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# Effect of High Cation Concentrations on Photosystem II Activities[1]

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## ABSTRACT

**The effects of wide concentration ranges of NaCl, KCl, and $MgCl_2$ on ferricyanide reduction and the fluorescence induction curve of isolated spinach *(Spinacia oleracea)* chloroplasts were investigated. Concentrations of the monovalent salts above 100 mM and $MgCl_2$ above 25 mM produced a decrease in the rate of ferricyanide reduction by thylakoids uncoupled with 2.5 mM $NH_4Cl$ which cannot be attributed to changes in the primary photochemical capacity of photosystem II. Salt-induced decreases in the effective concentration of the secondary electron acceptor of photosystem II, plastoquinone, reduce the capacity for secondary photochemistry of photosystem II and this could contribute to the reduction in ferricyanide reduction by uncoupled thylakoids at high salinities. The rate of ferricyanide reduction by coupled thylakoids is little affected by salinity changes, indicating that the rate-limiting phosphorylation mechanism in electron flow from water to ferricyanide in coupled thylakoids is salt-tolerant, whereas the rate-limiting reaction in uncoupled ferricyanide reduction is considerably affected by salinity changes. Salt-induced changes in the fluorescence induction curve are interpreted in terms of changes in the rate constants for excitation decay by radiationless transitions, exciton transfer from photosystem II chlorophylls to other associated chlorophyll species, and photochemistry.**

The ionic environment of the thylakoid membrane plays an important role in regulating many of the physicochemical reactions involved in the primary photosynthetic processes. Changes in both the conformational and energetic states of the thylakoid membrane can be induced by changes in the external cation concentration. Divalent cations can affect the degree of stacking of the thylakoids; stacking increases as the external cation concentration increases (10). Such changes in membrane stacking have been related to changes in energy transfer between PSII and PSI (16), although recently it has been shown that changes in stacking can occur unrelated to changes in fluorescence yield and energy transfer (23). The majority of studies on cation regulation of the primary photosynthetic processes have been made with monovalent cation concentrations in the range 0 to 150 mM, and 0 to 20 mM for divalent cations. Both 100 mM monovalent and 5 mM divalent cations produce an increase in PSII activity at the expense of PSI due to a decrease in energy spillover from PSII to PSI (2). Changes in energy distribution within the thylakoid are thought to be related to cation-induced changes in membrane ultrastructure, which produce a spatial separation of PSI and PSII Chl matrices, thus decreasing the possibility of exciton migration from PSII to PSI (8, 18). Cations can also affect the rate of electron flow through PSI, PSII, and both PSII and PSI in series (2). Such effects can often be attributed to changes in the distribution of energy within the photosynthetic apparatus (2). Coupling of phosphorylation to electron transport can also be cation-controlled (11, 22).

A knowledge of the ionic contents of the chloroplast is essential to understand fully the role of cations in the regulation of *in vivo* thylakoid function. Estimates of ion concentrations in chloroplasts of a number of species ranged from 3 to 550 mM for $Na^+$, 80 to 400 mM for $K^+$, and 30 to 110 mM for $Mg^{2+}$ (9, 17, 19). The effects of cation concentrations above 20 mM for divalent and 150 mM for monovalent species on the structure and activity of higher plant thylakoid membranes have not been widely studied, although it is possible that cation concentrations in the chloroplasts *in vivo* exceed these values. Studies have been made on intact algal cells; however, it has been difficult to determine whether the results reported are due to cation or osmotic effects (5). The work described in this paper is an initial study of the effects of high salinity on the photosynthetic membranes of higher plant; changes in the functioning of PSII are examined using electron transport and fluorescence kinetic studies on broken chloroplast preparations of spinach.

## MATERIALS AND METHODS

**Plant Material.** Spinach plants (*Spinacia oleracea* L., hybrid 102, from Samuel Yates, Macclesfield, U.K.) were grown from seed in a glasshouse at 20 C. Seeds were sown in John Innes No. 1 potting compost. Leaves were harvested approximately 6 weeks after sowing.

