The effects of high concentrations of salts on photosynthetic electron transport in spinach (*Spinacia oleracea*) chloroplasts

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1. Photosynthetic electron transport from water to lipophilic Photosystem II acceptors was stimulated 3–5-fold by high concentrations (≥ 1 M) of salts containing anions such as citrate, succinate and phosphate that are high in the Hofmeister series. 2. In trypsin-treated chloroplasts, $K_3Fe(CN)_6$ reduction insensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea was strongly stimulated by high concentrations of potassium citrate, but there was much less stimulation of 2,6-dichloroindophenol reduction in Tris-treated chloroplasts supplied with 1,5-diphenylcarbazine as artificial donor. The results suggest that the main site of action of citrate was the O_2 -evolving complex of Photosystem II. 3. Photosystem I partial reactions were also stimulated by intermediate concentrations of citrate (up to 2-fold stimulation by 0.6–0.8 M-citrate), but were inhibited at the highest concentrations. The observed stimulation may have been caused by stabilization of plastocyanin that was complexed with the Photosystem I reaction centre. 4. At 1 M, potassium citrate protected O_2 evolution against denaturation by heat or by the chaotropic agent $NaNO_3$. 5. It is suggested that anions high in the Hofmeister series stimulated and stabilized electron transport by enhancing water structure around the protein complexes in the thylakoid membrane.

It has been known for some time that, when present in high concentrations (>0.1 M), salts containing anions high in the so-called Hofmeister or lyotropic series have a tendency to stabilize native protein structure and to protect proteins against denaturation (Jencks, 1969). For example, high concentrations of sulphate and phosphate protect ribonuclease against thermal denaturation (Von Hippel & Wong, 1964; Ginsburg & Carrol, 1965), and similar stabilizing effects of sulphate, phosphate, citrate and acetate have been observed for collagen, gelatin and the mitochondrial complex II–succinate dehydrogenase multi-enzyme complex (Jencks, 1969; Davis & Hatefi, 1972). Most studies to date have been carried out on soluble proteins, which tend to be aggregated and precipitated by high

concentrations of certain ions in the phenomenon known as ‘salting out’, the most common example being the precipitation of proteins by high concentrations of $(NH_4)_2SO_4$. Less attention has been paid to the effect of such ions on membrane-bound or membrane-associated proteins, although Katoh & Gantt (1979) and Dilworth & Gantt (1981) found that although high concentrations of phosphate alone prevented disruption of the light-harvesting phycobilisome granules of red and blue-green algae, a combination of both phosphate and citrate (0.5 M and 0.3 M respectively) prevented detachment of the phycobilisomes from the thylakoids during membrane isolation and preserved energy transfer between the phycobiliproteins and Photosystem II. At the other end of the Hofmeister series, washing with 2 M-NaBr has been found to be an effective treatment for detaching coupling factor from thylakoid membranes (Nelson, 1980).

In the present study I report the effects of high concentrations of salts on the activity of the chloroplast electron-transport chain, particularly the components of Photosystem II and the O_2 -evolving system, and on the stability of O_2 evolution towards heat and chaotropic agents.

Abbreviations used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; DCDB, 3,6-dichloro-2,5-dimethoxy-1,4-benzoquinone; DMBQ, 2,6-dimethyl-1,4-benzoquinone; DBMIB, 2,5-dibromo-6-isopropyl-3-methyl-1,4-benzoquinone; TMPD, *NNN'*-tetramethylphenylene-1,4-diamine; DCIP, 2,6-dichloroindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Materials and methods

Chloroplast preparation

Fresh spinach (*Spinacia oleracea*) leaves were homogenized for 5 s at setting 5 in a Sorvall Omnimixer (Ivan Sorvall, Newtown, CT, U.S.A.), in ice-cold 30 mM-Tricine/KOH buffer, pH 8.0, containing 0.5 M-sorbitol, 5 mM-MgCl₂ and 5 mM-sodium D-isoascorbate. The brei was squeezed through one layer of Miracloth (Chicopee Mills, Milltown, NJ, U.S.A.) and filtered through a further two layers. The filtrate was centrifuged for 5 min at 2000 g, the chloroplasts washed once and finally resuspended in the above grinding buffer minus ascorbate. Chloroplasts were stored in the dark at 5°C and used within 2 h of preparation. Chlorophyll was assayed by the method of Arnon (1949).

