Biochimica et Biophysica Acta, 592 (1980) 536—545

BBA 47914

EFFECT OF SURFACE POTENTIAL ON *P*-700 REDUCTION IN CHLOROPLASTS

NORIAKI TAMURA, YASUSI YAMAMOTO and MITSUO NISHIMURA

Department of Biology, Faculty of Science, Kyushu University 33, Fukuoka 812 (Japan)

(Received January 28th, 1980)

Key words: Chloroplast membrane; P-700 reduction; Plastocyanin; Surface potential; Electron transfer

Summary

The effect of salt addition on the rate of reduction of *P*-700 oxidized by flash illumination was analyzed. In broken chloroplasts, the rate of *P*-700 reduction was accelerated by salts of mono-, di- and trivalent cations, with the increasing effectiveness in this order, in the presence of various artificial electron donors or acceptors. The rate was not dependent on the concentration and the valence of anions. On the other hand, in Photosystem I-enriched subchloroplast particles, added KCl did not induce the acceleration of direct reduction of *P*-700 by reduced DCIP.

At low KCl concentrations (below 10 mM), the rate of *P*-700 reduction was also accelerated by added KCl in sonicated chloroplasts to which purified plastocyanin was added. The curves of dependence of the reduction rate on plastocyanin concentration were not of the Michaelis-Menten type, but sigmoidal. The maximal of *P*-700 reduction was higher at higher salt concentrations and the half-maximal plastocyanin concentration for *P*-700 reduction became lower with increasing NaCl concentrations.

In broken chloroplasts treated with 50 mM glutaraldehyde, the rate of *P*-700 reduction was not accelerated by added KCl.

The Debye-Hückel theory and the Gouy-Chapman theory were applied to our data to analyze the electrostatic interaction between electron tranfer components on thylakoid membranes. It is suggested that the major factor determining the rate of *P*-700 reduction is the donation of electrons from plastocyanin to *P*-700. Most of the observed effect is probably due to the increase in the local concentration or accessibility of plastocyanin to the site of *P*-700 reduction which is expected when the negative surface potential rises when salt is added.

Abbreviations: DCIP, 2,6-dichloroindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Introduction

Salts induce significant changes in many reactions in the primary processes of photosynthesis [1—5]. Many of these effects are probably related to the changes of thylakoid membrane structure (configuration, conformation and grana stacking) [6—8]. The relationship between the changes of structure and physical parameters of thylakoid membranes and those of activities induced by salts has been investigated [9,10].

Nelson and Racker showed an inhibitory effect of high-concentration NaCl on photooxidation of cytochrome *f* in Photosystem I-enriched subchloroplast particles in the presence of plastocyanin [11]. Wood and Bendall applied the Debye-Hückel theory to explain the deceleration of *P*-700 reduction by added NaCl and suggested that the interaction of plastocyanin with *P*-700 was mainly electrostatic [12].

However, an opposite effect, the aceleration of *P*-700 reduction at low concentrations of added salt, is observed in broken chloroplasts, as will be analyzed in this paper.

By investigating the salt-induced change of fluorescence of 9-aminoacridine in the suspension of broken chloroplasts, Searle et al. [13] suggested that the electrical double layer on the negatively charged thylakoid membrane surface was regulating certain membrane-associated phenomena in chloroplasts. From the effects of salts and ionic detergents on electron transfer, Itoh indicated that the rates of *P*-700 reduction by ionic electron donors were determined by their concentrations at the surface of thylakoid membranes [14,15]. The importance of electrostatic interactions has been pointed out also for the reactions of plastocyanin in solution [16].

Plastocyanin may be involved in the rate-limiting step for *P*-700 reduction [17]. If plastocyanin is loosely bound on thylakoid membrane surfaces, the changes of physical parameters of the membrane surfaces by salt addition (e.g. by screening of negative surface charges by cations or charge neutralization by binding) would affect the interaction between plastocyanin and thylakoid membrane surfaces. In this paper, we examined whether the acceleration of *P*-700 reduction induced by added salts was due to the change of the electrostatic interaction between plastocyanin and *P*-700.

