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# The Effect of Chaotropic Agents on Photosynthetic Reactions

R. Barr and F.L. Crane

*Department of Biological Sciences, Purdue University*
*West Lafayette, Indiana 47907*

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## *Abstract*

The influence of a series of anions on photosynthetic reaction rates in spinach chloroplasts is described. For the most part, the stimulatory and inhibitory effects of these ions can be related to their chaotropic properties, although $F^-$, a nonchaotropic anion, inhibits photosystem II reactions and $SO_4^{2-}$ and $F^-$ inhibit photophosphorylation. Other exceptions include less severe effects of nitrate than expected and unusual sensitivity to iodide by photosystem I. Since free iodine inhibits photosystem I the iodine effect may be related to photooxidation of $I^-$ to $I^0$ by photosystem I. Cyclic and noncyclic photophosphorylation usually show greater sensitivity to each chaotrope than photosystems I and II activity, which suggests that phosphorylation factors, such as $CF_1$, are easily detached or dissociated. Bromide is unusual in that it appears to affect photophosphorylation and electron transport at similar low concentrations. The type of cation appears to influence the response to the chaotropic anion, especially as increased inhibition by chloride in the presence of magnesium in photophosphorylation reactions.


## *Introduction*

Hatefi and Hanstein [1, 2] found that chaotropic agents solubilize particulate proteins in mitochondria and that their action causes lipid oxidation in these membranes. Hanstein et al. [3] found that iron–sulfur proteins or their decomposition products are the catalysts responsible for lipid oxidation in the presence of chaotropic agents. Vainio [4], who studied the effect of chaotropic agents on drug-metabolizing enzymes in hepatic microsomes, found that drug oxidation was abolished by strong chaotropic agents or inhibited by weaker ones. Some enzymes showed a typical biphasic response: activation in the presence of low concentrations of chaotropic agents, inhibition at high concentrations.

The action of chaotropic agents on chloroplast membranes has been studied mainly on total electron transport by variable fluorescence yield and ESR spectroscopy by Lozier et al. [5]. Some chaotropic agents have also been used to solubilize individual chloroplast components [6, 7]. In this study it was decided to investigate the effect of chaotropic agents on photosynthetic reactions in more detail. Since chloroplasts contain lipids and proteins, oxidation of the former and solubilization of the latter, or combinations of both, could result in unforseen effects on the chloroplast electron-transport chain. The experiments reported in this paper proved these assumptions to be correct.

## *Materials and Methods*

Chloroplasts were made from market spinach according to Jagendorf and Avron [8]. Chlorophyll concentrations were determined by Arnon's method [9]. Chloroplasts containing 1 mg chlorophyll/ml solution were suspended in 0.4 M sucrose with 0.05 M NaCl for assays.

Photosystem I (PS I) activity was measured polarographically with a Clark-type electrode using ascorbate plus TMPD methyl viologen according to Brand et al. [10]. Reactions were buffered at pH 8 with 0.25 M Trizma-Mes. Reaction rates were calculated as $\mu$moles acceptor reduced/mg chlorophyll/h.

Photosystem II (PS II) activity was measured spectrophotometrically at 620 nm using the diphenyl carbazide–dichlorophenol-indophenol assay as performed by Brand et al. [10]. Reactions were run at pH 7.0. Reaction rates were calculated from the $\Delta$ (oxidized–reduced) using an extinction coefficient of 24.5 for dichlorophenolindophenol. The reduction of silicomolybdic acid by PS II was measured according to Giaquinta et al. [11] and Barr and Crane [12].

Chaotropic agents (0–1 M concentrations) were added to reaction cuvettes after all the other components. Total volume was 3 ml in all cases. Sulfate or fluoride ions were used as controls in this study to compare chaotropic and nonchaotropic effects. Addition of $K_2SO_4$ and NaF were limited to 0.5 M and 1 M stock solutions due to solubility problems.

Cyclic and noncyclic photophosphorylation rates were measured by the acid–base transition method on a pH meter according to Dilley [13].