Electron-transport assays

Electron transport was assayed at 20°C by using a Rank oxygen electrode (Rank, Bottisham, Cambridge, U.K.) calibrated with air-saturated distilled water. Saturating light (approx. 700 μ E/s per m²) was provided by a 250 W quartz/iodine lamp and filtered through a Corning CS 1-75 glass heat filter and a Corning 2-62 red glass filter.

The assay buffer for electron transport contained either 0.5 M-sorbitol / 5 mM-MgCl₂ / 20 mM-Hepes / KOH buffer, pH 7.5 ('sorbitol/Hepes buffer') or 20 mM-Hepes/KOH buffer, pH 7.5, plus various concentrations of potassium citrate or other salts. The buffer was made up and the pH adjusted separately for each concentration of salt used. It should be noted that errors can be incurred in the measurement of pH using glass electrodes in the presence of high concentrations of salt, particularly when the cation is Na⁺. However, the error only becomes significant at pH values greater than 8, and at pH 7.5 it is negligible (<0.05 pH unit in the presence of 3 M-Na⁺) (Bates, 1964).

The reaction mixture for reduction of lipophilic Photosystem II acceptors contained, in 3.0 ml, 0.5 mM-DMBQ or phenylene-1,4-diamine or DCDB, plus 1 mM-K₃Fe(CN)₆ and chloroplasts equivalent to 50 μ g of chlorophyll. DBMIB, when used, was at a final concentration of 1.0 μ M. For K₃Fe(CN)₆ reduction, the mixture contained 1 mM-K₃Fe(CN)₆ and chloroplasts equivalent to 50 μ g of chlorophyll.

For the water-to-Methyl Viologen reaction the mixture contained 0.1 mM-Methyl Viologen plus 0.5 mM-NaN₃ and chloroplasts equivalent to 50 μ g of chlorophyll. The concentration of NH₄Cl, when used, was 3 mM.

For Photosystem I assays the mixture contained 0.1 mM-Methyl Viologen, 0.5 mM-NaN₃, chloroplasts equivalent to 15 μ g of chlorophyll, and either 0.5 mM-duroquinol or 0.1 mM-TMPD plus 5 mM-sodium D-isoascorbate. Superoxide dismutase was

not routinely included in the reaction mixture, but when added [Sigma type I, 300 units (μ mol/min per ml) in 3 ml of reaction mixture], it decreased the rates of both Photosystem I-catalysed reactions by about 20%.

DBMIB, duroquinol and DCDB (generously given by Professor A. Trebst, Lehrstuhl für Biochemie der Pflanzen, Ruhr-Universität Bochum, 4630 Bochum 1, Germany) and DMBQ (Aldrich Chemicals) were prepared as stock solutions in 100% ethanol, whereas phenylene-1,4-diamine (Eastman Chemicals) was dissolved in 1 mM-ethanol. TMPD (BDH Chemicals) and Methyl Viologen were dissolved in distilled water.

Tris treatment

A portion (0.5 ml) of chloroplast suspension was diluted to 40 ml with 0.8 M-Tris/HCl, pH 8.0 at 20°C, and incubated for 20 min at 20°C in the light. Chloroplasts were pelleted by centrifuging (2000 g, 10 min), washed once and resuspended in 0.5 M-sorbitol/5 mM-MgCl₂/30 mM-Tricine/KOH buffer, pH 8.0. DCPIP reduction was assayed using a dual-wavelength difference spectrophotometer (Johnson Foundation, University of Pennsylvania, Philadelphia, PA, U.S.A.). The reaction mixture contained, in 1.5 ml, 17 μ M-DCIP, 1 mM-*sym*-1,5-diphenylcarbazide and chloroplasts equivalent to 5 μ g of chlorophyll. The measuring and reference wavelengths were 570 and 520 nm respectively. Actinic light (500 μ E/s per m²) was provided by a 150 W projector lamp and filtered through a Corning 1-75 heat filter and a Corning 2-60 red glass filter. The photomultiplier was shielded from the actinic light by a Corning 4-96 blue glass filter.