**Chloroplast Isolation.** Leaves were deribbed and homogenized at 4 C in 0.33 M sorbitol, 1 mM $MgCl_2$, 5 mM TES[2] at pH 7.0 using an M.S.E. Atomix at maximum speed for 10 sec. The homogenate was filtered through eight layers of butter muslin and six layers of nylon bolting cloth (25 $\mu$m pore size) and centrifuged for 90 sec at 3,000*g*. The resulting pellet was resuspended in 1 mM $MgCl_2$, 5 mM TES at pH 7.0. Chl contents were determined using the method of Arnon (1).

**Ferricyanide Reduction.** This was determined polarographically by following light-stimulated $O_2$ evolution in the presence of ferricyanide. The 3-$cm^3$ reaction mixture contained 1.5 mM potassium ferricyanide, 1 mM $MgCl_2$, 5 mM TES at pH 7.0, and less than 0.05 $cm^3$ of chloroplast preparation containing about 15 $\mu$g of Chl. Assays were performed at 25 C using a saturating irradiance of 2.94 mE $m^{-2}$ $sec^{-1}$ of white light produced from a quartz-iodine source. Radiant flux density was determined using a quantum sensor (model LI-PIOS, Lambda Instruments Corporation, Lincoln, Nebr.). Uncoupled electron transport rates were determined by addition of 2.5 mM $NH_4Cl$.

**Fluorescence Kinetics.** Fluorescence kinetics of the chloroplast

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[2] Abbreviations: TES: N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; PQ: plastoquinone.

preparation were determined at 25 C in a 4-cm$^3$ reaction mixture containing 1.5 mM potassium ferricyanide, 1 mM $MgCl_2$, 2.5 mM $NH_4Cl$, 5 mM TES at pH 7.0, and about 20 $\mu$g of Chl. Fluorescence of the dark-adapted chloroplast preparation was excited with broad band blue light (400–500 nm, 0.15 mE $m^{-2}$ $sec^{-1}$) and detected at right angles from the exciting beam with a Hamamatsu R446 phototube through a 690 nm filter package (Balzer 690 nm interference filter, Corning 9830 filter) giving a peak transmission of 690 nm and a 7 nm half-bandwidth. Fluorescence kinetic data were recorded on a Telequipment DM64 storage oscilloscope. In some assays 15 $\mu$M DCMU was added to the reaction mixture.

**Low Temperature Absorption Spectroscopy.** Absorption spectra of chloroplast preparations in 1 mM $MgCl_2$, 5 mM TES at pH 7.0 were measured at −196 C using a computerized single-beam spectrophotometer (3).

## RESULTS

The effect of a range of NaCl concentrations on the rate of ferricyanide reduction by coupled and uncoupled thylakoids is shown in Figure 1. Although NaCl had little effect on the rate of coupled electron flow, thylakoids which had electron transport uncoupled from phosphorylation by the addition of 2.5 mM $NH_4Cl$ showed marked changes in the rate of ferricyanide reduction on the addition of NaCl. Concentrations of 50 to 400 mM NaCl stimulated uncoupled electron transport, higher concentrations were inhibitory. Similar results were observed with KCl. In the presence of 700 mM NaCl and 600 mM KCl the rate of coupled ferricyanide reduction was the same as the uncoupled rate, suggesting that the rate-limiting reaction in the coupled thylakoids at these high salinities is not the phosphorylation mechanism but a component reaction of the electron transport chain between $H_2O$ and ferricyanide. This rate-limiting electron transport reaction in uncoupled thylakoids is obviously much more salt-sensitive than the normally rate-limiting phosphorylation process in coupled electron flow. Figure 2 shows the effect of $MgCl_2$ on the rate of ferricyanide reduction by coupled and uncoupled thylakoids. Inhibitory effects were observed with $MgCl_2$ at far lower concentrations than were found with monovalent cation salts; 100 mM $MgCl_2$ inhibited coupled electron transport, whereas 50 mM inhibited uncoupled electron flow.