*Results*

Figures 1 and 2 show the effect of various concentrations of chaotropic agents on PS I rates compared to a normal PS I rate in spinach

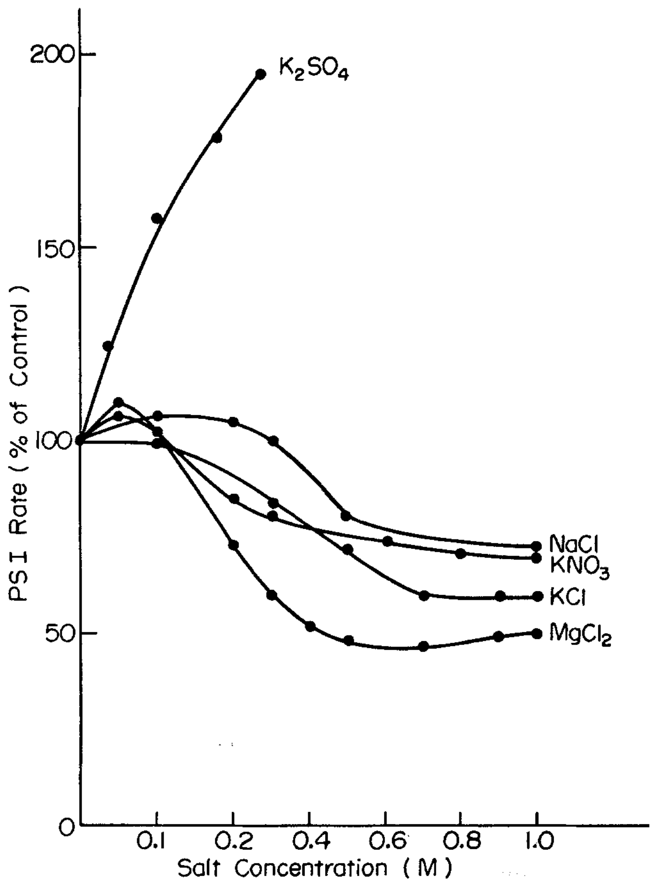


Figure 1. The effect of various concentrations of $Cl^-$ and $NO_3^-$ ions on Photosystem I reaction rates in spinach chloroplasts. Note the influence of the cation on the response to $Cl^-$ and the stimulatory effect of $K_2SO_4$. Control rates varied from 1800-2000 μmole acceptor reduced/mg chlorophyll/h in the ascorbate plus TMPD→methyl viologen reaction at 25°C. Reaction mixture contained in 4 ml: chloroplasts with 100 μg chlorophyll, 200 μmole sodium ascorbate, 0.03 mg TMPD, 1.6 μmole methyl viologen, 600 μmole Trizma-Mes, pH 8, and 1.2 μmole DCMU.

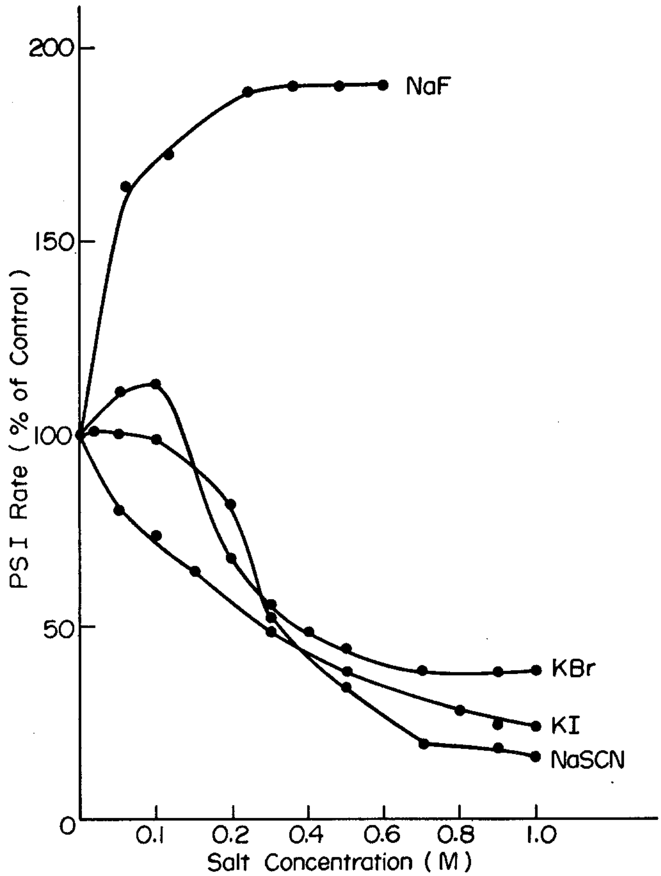


Figure 2. The effect of various concentrations of $Br^-$, $I^-$, and $SCN^-$ ions on Photosystem I reaction rates in spinach chloroplasts. Note the severe inhibition of PS I activity by KI which cannot be explained by chaotropic effects alone and stimulation of the reaction by NaF. Control reaction rates as in Fig. 1.

chloroplasts. It can be seen that various chlorides and nitrate showed less inhibition than bromide, iodide, or thiocyanate. At low concentrations (10–200 mM) most of these salts except NaSCN stimulated electron transport, while in high concentrations (1 M) 25–80% inhibition of PS I rates was seen. $K_2SO_4$ and NaF nearly doubled PS I rates.