Materials and Methods

About 50 g of washed and deveined spinach leaves were homogenized for 7—8 s in 100 ml of a mixture of 50 mM Tris-HCl (pH 7.8), 30 mM NaCl and 0.4 M sucrose in a Waring Blendor. The homogenate filtered through eight layers of gauze was centrifuged at $200 \times g$ for 10 s. The supernatant was then centrifuged at $2000 \times g$ for 15 min and the resulting pellet was resuspended in 10 mM Tris-HCl (pH 7.4). After centrifugation of the suspension at $10\,000 \times g$ for 15 min, the pellet was resuspended in the same buffer. Total chlorophyll was determined using absorption coefficients reported by Mackinney [18].

Photosystem I-enriched subchloroplast particles solubilized with digitonin were prepared according to the procedure of Anderson and Boardman [19].

To obtain plastocyanin-depleted thylakoid membranes, broken chloroplasts

were sonicated for 3 min in an ice bath. The sonicated broken chloroplasts were centrifuged at 10 000 × *g* for 15 min. The supernatant was then centrifuged at 144 000 × *g* for 1 h and the resulting pellet was resuspended in 10 mM Tris-HCl (pH 7.4).

Plastocyanin was prepared by the method of Katoh [20].

Absorbance changes at 705 nm due to the oxidation-reduction reactions of *P*-700 were measured using a Union Giken rapid reaction analyzer RA 1201, a rapid single-beam spectrophotometer combined with temperature-jump and pulse-flash-excitation devices. Illumination (duration 7.6 μs) was supplied by a xenon flash perpendicular to the measuring beam. A Hoya glass filter B-440 (maximum transmission at 440 nm) and a Corning 9782 filter were placed between the flash and the sample cuvette. An interference filter (maximum transmission at 703 nm) and a Hoya glass filter R-70 (red light transmission, >680 nm) were placed on the photomultiplier as guard filters. The reaction mixture contained broken chloroplasts equivalent to 30—100 μg of chlorophyll, a salt at a given concentration and other reagents as indicated in figure legends in a final volume of 3 ml at pH 6.0—6.5.

For glutaraldehyde treatment, broken chloroplasts were incubated, at a concentration of 100 μg of chlorophyll per ml, in 10 mM Tris-HCl (pH 7.4) containing a given concentration of glutaraldehyde for 20 min in an ice bath. Then glutaraldehyde was removed by diluting the suspension eight times with 10 mM Tris-HCl (pH 7.4) and centrifuging at 10 000 × *g* for 15 min to sediment broken chloroplasts. This washing was repeated again and the precipitated chloroplasts were resuspended in 10 mM Tris-HCl (pH 7.4). The 20 min incubation was sufficient for the maximal effects by glutaraldehyde treatment on flash-induced H^+ uptake, the 515-nm absorbance change and electron transfer.

Results and Discussion

Fig. 1A shows the effect of KCl on the rate of *P*-700 reduction with various artificial electron donors or acceptors. Half-recovery times after the flash-induced oxidation are compared. The slow dark recovery after the flash illumination in the absence of added KCl was significantly accelerated by KCl at low concentrations. Either addition of phenazine methosulfate or reduced DCIP in the presence of DCMU, where the electron donation by Photosystem II was inhibited, half-recovery time of *P*-700 reduction in the presence of 10 mM KCl (2 ms) was smaller in two orders of magnitude than that without KCl. Under the conditions where H_2O was the electron donor and methyl viologen the electron acceptor, a similar effect was observed (data not shown). The same acceleration effect by added KCl was observed when no externally-added donors or acceptors were added, both in the presence and absence of DCMU. Recently, a similar effect of salts on *P*-700 reduction, in the presence of diaminodurene and anthraquinone 2-sulfonate, was reported by Lockau [21].

As shown in Fig. 1B, the addition of KCl to the Photosystem I-enriched subchloroplast particles had little effect on the half-recovery time of oxidized *P*-700 in the presence of reduced DCIP at a sufficient level (133 μM). In the absence of KCl, the rate of *P*-700 reduction by reduced DCIP in Photosystem I-enriched subchloroplast particles was similar to that in broken chloroplasts.