The possibility that salt-induced changes in the electron transport rates of thylakoids are due to osmotic stress was examined by studying the effect of mannitol on the rate of ferricyanide reduction by coupled and uncoupled thylakoids. Increasing osmotic pressure produced a slight stimulation of the uncoupled rate of ferricyanide reduction, but no effect was observed on the coupled electron transport. Clearly the observed salt-induced changes in ferricyanide reduction by coupled and uncoupled thylakoids are not osmotic in nature. Similar experiments using $Na_2SO_4$ and

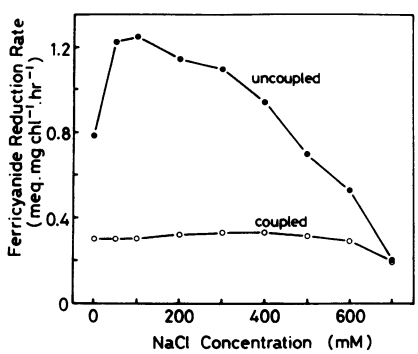


FIG. 1. Effect of NaCl on rate of ferricyanide reduction by coupled and uncoupled spinach thylakoids. Electron transport was uncoupled from phosphorylation with 2.5 mM $NH_4Cl$. Each point represents mean of four determinations.

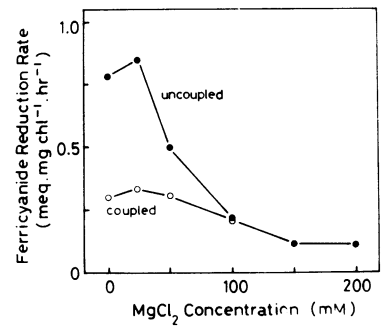


FIG. 2. Effect of $MgCl_2$ on rate of ferricyanide reduction by coupled and uncoupled spinach thylakoids. Electron transport was uncoupled from phosphorylation with 2.5 mM $NH_4Cl$. Each point represents mean of four determinations.

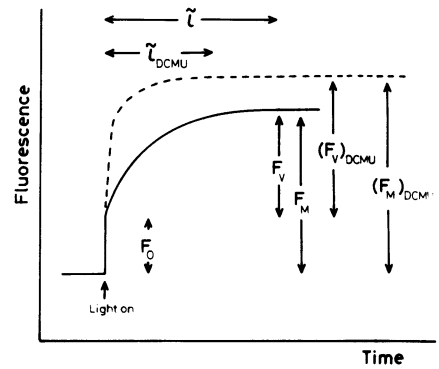


FIG. 3. Fluorescence induction curves for spinach thylakoids in the absence (——) and presence (– – –) of 15 $\mu$M DCMU. $F_0$ and $F_M$ represent minimal and maximal levels of fluorescence, respectively; $F_V$ is fluorescence of variable yield ($F_V = F_M - F_0$); $\tau$ is rise time of fluorescence induction.

$MgSO_4$ indicated that these salt-induced effects were dependent upon the concentration and nature of the cation species of the salt, *i.e.* the concentration range 0 to 350 mM $Na_2SO_4$ produced the same effect as 0 to 700 mM NaCl, and the effects of 0 to 200 mM $MgSO_4$ were similar to that range of $MgCl_2$.

Fluorescence induction curves obtained from dark-adapted chloroplast preparations at room temperature can provide useful information on PSII activities. Figure 3 shows typical fluorescence induction curves for thylakoids with and without DCMU present. The basic parameters of fluorescence used in this study are the minimal level of fluorescence, $F_0$, the maximal level, $F_M$, and the fluoresence of variable yield, $F_V$, ($F_V = F_M - F_0$). $F_0$ remains constant with time and represents the level of Chl fluorescence when all of the primary acceptor of PSII, Q, is in the oxidized (open) state (20). The rise in the fluorescence level from $F_0$ with irradiation time is directly related to the redox stater of Q (6, 7). $F_V$ determined in the presence of DCMU, $(F_V)_{DCMU}$, which ensures that all of the Q is reduced at the $F_M$ level, is directly determined by the concentration of Q in the preparation.