Figure 3 shows the effect of chaotropic agents on silicomolybdic acid reduction by PS II. Reaction rates were strongly inhibited by low concentrations ($< 0.1$ M) of $I^-$ and $SCN^-$ but $Br^-$ had less effect even in high concentrations (1 M). The nonchaotropic salt $K_2SO_4$ recovered from inhibition at higher concentrations.

Figures 4 and 5 show the effect of chaotropic agents and PS II rates in the reaction diphenylcarbazide→dichlorophenol indophenol dye. In this case, chaotropically induced stimulation of reaction rates was shown only by low concentrations of NaCl. All other chaotropic agents used inhibited reaction rates at low or high concentrations according to an

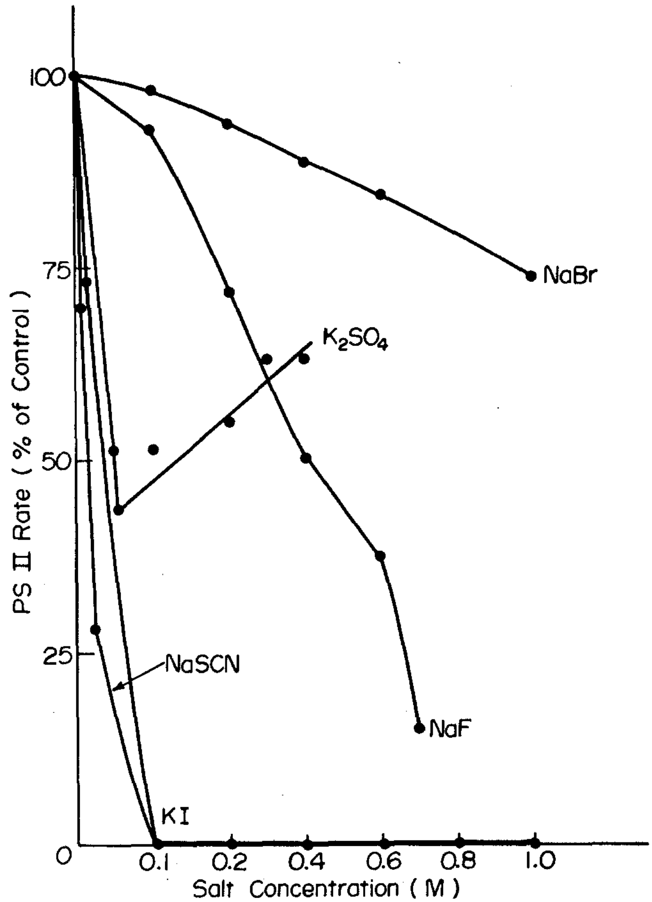


Figure 3. The effect of various ions on the diphenylcarbazide→silico-molybdic acid pathway in Photosystem II of spinach chloroplasts. Note the opposite effects of $K_2SO_4$ and NaF. Control rates varied from 250–360 μmole silicomolybdic acid reduced/mg chlorophyll/h. Reaction mixture contained in 3 ml: chloroplasts containing 50 μg chlorophyll, 0.05 M Trizma-Mes, pH 7, 4 mM $NH_4Cl$, 4 mM $MgCl_2$, 0.02 mg silicomolybdic acid, 0.1 mg DCMU, and 0.12 mg DPC.