Trypsin treatment

A method similar to that of Renger (1976) resulted in 100% inhibition of electron transport from water to Methyl Viologen. Chloroplasts containing 55 μ g of chlorophyll were incubated for 5 min at room temperature in 1.1 ml of buffer containing 30 mM-Tricine/KOH buffer, pH 7.5, and 100 μ g of trypsin (Sigma type XI, 7900 units/mg). The reaction was stopped by a 5-fold excess of soya-bean trypsin inhibitor (500 μ g, Sigma Type 1-S). O₂ evolution was assayed in the presence of 10 mM-K₃Fe(CN)₆ and 5 μ M-DCMU.

Chaotrope treatment

Chloroplasts were treated with the chaotrope NaNO₃, essentially by the method of Lozier *et al.* (1971).

Results

Effects of high concentrations of salts on rates of electron transport

Photosystem II partial reactions. Electron transport from water to the lipophilic Photosystem II

acceptor DMBQ was stimulated 2–3-fold in the presence of potassium citrate at concentrations of approx. 0.7 M and above (Fig. 1). The degree of stimulation was greater than that caused by the uncoupler NH_4Cl . Up to 5-fold stimulation by citrate was observed in the presence of $1\mu\text{M}$ -DBMIB, which blocks electron transport from plastoquinone to Photosystem I (Trebst *et al.*, 1970). O_2 evolution in the presence of 1.0 M-potassium citrate was light-dependent and DCMU-sensitive, and also showed the normal pH profile and DMBQ-concentration-dependence for a Photosystem II partial reaction with a lipophilic acceptor (Gould & Izawa, 1973) (results not shown).

Stimulation of O_2 evolution by high concentrations of citrate was also observed when other Photosystem II acceptors, phenylene-1,4-diamine and DCDB, were used instead of DMBQ (Table 1). In contrast, whereas O_2 evolution with 1mM - $\text{K}_3\text{Fe}(\text{CN})_6$ was stimulated to 20–40% above the uncoupled rate by citrate at concentrations between 0.7 M and 1.0 M, the reaction was highly sensitive to DBMIB. In the presence of DBMIB there was less stimulation by 1.0 M-potassium citrate than was

the case with lipophilic acceptors, presumably because of low accessibility of Photosystem II to $\text{K}_3\text{Fe}(\text{CN})_6$. This interpretation was supported by the results of experiments on trypsin-treated chloroplasts. Mild trypsin treatment removes a proteinaceous component overlying the acceptor complex of Photosystem II (Renger, 1976) and thus renders the site more accessible to hydrophilic oxidants such as $\text{K}_3\text{Fe}(\text{CN})_6$, which can then be rapidly reduced by Photosystem II in a DCMU-insensitive reaction. As shown in Fig. 2, after mild trypsin treatment DCMU-insensitive O_2 evolution with 10mM - $\text{K}_3\text{Fe}(\text{CN})_6$ as acceptor was strongly stimulated by high concentrations of citrate.

The results of the experiments reported so far suggest that the effect of citrate on Photosystem II could be localised on the oxidizing side of the reaction centre. An attempt was made to locate the site of action more precisely, by examining the effects of citrate on Photosystem II reactions in Tris-washed chloroplasts, which cannot oxidize water but can perform electron transport through Photosystem II if supplied with an artificial donor. In Tris-washed chloroplasts, DCIP reduction with *sym*-1,5-diphenylcarbazide as donor was stimulated approx. 50% by 0.7 M-potassium citrate, but inhibited by 1 M-citrate (Table 2). This result may indicate a site of stimulation on the oxidizing side of Photosystem II between the O_2 -evolving system and the Photosystem II reaction centre. However, since the degree of stimulation in untreated chloroplasts was much greater (up to 500%), it seems probable that the main effect of citrate in Photosystem II was