The salt-induced changes in $F_0$, $F_M$ and $F_V$ for thylakoids with and without 15 $\mu$M DCMU in the presence of ferricyanide are given in Figure 4. KCl was found to produce similar results to NaCl. The results of similar experiments using $MgCl_2$ are presented in Figure 5. The quantum yield of fluorescence at the $F_0$ level, $\phi_{F_0}$, can be defined as:

$$\phi_{F_0} = \frac{k_f}{k_f + k_h + k_t + k_p P} \quad (1)$$

where $k_f$, $k_h$, $k_t$, and $k_p$ are the rate constants for excitation decay by fluorescence, radiationless decay, transfer to associated Chl, and photochemistry respectively; P is the fraction of open PSII

traps (2). In this situation P can be equated to the fraction of PSII primary acceptor, Q, molecules in the oxidized form. At the $F_M$ level in the presence of DCMU, all of Q is reduced hence all of the PSII traps are closed, thus $k_pP = 0$, and the fluorescence yield at the $F_M$ level, $\phi_{F_M}$, can be defined as:

$$\phi_{F_M} = \frac{k_f}{k_f + k_h + k_t} \quad (2)$$

Since $F_V = F_M - F_0$, the fluoresence yield of $F_V$, $\phi_{F_V}$, is defined as:

$$\phi_{F_V} = \left(\frac{k_f}{k_f + k_h + k_t}\right) - \left(\frac{k_f}{k_f + k_h + k_t + k_pP}\right) \quad (3)$$

For any given sample $k_f$, $k_h$, $k_t$, and $k_p$ can be assumed to be the same at both $F_0$ and $F_M$ levels, hence $F_V$ at any time can give an indication of the concentration of reduced Q; $F_V$ increases with increase in the reduced Q pool. For thylakoids treated with DCMU, $(F_V)_{DCMU}$ provides an estimate of the effective Q pool. It is impossible to interpret salt-induced changes in $(F_V)_{DCMU}$ in this manner because as salinity affects both $F_0$ and $F_M$, it is obvious that the rate constants $k_f$, $k_h$, and $k_t$ for thylakoids exposed to a given salt concentration are not necessarily the same for other concentrations. The ratio $F_V/F_M$ provides a useful indicator of photochemical capacity, $k_pP$, *i.e.*

$$\frac{F_V}{F_M} = \frac{k_pP}{k_f + k_h + k_t + k_pP} \quad (4)$$

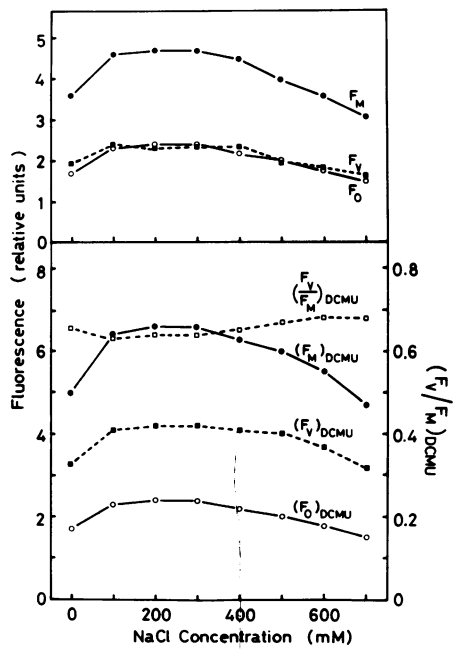


FIG. 4. Effect of NaCl on the fluorescence parameters $F_0$, $F_M$, $F_V$, $(F_0)_{DCMU}$, $(F_M)_{DCMU}$, $(F_V)_{DCMU}$, and $(F_V/F_M)_{DCMU}$ for spinach thylakoids uncoupled with 2.5 mM $NH_4Cl$ in presence of 1.5 mM potassium ferricyanide. In experiments involving DCMU, thylakoids were treated with 15 μM DCMU. Each point represents mean of four determinations.

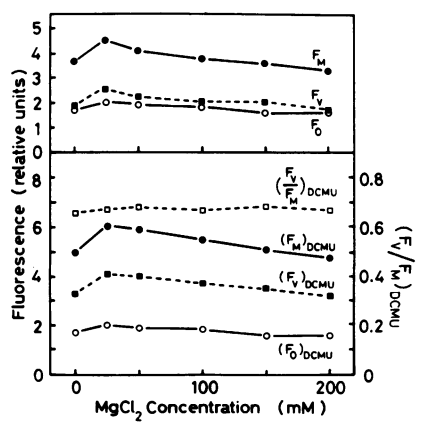


FIG. 5. Effect of $MgCl_2$ on fluorescence parameters $F_0$, $F_M$, $F_V$, $(F_0)_{DCMU}$, $(F_M)_{DCMU}$, $(F_V)_{DCMU}$, and $(F_V/F_M)_{DCMU}$ for spinach thylakoids uncoupled with 2.5 mM $NH_4Cl$ in presence of 1.5 mM potassium ferricyanide. In experiments involving DCMU, thylakoids were treated with 15 μM DCMU. Each point represents mean of four determinations.