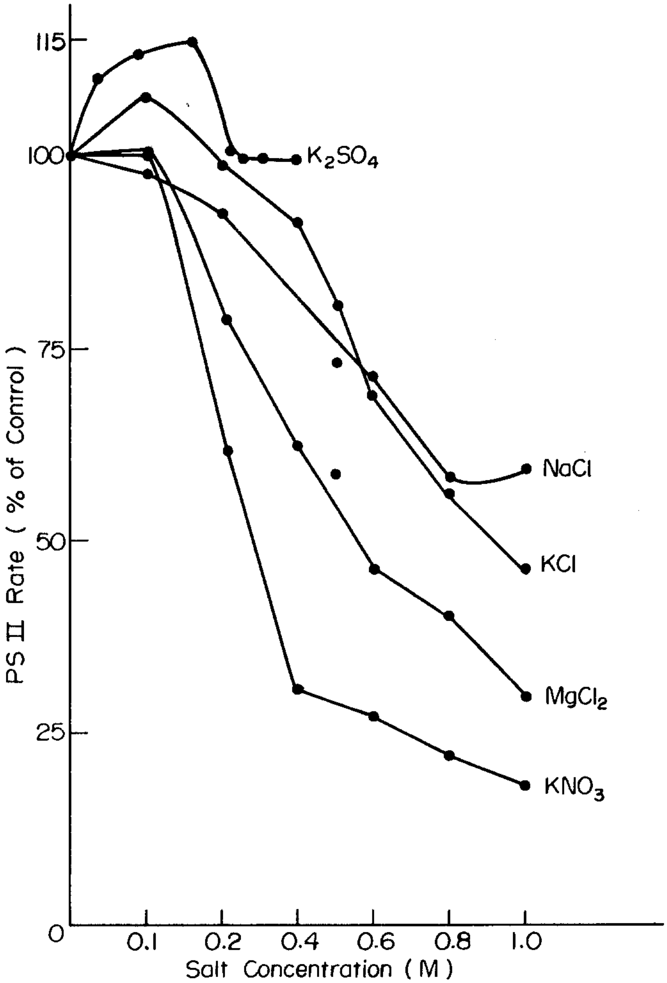


Figure 4. The effect of various concentrations of $Cl^-$ and $NO_3^-$ ions on Photosystem II reaction rates in spinach chloroplasts. Note the influence of the cation on the response to $Cl^-$ and the effect by $K_2SO_4$. Control rates in the DPC→DCPIP reaction varied from 150–200 $\mu$mole acceptor reduced/mg chlorophyll/h. Reaction mixture contained in 3 ml: chloroplasts containing 50 $\mu$g chlorophyll, 0.05 M Trizma-Mes, pH 7, 4 mM $NH_4Cl$, 4 mM $MgCl_2$, 0.12 mg DPC, and 2.5 mM DCPIP.

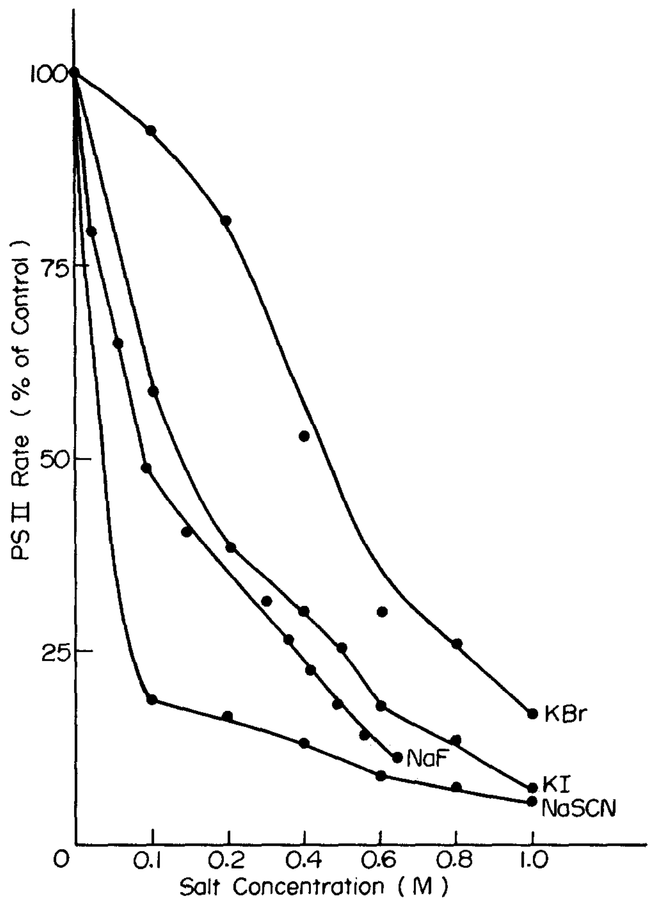


Figure 5. The effect of various concentrations of $Br^-$, $I^-$, and $SCN^-$ ions on Photosystem II reaction rates in spinach chloroplasts. Note the low concentrations of $SCN^-$ necessary for more than 50% inhibition of activity by NaSCN. The reaction was also inhibited by NaF. Control reaction rates as in Fig. 4.