Fig. 1. Effect of citrate on the rate of electron transport from water to DMBQ

O_2 evolution was assayed at 20°C in the oxygen electrode in saturating red light, with 0.5mM -DMBQ [plus 1mM - $\text{K}_3\text{Fe}(\text{CN})_6$] as electron acceptor. O, Rate of O_2 evolution in 20mM -Hepes/KOH buffer, pH 7.5, containing the concentrations of citrate shown; □, in 20mM -Hepes/KOH buffer, pH 7.5, containing $1.0\mu\text{M}$ -DBMIB and the concentrations of citrate shown; Δ, in sorbitol/Hepes buffer, pH 7.5; ▲, in sorbitol/Hepes buffer, pH 7.5, plus 3mM - NH_4Cl ; ■, in sorbitol/Hepes buffer, pH 7.5, plus $1.0\mu\text{M}$ -DBMIB.

Table 1. Effect of 1.0 M-potassium citrate on rates of electron transport with different Photosystem II acceptors
 O_2 evolution was assayed using the oxygen electrode as described in the Materials and methods section. The concentrations of the various reagents were: 0.5mM -phenylene-1,4-diamine (PD), 0.5mM -DCDB, $1.0\mu\text{M}$ -DBMIB, 1mM - $\text{K}_3\text{Fe}(\text{CN})_6$, 3mM - NH_4Cl .

Reaction system	Rate of O_2 evolution (μmol of O_2 /h per mg of chlorophyll):	
	In sorbitol/ Hepes buffer, pH 7.5	In 1.0 M-potassium citrate/20 mM-Hepes/ KOH, pH 7.5
$\text{H}_2\text{O} \rightarrow \text{PD}$	177	500
$\text{H}_2\text{O} \rightarrow \text{PD}$ (+DBMIB)	105	310
$\text{H}_2\text{O} \rightarrow \text{DCDB}$	110	222
$\text{H}_2\text{O} \rightarrow \text{DCDB}$ (+DBMIB)	92	196
$\text{H}_2\text{O} \rightarrow \text{K}_3\text{Fe}(\text{CN})_6$	20	200
$\text{H}_2\text{O} \rightarrow \text{K}_3\text{Fe}(\text{CN})_6$ (+NH ₄ Cl)	160	200
$\text{H}_2\text{O} \rightarrow \text{K}_3\text{Fe}(\text{CN})_6$ (+DBMIB)	8	13

Fig. 2. Effect of citrate on the rate of electron transport from water to $K_3Fe(CN)_6$ in trypsin-treated chloroplasts. Chloroplasts containing $55\mu g$ of chlorophyll were treated with $100\mu g$ of trypsin for 5 min as described in the Materials and methods section. O_2 evolution was assayed in 3 ml of medium containing treated chloroplasts equivalent to $30\mu g$ of chlorophyll, $10\text{ mM-}K_3Fe(CN)_6$ and $5\mu\text{M-DCMU}$. ○, Rate of O_2 evolution in assay medium containing 1 ml of 30 mM-Tricine/KOH , pH 7.5, plus 2 ml of 20 mM-Hepes/KOH , pH 7.5, and the concentrations of citrate shown; △, in assay medium containing 1 ml of 30 mM-Tricine/KOH , pH 7.5, plus 2 ml of sorbitol/Hepes buffer.

Table 2. Effect of high concentrations of potassium citrate on electron transport through Photosystem II in Tris-treated chloroplasts

Chloroplasts were treated with 0.8 M-Tris/HCl , pH 8.0, and DCIP reduction was assayed spectrophotometrically as described in the Materials and methods section. The reaction mixture contained $17\mu\text{M-DCIP}$, treated chloroplasts equivalent to $5\mu g$ of chlorophyll, $1.0\text{ mM-sym-1,5-diphenylcarbazide}$ and 10 mM-Tris/HCl , pH 7.1, plus the additions shown.

Additions to 10 mM-Tris/HCl , pH 7.1	Rate of DCIP reduction ($\mu\text{mol of DCIP/h per mg of chlorophyll}$)
Sorbitol (0.5 M) and $MgCl_2$ (5 mM)	42
Sorbitol (0.5 M), $MgCl_2$ (5 mM) and NH_4Cl (3 mM)	67
Potassium citrate (0.7 M)	100
Potassium citrate (1.0 M)	38

on the O_2 -evolving system itself. In addition, since in undamaged chloroplasts DCIP accepts electrons mainly from Photosystem I (Izawa, 1980), the 50% stimulation of DCIP reduction in Tris-washed chloroplasts may be attributable to an effect on Photosystem I. The effect of citrate on Photosystem I-catalysed reactions will be discussed further below.