Because it is generally assumed that $k_pP$ is relatively large compared to $k_f$, $k_h$, and $k_t$, the ratio $F_V/F_M$ will be less affected by changes in $k_f$, $k_h$, or $k_t$ than the parameter $F_V$ (see equation 3). In the presence of DCMU, where P is the total Q pool, $F_V/F_M$ is representative of the primary photochemical capacity of PSII. The effects of NaCl and $MgCl_2$ on $(F_V/F_M)_{DCMU}$ are shown in Figures 4 and 5; changes in salt concentration had little effect on this ratio. The removal of 1 mM $MgCl_2$ from the chloroplast resuspension and assay media resulted in a decrease in $(F_V/F_M)_{DCMU}$ in the absence of NaCl, suggesting that a decrease occurs in the primary photochemical capacity of thylakoids deprived of 1 mM $MgCl_2$. The resuspension and assay media were supplemented with 1 mM $MgCl_2$ to reduce the effects of chloroplast aging and increase reproducibility of results in the electron transport studies. The absence of large changes in $(F_V/F_M)_{DCMU}$ with changes in salinity initially suggests that the photochemical capacity, $k_pP$, of the thylakoids is not affected by cations. However, examination of equation 4 shows that $k_pP$ could change with no resultant change in $(F_V/F_M)_{DCMU}$ provided changes in any or all of the other rate constants ($k_f$, $k_h$, $k_t$) occurred. Previously it has been suggested that cations significantly affect both $k_h$ and $k_t$ (2, 13, 15), these constants decreasing with the addition of low cation concentrations to cation-free thylakoids. The complication of $k_h$ and/or $k_t$ showing cation-induced changes and interfering in interpretations of cation induced effects on the primary photochemical capacity, can be overcome by examining the ratio $(F_V/F_0 \cdot F_M)_{DCMU}$. From equations 1, 2 and 3 it can be deduced that:

$$\left(\frac{F_V}{F_0 \cdot F_M}\right)_{DCMU} = \frac{k_pP}{k_f} \quad (5)$$

Salt-induced changes in $(F_V/F_0 \cdot F_M)_{DCMU}$ will be dependent only upon changes in $k_f$ and/or $k_pP$; changes in $k_h$ or $k_t$ will not influence this ratio. However, since $(F_M)_{DCMU}$ changes with increasing salinity (see Figs. 4 and 5), it is necessary to normalize the $(F_M)_{DCMU}$ values at the different salt concentrations before calculating and comparing values of $(F_V/F_0 \cdot F_M)_{DCMU}$. Table I shows the effect of NaCl and $MgCl_2$ on $(F_V/F_0 \cdot F_M)_{DCMU}$ after normalization of the $(F_M)_{DCMU}$ values. Concentrations of NaCl above 400 mM produce a small increase in this ratio. No significant changes are observed with $MgCl_2$. Previously it has been assumed that $k_f$ is negligible compared with $k_h$ and $k_t$, and is unaffected by cations (15). This assumption is supported by a comparison of the low temperature absorption spectra and their fourth derivatives of thylakoids treated with a range of NaCl and $MgCl_2$ concentrations. No spectral shifts or changes in the Chl species composition of the thylakoids were observed with salt. Inasmuch as $k_f$ is directly related to the absorption coefficient of the thylakoids, which is dependent upon the Chl species present (15), it is probable that $k_f$ is not changing with salt treatment. Any changes in $(F_V/F_0 \cdot F_M)_{DCMU}$ can probably be attributed to changes in $k_pP$, and the observed increase in the ratio induced by NaCl concentrations above 400 mM is due to an increase in the photochemical capacity of the thylakoids. Changes in $(F_M)_{DCMU}$ with increasing salinity

cannot be attributed to changes in $k_pP$ (see equation 2); such changes must be due to changes in $k_h$ and/or $k_t$. One or both of these rate constants must be increasing with increasing NaCl concentrations above 300 mM.