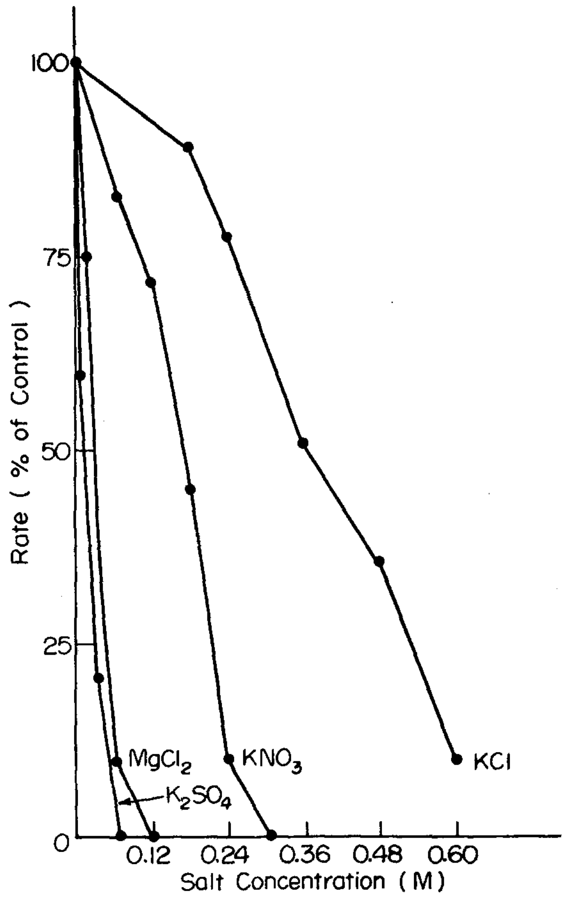


Figure 6. The effect of various concentrations of $Cl^-$ and $NO_3^-$ ions on noncyclic photophosphorylation rates in spinach chloroplasts. Note the low concentrations of $MgCl_2$ necessary for more than 50% inhibition of noncyclic photophosphorylation. This shows the influence of the cation, such as $Mg^{++}$, on the action of the $Cl^-$ anion. Inhibition was also given by $K_2SO_4$. Control reaction rates varied from 40–50 μmole ATP formed/mg chlorophyll/h at 18°C. Reaction mixture contained in 5 ml: chloroplasts containing 100 μg chlorophyll, 100 mM KCl, 5 mM $MgCl_2$, 0.8 mM ADP, 0.8 mM $K_2HPO_4$ and 0.4 mM methyl viologen.

increased degree of severity as follows: NaCl, KCl, $MgCl_2$, KBr, $KNO_3$, KI, and NaSCN. Fluoride also inhibited indophenol reduction while sulfate did not affect it to a large degree.

Both cyclic photophosphorylation with PMS and noncyclic photophosphorylation with methyl viologen were also affected by chaotropic agents. Figures 6 and 7 show the effect of the various salts on noncyclic photophosphorylation in concentrations ranging from 6–600 mM. All salts inhibited noncyclic photophosphorylation up to