When other salts were tested it was found that the sodium and potassium salts of succinate, phosphate, sulphate and tartrate were also very effective in stimulating Photosystem II partial reactions (Table 3). In each case, maximum stimulation was obtained at high salt concentration, often corresponding to the maximum concentration achievable in a saturated solution of the salt. All of the effective salts contained anions high in the Hofmeister series. In contrast, chloride, which has a lower position in the series, did not stimulate activity, as noted previously by Ben-Amotz & Avron (1972). Although millimolar concentrations of chloride have been reported to be required for O_2 evolution (Izawa

Table 3. Effect of high concentrations of salts on electron transport from water to DMBQ

O_2 evolution was assayed as described in the legend to Fig. 1. Control rates of O_2 evolution ($\mu\text{mol of } O_2/\text{h per mg of chlorophyll}$) were 97 in sorbitol/Hepes buffer and 171 in sorbitol/Hepes buffer plus 3 mM-NH_4Cl .

Additions to 20 mM-Hepes/KOH , pH 7.5	Concentration of addition (M)	Rate of O_2 evolution ($\mu\text{mol of } O_2/\text{h per mg of chlorophyll}$)
Potassium phosphate	0.5	164
	1.0	233
	2.0	438
Potassium succinate	0.5	188
	1.0	267
	2.0	433
Sodium citrate	1.0	448
Potassium phosphate plus sodium citrate	0.5 plus 0.3	278
Sodium potassium tartrate	0.5	199
	0.75	254
	1.0	300
Sodium sulphate	0.5	159
	0.75	255
	1.0	396
Sodium acetate	1.0	149
	2.0	198
	3.0	142
Magnesium succinate	0.8	193
	1.0	184
Magnesium sulphate	0.5	89
Ammonium sulphate	1.0	95
	2.0	73
Potassium chloride	1.0	52
	2.0	26

et al., 1969), much higher concentrations had a deleterious effect on electron transport.

There were some exceptions to the results predicted from the Hofmeister series, when specific chemical effects of some ions prevailed over the stimulation expected for the salt. Thus magnesium salts, particularly MgSO_4 , did not stimulate, and gradually inhibited O_2 evolution over longer time periods (5–10 min), probably because high concentrations of Mg^{2+} removed manganese from the O_2 evolving enzyme (Chen & Wang, 1974). High concentrations of NH_4^+ were also inhibitory (Cheniae & Sayre, 1980), and acetate, although not inhibitory, was less effective than predicted from its position in the Hofmeister series. These discrepancies are perhaps not surprising, since the series was originally worked out as the order of effectiveness of anions in precipitating serum proteins, and Jencks (1969) has listed examples to show that there can be some variation in the order of the series for different types of proteins or when different types of

response to the ions (e.g. stabilization/denaturation or aggregation/dissociation) are considered. Nevertheless, the *general* trend that ions high on the series tend to stabilize and aggregate has been found to apply to a remarkably wide range of proteins.

Since those salts that were effective in stimulating O_2 evolution appeared to behave in a similar manner to one another, potassium citrate was used as a representative salt in subsequent experiments.

Photosystem I partial reactions and electron transport through the complete chain. Electron transport through both Photosystem I and Photosystem II, from water to the low-potential dye Methyl Viologen, was also stimulated by high concentrations of citrate, but the extent of stimulation was smaller than in the Photosystem II partial reactions, and rates fell steeply at citrate concentrations above 1.0 M (Fig. 3). Similar results were obtained for the partial reaction duroquinol to Methyl Viologen (Fig. 4), which proceeds via electron donation either to the cytochrome b_6/f -Rieske-centre complex or to plastoquinone (Izawa & Pan, 1978). Thus there appeared to be a citrate-sensitive site in Photosystem I, as well as in Photosystem II, although the stimulatory effect was