For DCMU-treated thylakoids the area above the fluorescence induction curve provides an estimate of the concentration of PSII primary acceptor, Q (4). By normalizing the induction curves for thylakoids with and without salt it is possible to ascertain whether salinity affects the effective Q pool. No differences were observed in the normalized areas over the induction curves for thylakoids treated with NaCl (0–700 mM) and $MgCl_2$ (0–200 mM); as the concentration of Q is remaining constant with increasing salinity, changes in the primary photochemical capacity, $k_pP$, of the thylakoids are due to changes in $k_p$ and not the number of PSII traps.

The area above the fluorescence induction curve of thylakoids in the absence of DCMU is proportional to the concentration of the secondary acceptor of PSII, PQ (4, 14). After normalization of the fluorescence induction curves it was found that the PQ pool was affected by salt (Table II). Low salt concentrations produced a significant decrease in the PQ pool, reflecting a large reduction in the number of PQ molecules linked to any one reaction center-Q system.

The rise time, $\tau$, of the fluorescence induction for thylakoids treated with DCMU gives an indication of the rate at which Q is reduced. Table III shows the effect of salinity changes on this parameter. Concentrations of NaCl above 100 mM and of $MgCl_2$ above 25 mM increase $\tau$. Since the effective concentration of Q is not affected by salinity, changes in $\tau$ can be attributed directly to changes in the rate of primary photochemistry of PSII.

Similar fluorescence induction studies using $Na_2SO_4$, $MgSO_4$, and mannitol indicated that the observed changes in fluorescence induction curves with NaCl and $MgCl_2$ are cation-induced and not anionic or osmotic in nature.

Table I. Effect of NaCl and $MgCl_2$ on $\left(\frac{F_V}{F_O \cdot F_M}\right)_{DCMU}$ for spinach thylakoids

| NaCl Conc | $\left(\frac{F_V}{F_O \cdot F_M}\right)_{DCMU}$ | $MgCl_2$ Conc | $\left(\frac{F_V}{F_O \cdot F_M}\right)_{DCMU}$ |
|---|---|---|---|
| mM | | mM | |
| 0 | 0.39 | 0 | 0.39 |
| 100 | 0.36 | 25 | 0.41 |
| 200 | 0.36 | 50 | 0.43 |
| 300 | 0.36 | 100 | 0.41 |
| 400 | 0.36 | 150 | 0.44 |
| 500 | 0.39 | 200 | 0.40 |
| 600 | 0.43 | | |
| 700 | 0.43 | | |

Table II. Effect of NaCl and $MgCl_2$ on the effective concentration of the secondary acceptor of PSII (PQ) in spinach thylakoids

The method of estimating PQ concentration is described fully in the text. The concentration of PQ for salt-treated thylakoids is expressed as a fraction of the PQ concentration in thylakoids suspended in basal reaction medium (1.5mM potassium ferricyanide, 1mM $MgCl_2$, 5mM TES, pH 7.0).

| Salt Conc | PQ Conc |
|---|---|
| 0 | 1 |
| 50 mM NaCl | 0.59 |
| 100 mM NaCl | 0.55 |
| 200 mM NaCl | 0.52 |
| 400 mM NaCl | 0.48 |
| 600 mM NaCl | 0.32 |
| 25 mM $MgCl_2$ | 0.57 |
| 100 mM $MgCl_2$ | 0.37 |
| 200 mM $MgCl_2$ | 0.25 |

Table III. Effect of NaCl and $MgCl_2$ on the rise time of the fluorescence induction curve of spinach thylakoids treated with 15 μM DCMU

| Salt Conc | Rise Time |
|---|---|
| | sec |
| 0 | 0.55 |
| 100 mM NaCl | 0.55 |
| 400 mM NaCl | 0.64 |
| 700 mM NaCl | 0.85 |
| 24 mM $MgCl_2$ | 0.55 |
| 100 mM $MgCl_2$ | 0.65 |
| 200 mM $MgCl_2$ | 0.80 |