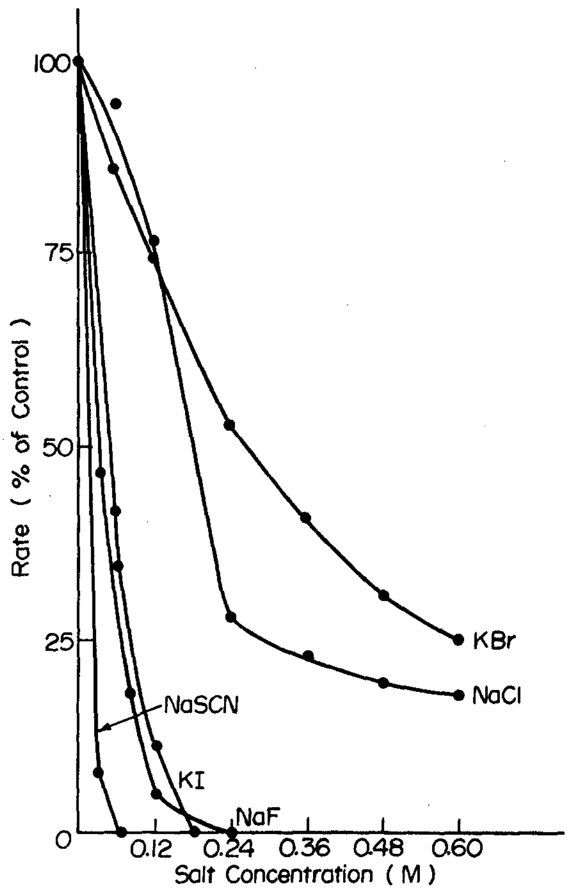


Figure 7. The effect of various concentrations of $Cl^-$, $Br^-$, $I^-$, and $SCN^-$ anions on noncyclic photophosphorylation rates in spinach chloroplasts. Note the low concentrations of KI and NaSCN necessary for more than 50% inhibition of noncyclic photophosphorylation. NaF also gave strong inhibition. Control reaction rates as in Fig. 6.

100% in concentrations lower than those required to get complete inhibition of PS I and II activities. $K_2SO_4$ and NaF, salts used for their nonchaotropic properties, also inhibited noncyclic photophosphorylation.

Figures 8 and 9 show how chaotropic agents affect cyclic photophosphorylation. None of the salts used in this study show a typical chaotropic stimulation when used in low concentrations. Complete inhibition was given by low concentrations of $MgCl_2$ and

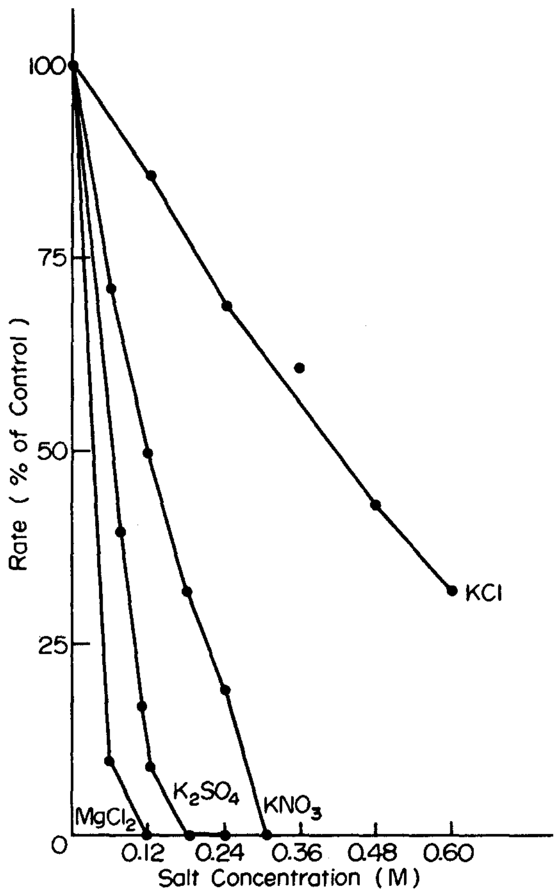


Figure 8. The effect of various concentrations of $Cl^-$ and $NO_3^-$ ions on cyclic photophosphorylation. Note the influence of the cation on the response to $Cl^-$ and inhibition by $K_2SO_4$. Control reaction rates varied from 50–75 μmole ATP formed/mg chlorophyll/h at 18° C. Reaction mixture as in Fig. 6 except 0.4 mM PMS in place of methyl viologen.