Fig. 3. Effect of citrate on the rate of electron transport from water to Methyl Viologen

Electron transport was assayed as O_2 uptake in the presence of 0.1 mM-Methyl Viologen and 0.5 mM- NaN_3 . $\circ$, Rate of O_2 uptake in 20 mM-Hepes/KOH buffer, pH 7.5, containing the concentrations of citrate shown; $\bullet$, in 20 mM-Hepes/KOH buffer, pH 7.5, containing 3 mM- NH_4Cl and the concentrations of citrate shown; $\triangle$, in sorbitol/Hepes buffer, pH 7.5; $\blacktriangle$, in sorbitol/Hepes buffer, pH 7.5, plus 3 mM- NH_4Cl .

Fig. 4. Effect of citrate on the rate of electron transport from duroquinol to Methyl Viologen

Electron transport was assayed as O_2 uptake in the presence of 0.1 mM-Methyl Viologen, 0.5 mM- NaN_3 and 0.5 mM-duroquinol. $\circ$, Rate of O_2 uptake in 20 mM-Hepes/KOH buffer, pH 7.5, containing the concentrations of citrate shown; $\bullet$, in 20 mM-Hepes/KOH buffer, pH 7.5, containing 3 mM- NH_4Cl and the concentrations of citrate shown; $\triangle$, in sorbitol/Hepes buffer, pH 7.5; $\blacktriangle$, in sorbitol/Hepes buffer, pH 7.5, plus 3 mM- NH_4Cl ($\blacktriangle$).

Fig. 5. Effect of citrate on the rate of electron transport from TMPD (reduced) to Methyl Viologen

Electron transport was assayed as O₂ uptake in the presence of 0.1 mM-Methyl Viologen, 0.5 mM-NaN₃, 5 mM-sodium D-isoascorbate and 0.1 mM-TMPD. O, Rate of O₂ uptake in 20 mM-Hepes/KOH buffer, pH 7.5, containing the concentrations of citrate shown; Δ, in sorbitol/Hepes buffer, pH 7.5.

less marked. Possibly citrate stabilized a functional complex which has been found to be formed by plastocyanin and the Photosystem I reaction centre (Haehnel *et al.*, 1980). This suggestion was supported by the stimulation of electron transport from reduced TMPD to Methyl Viologen (Fig. 5), a reaction that involves only a small section of the electron-transport chain between plastocyanin and the Photosystem I primary acceptor (Izawa, 1980).

Protection of O₂ evolution against heat and chaotropes

The strong stimulation of O₂ evolution by high concentrations of citrate and other ions raised the question of whether these ions also stabilized the O₂-evolving complex, which is well-known to be the most readily denatured component of the electron-transport chain. It was found that 1.0 M-potassium citrate did protect O₂ evolution against inactivation by heat (Fig. 6) and by the chaotrope NaNO₃ (Fig. 7). Significantly, nitrate is an ion that is low in the Hofmeister series.

Discussion

It has been noted previously that moderate concentrations of ions such as citrate and phosphate (approx. 0.1–0.5 M) uncouple photosynthetic

Fig. 6. Effect of heating in sorbitol/Hepes buffer or in 1.0 M-potassium citrate/20 mM-Hepes/KOH, pH 7.5, on O₂-evolution activity in spinach chloroplasts

Samples (0.5 ml) of chloroplasts in the appropriate buffer were heated at 50°C for the times shown, then cooled on ice. O₂ evolution [0.5 mM-DMBQ plus 1 mM-K₃Fe(CN)₆ as acceptor] was assayed immediately at 20°C in 1.0 M-potassium citrate/20 mM-Hepes/KOH, pH 7.5. Δ, Chloroplasts heated in sorbitol/Hepes buffer, pH 7.5; O, chloroplasts heated in 1.0 M-potassium citrate/20 mM-Hepes buffer, pH 7.5.

electron transport (Good *et al.*, 1966). However, in the present study, at much higher concentrations of the ions, further stimulation was observed that could no longer be attributed to uncoupling alone. The high concentrations of citrate required for maximal stimulation, the similar effectiveness of sodium and potassium phosphates, succinates, sulphates and tartrates and the opposite effect of high concentrations of chloride and nitrate suggest a relationship with the Hofmeister series, although, as mentioned previously, there were some discrepancies.