Table IV. Summary of $Na^+$-induced effects on activities associated with PSII of spinach thylakoids

| Parameter | Effect induced by increasing salinity | |
|---|---|---|
| | 0–100 mM NaCl | 100–700 mM NaCl |
| $k_h$, $k_t$ | decrease in one or both rate constants | increase in one or both rate constants |
| $k_p$ | small decrease | small increase |
| Effective concentration of PSII primary acceptor, Q | no effect | no effect |
| Rate of reduction of Q | no effect | decrease |
| Effective concentration of PSII secondary acceptor, PQ | large decrease | decrease |
| Rate of ferricyanide reduction by uncoupled membranes | increase | decrease |
| Rate of ferricyanide reduction by coupled membranes | no effect | small increase with 400 mM NaCl, then decrease |

## DISCUSSION

Cations are considered to produce an increase in hydrophobic interactions within the thylakoids, resulting in changes in water-lipid interfaces and membrane microstructure and conformation (20, 21). Such physical changes would be expected to produce a wide range of changes in thylakoid function. This present investigation has shown that cations can induce changes in membrane function ranging from excitation transfer to coupled electron transport. A summary of the changes induced by $Na^+$ in membrane activity is given in Table IV.

Increasing cation concentrations are thought to increase the physical separation of photosystem units and of their constituent Chl molecules (20). The Förster inductive resonance excitation exchange theory predicts that increasing intermolecular distances between Chl molecules will produce a decrease in $k_t$ (12), however an increase in $k_h$ will be expected (14). The observed stimulation of $(F_M)_{DCMU}$ by 100 mM NaCl must be attributed to a decrease in $k_t$, since an increase in $k_h$ would decrease $(F_M)_{DCMU}$. The salt-induced decrease in $k_t$ must be greater than the increase in $k_h$. Concentrations of NaCl over the range 400 to 700 mM produce a small increase in $k_p$, suggesting that the physical separation of Chl species within PSII units does not increase over this salinity range. However, this does not exclude a physical separation of PSI and PSII units occurring, which would result in a decrease in $k_t$. As the number of PSII traps is unaffected by salinity, $k_p$ can be used as an indicator of the primary photochemical capacity of PSII; salt appears to have little effect on this capacity, although the rate at which Q is reduced is considerably decreased by increasing salinity. The secondary acceptor (PQ) pool is markedly reduced

by increasing salinity, thus reducing the capacity for secondary photochemistry *i.e.* the reduction of PQ.

The stimulation of ferricyanide reduction by 100 mM NaCl cannot be attributed to changes in either the primary or secondary photochemical capacity of PSII; 100 mM NaCl produces a slight decrease in the capacity of primary photochemistry and a large decrease in the secondary photochemical capacity. Under nonsaturating light conditions this stimulation of ferricyanide reduction is generally explained by a decrease in energy spillover from PSII to PSI (2). In the experiments reported here ferricyanide reduction was determined under light-saturating conditions and the observed stimulation is the result of increased efficiency of an electron transport reaction(s) between $H_2O$ and $P_{680}$, or between PQ and ferricyanide. Salt-induced membrane conformational changes could produce this effect by increasing the proximity of the components of the rate-limiting electron transport reaction(s). Inhibition of uncoupled ferricyanide reduction by salinities above 100 mM NaCl could be due to decreases in the capacity for secondary photochemistry. Currently attempts are being made to locate the salinity-affected rate-limiting electron transport reaction(s) involved in uncoupled ferricyanide reduction.

The rate of ferricyanide reduction by coupled thylakoids is less affected by salinity changes than for uncoupled thylakoids, implying that the rate-limiting step in coupled electron transport (phosphorylation mechanism) is more salt-tolerant than its counterpart in uncoupled electron flow. The rate-limiting reaction of coupled thylakoids is physiologically important in determining energy production by the membrane and it is significant that this reaction is little affected by salinity changes, whereas many other membrane activities are markedly affected. If energy production by spinach thylakoids is not affected by increasing salinity, then the observed decrease in productivity of spinach plants with increasing salinity of the culture medium (Dominy and Baker, unpublished data) cannot be attributed to a decrease in primary photosynthetic energy production. Other aspects of cell metabolism, possibly the dark reactions of photosynthesis, must be limiting productivity.

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