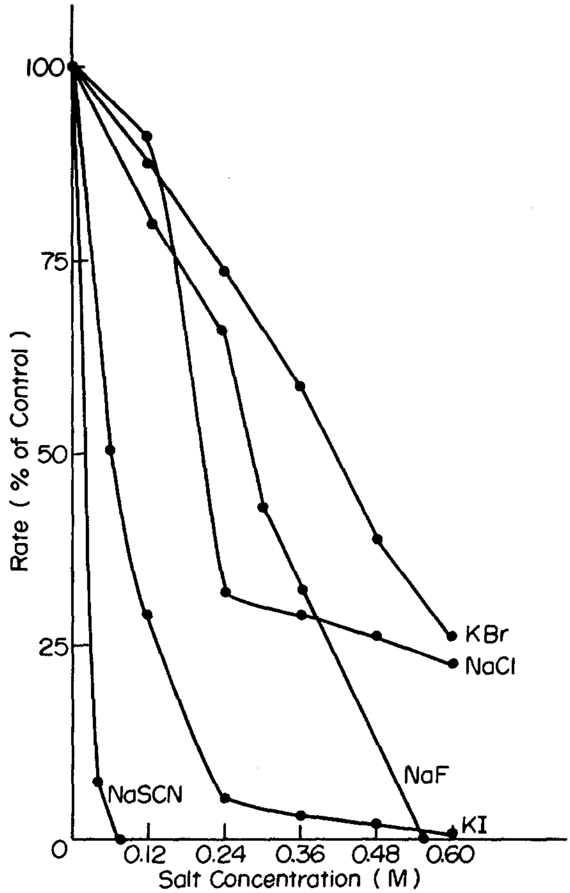


Figure 9. The effect of various concentrations of $Cl^-$, $Br^-$, $I^-$, and $SCN^-$ ions on cyclic photophosphorylation rates in spinach chloroplasts. Note the low concentration of KI and NaSCN necessary for more than 50% inhibition of cyclic photophosphorylation. NaF also inhibited this reaction. Control reaction rates as in Fig. 8.

NaSCN (120 mM). Cyclic photophosphorylation was least affected by KCl, $KNO_3$, and NaCl. It was also inhibited by low concentrations of $K_2SO_4$ and NaF.

## *Discussion*

Lozier et al. [5] found that incubating spinach chloroplasts with some chaotropic agents resulted in the inhibition of photosynthetic electron transport between water oxidation and PS I. This inhibition appeared to be smaller to that caused by washing chloroplasts with high concentrations of Tris buffer. The inhibitory effects were caused by the release of manganese from its bound state in the chloroplast. Partial restoration of NADP photoreduction and fluorescence of variable yield could be achieved by adding hydroquinone or $Mn^{2+}$.

The release of manganese from chloroplast membranes cannot explain the loss of PS II activity encountered in this study after the addition of chaotropic agents to PS II activity assays because diphenylcarbazide was used as an electron donor, but it may not be the only effect created by the presence of chaotropic agents. When Cox and Bendall [14] treated chloroplast suspensions with perchlorate and KI, among other substances, they found that cytochrome-*b*-559HP, which may be associated with PS II, was converted to a low potential form not reducible by hydroquinone. However, they concluded that cytochrome-*b*-559HP was not directly involved in the process of water oxidation or the oxidation of diphenyl carbazide through PS II. Yet, the fact that the conformation of the cytochrome was altered by treatment with chaotropic agents suggested to them that the redox potential was dependent in part on hydrophobic lipid–protein interactions which had been altered by treatment with chaotropic agents.

As Figs. 3, 4, and 5 show, the inhibition of PS II activity was more severe with NaSCN, KI, and $KNO_3$ than with the chlorides, indicating a basic chaotropic effect. A nonchaotropic agent, such as $K_2SO_4$, did not permanently disorganize chloroplast membranes, although NaF inhibited PS II activity as did typical chaotropic agents. This difference may be due to reasons other than chaotropic effects on the structure of water. As Jencks and associates [15, 16], who studied the solubilization of a model peptide and F-actin in the presence of chaotropic agents, concluded, solubilization could not be explained by any of the following criteria alone: breaking of covalent bonds, hydrophobic bonds, binding of anions to cationic sites, or effects on the structure of water. Rather, the effect of salts was on the peptide and amide groups which became exposed to the solvent. There was an ordinary "salting-out" effect which can be described in terms of the average cohesive energy or internal pressure of the solvent. The other force was a direct interaction between certain large anions and the amide dipole. Therefore, it is likely that the effect of chaotropic agents and other salts on chloroplast PS II is a multiple effect including chaotropic interaction between chloroplast proteins and water structure.

According to von Hippel and Schleich [17] KSCN and $CaCl_2$ are potent destabilizers of macromolecular ordered structure, $KH_2PO_4$ and $(NH_4)_2SO_4$ are potent stabilizers of ordered form, while KCl and NaCl are inert. An examination of PS I data for spinach chloroplasts in this study (Figs. 1 and 2) shows that such a classification of salts by their effect on macromolecular structure is consistent with their effects on photosynthetic activity. Sulfate, a stabilizer of ordered structure and a nonchaotrope-like fluoride, both stimulated PS I activity nearly two-fold, while the inert salts NaCl and KCl inhibited PS I rates less than NaSCN.