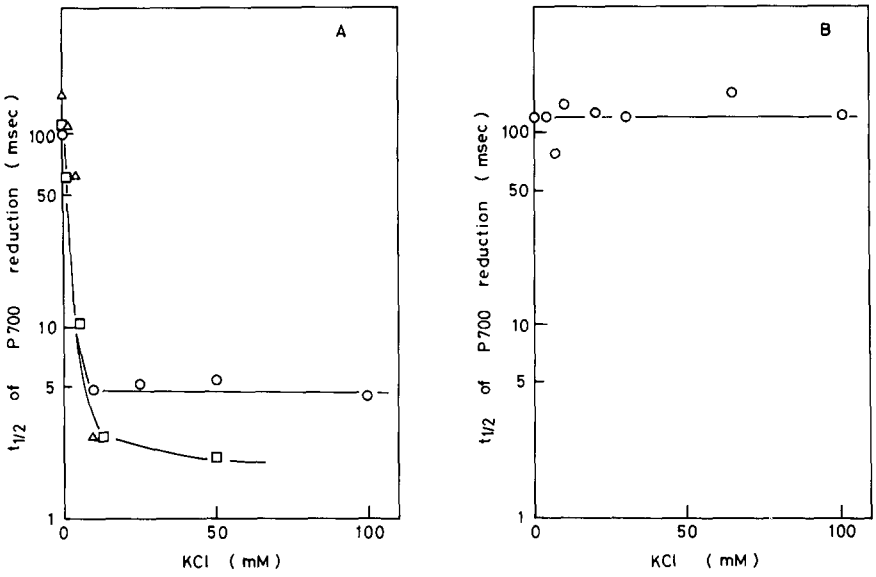


Fig. 1. Effect of KCl on *P*-700 reduction in (A) broken chloroplasts and (B) Photosystem I-enriched subchloroplast particles. The rates of *P*-700 reduction were compared in half-recovery times at various KCl concentrations. (A) ○, no addition, broken chloroplasts equivalent to 100 μg of chlorophyll in 3 ml; △, in the presence of 20 μM phenazine methosulfate and 10 μM DCMU; □, in the presence of 133 μM DCIP, 1 mM Na ascorbate, 133 μM methylviologen and 10 μM DCMU. (B) The reaction mixture contained 133 μM DCIP, 1 mM sodium ascorbate, 133 μM methylviologen and Photosystem I-enriched subchloroplast particles equivalent to 30 μg of chlorophyll, in 3 ml.

Fig. 2 shows effects of various salts on the half-recovery time of *P*-700 reduction in broken chloroplasts in the presence of phenazine methosulfate. In general, the effectiveness was dependent on the valence and concentrations of cations. Half-maximal effects of mono-, di- and trivalent cations were observed at concentrations of about 5 mM, 0.5 mM and 100 μM, respectively (Fig. 2A). The effect was not specific to cation or anion species. The concentration dependences with $MgCl_2$ and $MgSO_4$ were identical (Fig. 2A) and NaCl and