The most strongly affected component in the electron-transport chain appeared to be the O₂-evolving complex. Not only was the greatest stimulation observed in partial reactions involving this region of the chain, but it was also found that citrate afforded protection to Photosystem II activity against denaturation by heat and a chaotropic agent, both of which are known to inhibit O₂ evolution by disrupting the manganese-binding site of the O₂-evolving enzyme (Cheniae & Martin, 1966; Lozier *et al.*, 1971). However, since citrate also affected to a lesser extent other regions of the electron-trans-

Fig. 7. Effect of chaotropic agent NaNO_3 on the O_2 -evolution capacity of chloroplasts suspended in sorbitol/Hepes buffer or in 1.0M-potassium citrate/20 mM-Hepes/KOH, pH 7.5

Samples (200 μl) of chloroplasts in the appropriate buffer were treated with various concentrations of NaNO_3 for 20 min in the dark, then O_2 evolution was assayed immediately in 1.0M-potassium citrate/20 mM-Hepes/KOH buffer, pH 7.5, plus 0.5 mM-DMBQ and 1 mM- $\text{K}_3\text{Fe}(\text{CN})_6$. Δ , Chloroplasts treated with NaNO_3 in sorbitol/Hepes buffer, pH 7.5; $\circ$, chloroplasts treated with NaNO_3 in 1.0M-potassium citrate/20 mM-Hepes/KOH, pH 7.5.

port chain, including Photosystem I and possibly other component(s) on the oxidizing side of Photosystem II, a general explanation for the effect seems necessary.

Although the Hofmeister series has been known for almost a century, the effect has still not been explained fully, even in simple aqueous systems. Franks (1977) has suggested that the effects of high concentrations of ions on protein structure may be mediated via a shell of oriented water molecules surrounding the amphipathic protein molecule. Stabilizing ions would enhance the 'structure' of water in the shell, protecting native protein conformation, whereas destabilizing ions would disrupt it and thus force various groups on the protein into unfavourable contact with the bulk-phase water. In the case of the O_2 -evolving system, the effect reported in the present paper might shed some light on the problem of the instability of the system *in vitro*, a problem that has frustrated many attempts to extract and purify the O_2 -evolving complex of higher plants in active form. A number of the classical treatments that inhibit O_2 evolution, such as Tris treatment, mild heating and treatment with chaotropic agents, are known to release the man-

ganese of the complex from its functional site (Radmer & Cheniae, 1977). Of the treatments mentioned, both heating and chaotropic agents would be expected to disrupt structured water, which certainly covers extensive regions along membrane surfaces and around any surface-exposed membrane proteins (Clifford, 1975). Perhaps in the native thylakoid, the maintenance of manganese in its correct functional position depends critically on preserving the structure of water in the micro-environment of the complex, particularly in the light, when there is some evidence that membrane conformational changes alter the accessibility of the manganese-containing protein to the aqueous medium outside the membrane (Robinson *et al.*, 1980).

However, an explanation must be found not only for protection of the O_2 -evolving complex against inactivation, but also for the observed stimulation of O_2 evolution and other electron-transport reactions. It may be that, at least *in vitro*, the O_2 -evolving complex has a degree of 'looseness' in its configuration, possibly at the site of manganese binding, which impairs both its stability and the efficiency of its interaction with Photosystem II. There may also be some flexibility in other functional complexes of the thylakoids, such as the association between plastocyanin and the Photosystem I reaction centre. This idea would be consistent with the finding that most proteins and complexes are not usually in the most highly folded state possible. Up to 25% of possible internal hydrogen bonds may not be achieved (Franks, 1977), with this incomplete bonding leading to 'mobile defects' in the interior of the protein. Via their effects on the organization of water around the protein complexes in photosynthetic membranes, high concentrations of ions such as citrate may promote tighter, more efficient conformations of the complexes that are capable of catalysing higher reaction rates than those normally observed *in vitro*.

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