Other effects produced by chaotropic agents include their action on iron–sulfur proteins which lead to lipid oxidation in submitochondrial particles and microsomes [3]. Lipid photoperoxidation leading to the production of malondialdehyde as a decomposition product of tri-unsaturated fatty-acid hydroperoxides is known to exist in chloroplasts from the study of Heath and Packer [18]. If in mitochondria peroxidation in the presence of chaotropic agents is facilitated by iron–sulfur proteins or their decomposition products, the same processes may take place in chloroplasts which possess ferredoxin [19, 20], bound ferredoxin [21], or other iron–sulfur proteins. When Cammack et al. [22] studied the electron paramagnetic resonance and the optical rotatory dispersion signals from purified spinach ferredoxin in the presence of small amounts of chaotropic agents, they detected changes in these spectra, while at high concentrations more changes occurred. The observed changes were, however, reversible. They also found that with increased concentrations of chaotropic agents ferredoxin became unstable, especially in the reduced state. If similar reactions occur in isolated chloroplasts in the presence of chaotropic agents, lipid peroxidation would also be initiated leading to reduced electron-transport rates.

As Figs. 1–9 show, most effects of PS I and II activity or photophosphorylation rates in this study follow Hofmeister's lyotropic series [4] with a few exceptions. By severity of effect from the least damaging to the more disruptive this order of anions is as follows: $Cl^- < Br^- < NO_3 < I^- < SCN^-$. A notable exception to this series is shown by PS I activity in the presence of iodide (Fig. 2) where low concentrations of KI inhibit PS I rates as much as NaSCN in comparable concentrations. This may be explained by the direct photooxidation of $I^-$ to $I^0$ by PS I, with inhibition of activity arising from the toxic effect of $I^0$. As Table I shows, iodine had a similar effect on PS I activity when added to reaction mixtures in ethanol.

The other notable exception to Hofmeister's lyotropic series was the relatively more severe effect of NaCl on cyclic and noncyclic photophosphorylation over the effects of KBr and KCl (Figs. 6–9). $MgCl_2$ was even more inhibitory. This implies that the cation may also influence the action of the chaotropic anion. As von Hippel and Schleich [17] found in a model system, the binding of salts to a column was influenced by both the cation and the anion.

Cyclic and noncyclic photophosphorylation were inhibited more severely by the various chaotropic agents used in this study than electron

TABLE I. Inhibition of photosystem I activity in spinach chloroplasts by iodine

| | PS I rate (μmole acceptor reduced/ mg chlorophyll/h) |
|---|---|
| Chloroplasts | 1974 |
| Chloroplasts plus 0.2 mM iodine | 1974 |
| Chloroplasts plus 0.4 mM iodine | 1635 |
| Chloroplasts plus 0.8 mM iodine | 1578 |
| Chloroplasts plus 1 mM iodine | 1185 |
| Chloroplasts plus 1.6 mM iodine | 1014 |
| Chloroplasts plus 2 mM iodine | 903 |

transport. This may be explained by the action of chaotropic agents on the coupling factor, $CF_1$. In mitochondria, it has been shown by Hare and Crane [23] that washing with chaotropic agents can remove mitochondrial ATPase. When Lien et al. [6] studied the cold-activation of $CF_1$ of spinach chloroplasts at pH 6.5 in the presence of 0.4 M KCl, they found 89% loss of coupling factor activity due to its dissociation into subunits in the cold. Recently, Kamienietzky and Nelson [7] used NaBr to deplete chloroplasts of $CF_1$ and Ellefson and Krogmann (personal communication) found that concentrations less than 0.02 M of KI gave 50% inhibition of chloroplast $Mg^{+2}$ ATPase. This implies that inhibition of photophosphorylation by chaotropic agents may be caused by their action on the chloroplast coupling factor. However, the exceptions to a strict chaotropic series mentioned earlier—more severe inhibition by NaCl and $MgCl_2$ than expected—and the fact that the nonchaotropic salts used in this study also inhibited both cyclic and noncyclic photophosphorylation—may imply the influence of other factors. Baldry et al. [24] found that sulfate inhibited the oxygen evolution associated with $CO_2$ fixation. They concluded that sulfate did not influence $O_2$ evolution directly but affected phosphorylation or ATP utilization. It was shown by Hall and Telfer [25] that sulfate could replace phosphate in the energy-conserving mechanism of chloroplasts. Pick and Avron [26] described the action of inorganic sulfate in lettuce chloroplasts as an energy-transfer inhibitor. Montal et al. [27] and Gromet-Elhanan and Leiser [28] studied the inhibition of photophosphorylation by sulfate in *Rhodospirillum* chromatophores.

Inhibition of photophosphorylation by sulfate as an energy-transfer inhibitor in chloroplasts does not explain the inhibition shown by the nonchaotropic salt NaF. Further studies are necessary to elucidate the mechanism of NaF inhibition.

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