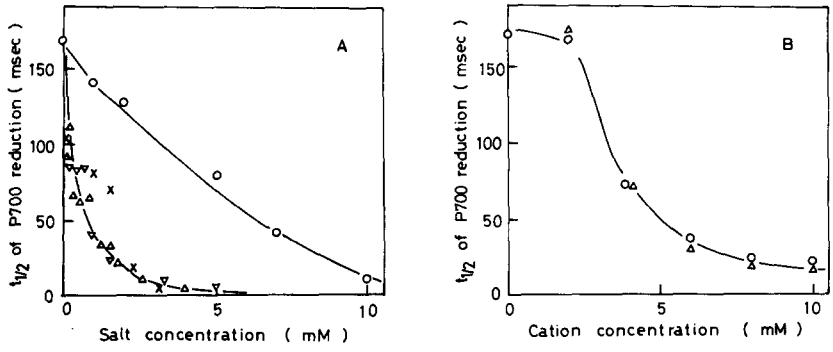


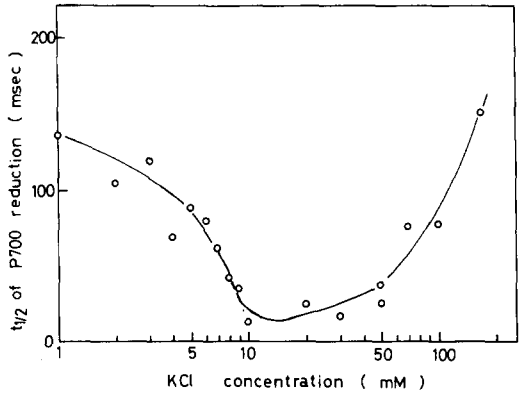
Fig. 2. Effect of various salts on *P*-700 reduction in broken chloroplasts in the presence of 20 μM phenazine methosulfate and 10 μM DCMU. (A) ○, NaCl; ▽, $MgSO_4$; △, $MgCl_2$; X, $CaCl_2$. (B) ○, NaCl; △, Na_2SO_4. A and B contained broken chloroplasts equivalent to 100 and 30 μg of chlorophyll, respectively, in 3 ml.

Na_2SO_4 gave almost identical results when compared on the cation concentration scale (Fig. 2B).

KCl had two types of effects on the half-recovery time of *P*-700 reduction in sonicated chloroplasts to which purified plastocyanin was added (Fig. 3). At low concentrations (below 10 mM), *P*-700 reduction was accelerated by adding KCl. On the other hand, in the concentration range above 10—20 mM, the half-recovery time of *P*-700 reduction increased as KCl increased. The former effect is consistent with the KCl-dependence of the half-recovery time of *P*-700 reduction in broken chloroplasts. Effects, similar to the latter effect (decrease of rate by salt at higher concentrations), were observed with other phenomena (chlorophyll fluorescence, thylakoid stacking and electron transport) [9,10].

The dependence of the apparent rate constant (= ln $2/t_{1/2}$) of *P*-700 reduction on the concentration of plastocyanin added to sonicated chloroplasts was studied at five concentrations of KCl (2, 4, 6, 8 and 10 mM) (Fig. 4). The curves were not of the Michaelis-Menten type. At lower concentrations of KCl, the rate did not saturate within the concentration range of plastocyanin used (up to 5.9 μM). The concentration of plastocyanin which gave the half-maximal stimulation became lower as the increase of concentration of added KCl. The same result was obtained for *P*-700 reduction after flash illumination when reduced plastocyanin was added to the Photosystem I-enriched subchloroplast particles obtained by treating chloroplasts with Triton X-100 (data not shown).

Fig. 5 shows the effect of KCl on the half-recovery time of *P*-700 reduction in chloroplast preparations treated with glutaraldehyde at various concentrations. Glutaraldehyde is known to crosslink protein molecules and block structural changes of membranes induced by light, salt, etc. [8]. A decrease of the extent of *P*-700 oxidation and an increase of the half-recovery time of *P*-700 reduction after flash illumination were observed in the glutaraldehyde-treated prepara-



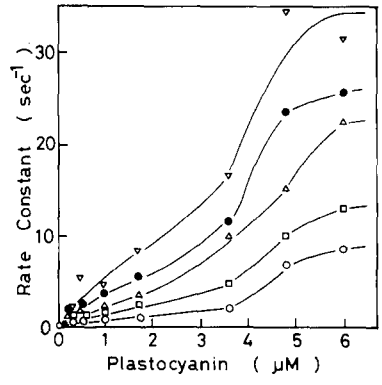


Fig. 3. Effect of KCl on *P*-700 reduction in sonicated chloroplasts to which purified plastocyanin was added. The reaction mixture contained sonicated chloroplasts equivalent to 90 μg of chlorophyll, 4 μM plastocyanin, 133 μM methylviologen and a given concentration of KCl, in 3 ml.

Fig. 4. Concentration dependence of effect of plastocyanin added to sonicated chloroplasts on *P*-700 reduction. Purified and ascorbate-reduced plastocyanin was added to sonicated chloroplasts equivalent to 30 μg of chlorophyll in 3 ml, in the presence of 10 μM DCMU. ○, 2 mM KCl; □, 4 mM KCl; △, 6 mM KCl; ●, 8 mM KCl; ▽, 10 mM KCl.

