly does not occur in the same manner.

Like PSII, PSI also seems to be slightly sensitive at high concentrations of salts (Table 4, true values). Another way to follow the electron through the photosynthetic chain is the study of non-cyclic phosphorylation. No stimulation is observed with citrate, and even sulphate seems to inhibit the reaction (Table 6). From Stewart's (1982) studies, potassium citrate seems to be the most efficient in protecting oxygen evolution against heat and chaotropes and in the restoration and stimulation of electron transport from water to the electron acceptors in untreated, Tris-washed or trypsin-treated thylakoids. As reported by Zilinskas & Glick (1981), citrate ion is more efficient than sulphate or phosphate in maintaining the phycobilisome integrity of *Porphyridium cruentum*.

In a general review, Hatefi & Hanstein (1974) showed that the addition of chaotropic ions (SCN^- , ClO_4^- , NO_3^- , $\text{CCl}_3\text{CO}_2^-$...) decreases water structures and induces a destabilization of membrane enzyme complexes and macromolecules. On the contrary, water-structure-forming ions, namely anti-chaotropes (PO_4^{3-} , SO_4^{2-} , $\text{C}_6\text{H}_5\text{O}_7^{3-}$...), tend to strengthen hydrophobic interactions in protein and multicomponent systems, and in particular restore the Complex II system (Davis & Hatefi, 1972). This slight apparent stimulation cannot be correlated with the relative ionic strength of these anions (Jencks, 1969; Davis & Hatefi, 1972; Figs 1a and 1b) and is not simply due to a countershielding of charges on proteins. In particular, low concentrations of bivalent cations can provide a more effective

countershielding than do univalent cations (Barber, 1980; Thomasset *et al.*, 1982).

The low accessibility of PSII to hydrophobic acceptors (ferricyanide or Methyl Viologen) can also explain this slight stimulation in oxygen evolution. Hardt & Kok (1976) have noticed that reactions are more sensitive to other lipophilic electron acceptors such as DMBQ, PD_{ox}, or DCDB.

It does not appear that a citrate-sensitive site in PSI and PSII exists, as indicated by Stewart (1982), but a destabilization of proteins can occur after the preparative treatment of organelles. Anti-chaotropes can really increase stability of the biological macrostructures (Hatefi & Hanstein, 1974).

From our experiments we have shown that addition of anti-chaotropes to untreated thylakoids does not greatly improve photosynthetic activity.

Thylakoids isolated in anti-chaotropic buffers

Another purpose of this work was to isolate the thylakoids directly in the presence of high concentrations of citrate or sulphate. We compared the activity with those of thylakoids isolated in sorbitol buffer.

In agreement with Zilinskas & Glick (1981), citrate is more efficient; nevertheless, high concentrations of citrate or sulphate partially inhibit electron transport (Table 2, true values; Table 5). The inhibition is less important when we study the electron transport through the complete chain (from water to Methyl Viologen; Table 4, true values). Non-cyclic photophosphorylation is strongly inhibited by high concentrations of salts (Table 6). Citrate and sulphate seem to have the same effect.

From this study the initial treatment of thylakoids seems to be of considerable importance for electron-transport efficiency. In conclusion, the preparation of thylakoids directly with anti-chaotropes does not improve initial photosynthetic activity. The initial treatment of thylakoids determines their responses to various environmental conditions. Results from these experiments suggest that a study of the stability of the two photosystems under continuous saturating illumination may prove worthwhile (Thomasset *et al.*, 1983).

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