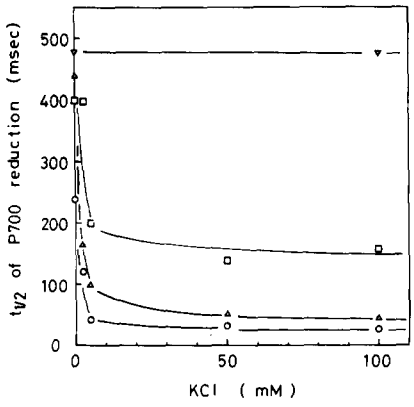


Fig. 5. Effect of KCl on *P*-700 reduction in chloroplasts treated at various glutaraldehyde concentrations. The reaction mixture contained chloroplast preparation equivalent to 100 μg of chlorophyll in 3 ml, 133 μM methylviologen and a given concentration of KCl. Glutaraldehyde concn.: ○, 0; △, 5 mM; □, 25 mM; ▽, 50 mM.

tions in the presence of methylviologen. A similar result was obtained in the presence of phenazine methosulfate. In the chloroplast preparation treated with 50 mM glutaraldehyde, the rate of *P*-700 reduction was not accelerated by adding KCl. This was confirmed by a separate experiment which had points corresponding to 10 and 50 mM KCl, where $t_{1/2}$ remained the same as the control (no KCl). The acceleration of *P*-700 reduction by $MgSO_4$ addition was also abolished in 50 mM glutaraldehyde-treated chloroplast preparations. In this type of preparation, the Photosystem II-activity was inhibited much less than the Photosystem I-activity (data not shown). Hardt and Kok suggested that the possible site of photosynthetic electron trasnfer blocked by glutaraldehyde was plastocyanin [22]. It may be noted that the charge density of membrane is not affected by glutaraldehyde treatment at neutral pH [23].

When salts are added to the suspension of thylakoid membranes, they may suppress membrane-macromolecule and membrane-membrane electrostatic interactions by screening the fixed negative charges of thylakoid membranes which have the isoelectric point at a weakly acidic pH [9,10,24,25]. In addition, salts induce the change of osmolarity in the suspension of thylakoid membranes. However, in the presence of 0.4 M sucrose, the rate of *P*-700 reduction was accelerated by adding low-concentration NaCl, as in the absence of sucrose (data not shown).

It was found that the flash-induced H^+ uptake and the 515-nm absorbance change were accelerated by adding salts (data not shown). By adding valinomycin, in the presence of low-concentration KCl, the recoveries of 515-nm absorbance change and H^+ uptake after the flash illumination were accelerated significantly, whereas the rate of *P*-700 reduction was not accelerated. Carbonyl cyanide *m*-chlorophenylhydrazone, which facilitates H^+ translocation across thylakoid membranes, was similar to valinomycin-plus-K^+ in its effects on the 515-nm absorbance change and *P*-700 reduction. These data indicated that the translocation of ions across thylakoid membranes did not induce the acceleration of *P*-700 reduction.

The difference observed between the effects of KCl on the reduction of

P-700 by reduced DCIP in the broken chloroplasts and the Photosystem I-enriched subchloroplast particles may depend on the types of electron donation to *P*-700. In the broken chloroplasts, *P*-700 is probably reduced via plastocyanin or cytochrome *f* by reduced DCIP. On the other hand, in the Photosystem I-enriched subchloroplast particles, it is probably reduced by reduced DCIP. Therefore, in the latter there will be no salt effect, since reduced DCIP carries no net charge.

Haehnel [17] suggested that at least 85% of the linear electron transfer ran from plastoquinone to *P*-700 via plastocyanin, not cytochrome *f* in situ. Since plastocyanin is a water-soluble protein and is negatively charged at pH 6.0—6.5 where our experiments were carried out, it is expected that plastocyanin responds to the change of ionic environment, giving a significant influence on the rate of *P*-700 reduction.

Wood and Bendall have indicated that the reaction between the Photosystem I-enriched subchloroplast particles and plastocyanin obeys the Brønsted theory at high salt concentrations [12]. Provided that the interaction between electron transfer components on thylakoid membranes is determined by electrostatic forces between molecules (rather than between membrane and molecules as discussed later), the following equation can be used to explain the ionic strength-dependent acceleration of *P*-700 reduction. In a dilute solution (below 10 mM of monovalent salts), the simplified Brønsted relationship applied to the Debye-Hückel theory will give the rate constant k for the reaction between two species with charges z_a and z_b, as a function of ionic strength I, at 298 K;

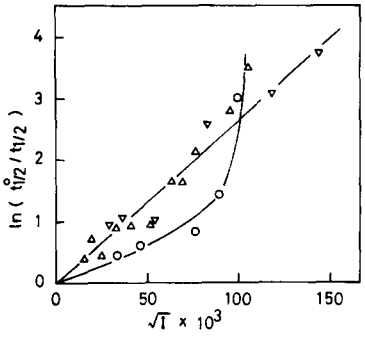
$$\log k = \text{constant} + 1.018 z_a z_b I^{1/2} \tag{1}$$

In the above discussion, the radii of charged reactants are assumed to be negligible. Logarithms of $(t^0_{1/2}/t_{1/2})$ are plotted against square root of ionic strength, where $t^0_{1/2}$ is the half-recovery time of *P*-700 reduction at zero-added-salt concentration (Fig. 6). In the cases of salts of mono- and divalent cations, the slopes of the lines were positive and different; their relative values were 12 and 24, respectively, at low salt concentrations (below 10 mM). The slopes were not dependent on the valence of anion species. Different contributions of cations and anions can not be explained by the Brønsted theory. In addition, the negatively charged membrane surfaces should induce unequal distributions of cations and anions near the site of *P*-700 reduction, where the application of Brønsted theory is difficult.

As an alternative explanation for our data, electrostatic interaction of plastocyanin and the surface of thylakoid membranes, both negatively charged at neutral pH, is examined. The contribution of negative charges on the membrane surface to the rate of *P*-700 reduction is analyzed by applying the electrical double layer theory developed by Gouy-Chapman. The relationship between membrane surface potential and charge density is given by the following;

$$q = \left(\frac{2RT\epsilon}{\pi}\right)^{1/2} C_b^{1/2} \sinh\left(\frac{Fz\psi_0}{2RT}\right) \tag{2}$$

where chloroplasts were suspended in a medium containing *z*-*z* electrolyte such as NaCl or $MgSO_4$ and q is the surface charge density in $\mu C/cm^2$, C_b the bulk



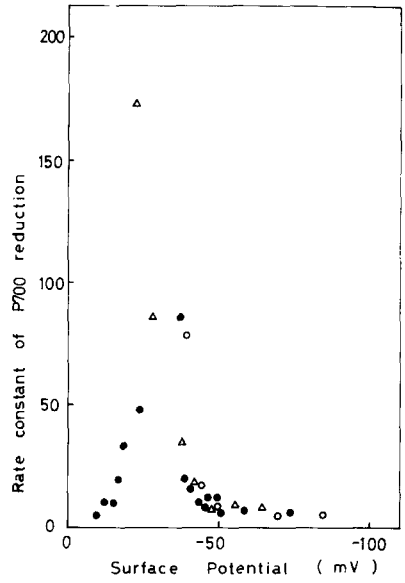


Fig. 6. The data of Fig. 3 replotted as a function of (ionic strength)$^{1/2}$. Natural logarithm of the rate of half-recovery time is plotted on the ordinate, where $t^0_{1/2}$ is the half-time of *P*-700 reduction at zero-added-salt concentration. ○, NaCl; △, $MgSO_4$; ▽, $MgCl_2$.

Fig. 7. Dependence of the rate constant for *P*-700 reduction on surface potential. NaCl (○) or $MgSO_4$ (△) was added to the suspension of broken chloroplasts (data of Fig. 3). KCl was added to sonicated chloroplasts to which plastocyanin was added (●) (data of Fig. 4).

salt concentration in M, ψ_0 the surface potential and ϵ the dielectric constant of the solution.

If the rate of *P*-700 reduction is determined by the surface potential, when the surface charge density and other physical parameters remain the same, q and ψ_0 can be calculated. In our data, the presence of 4.6 mM NaCl or 0.35 mM $MgSO_4$ gave the same rate of *P*-700 reduction, a level twice as fast as that in the absence of added salt. Using these data, the value of -0.93 $\mu C/cm^2$ is obtained as q, which is equivalent to about one negative elementary charge per 1720 Å^2. The obtained value is comparable to the previously reported ones [13—15]. Apparent rate constants for *P*-700 reduction in Figs. 2 and 3 are replotted against the surface potential calculated from Eqn. 2 using the value of -0.93 $\mu C/cm^2$ as q (Fig. 7). Though q is assumed to be constant at a given salt concentration, the surface potential ψ_0 indicated in Fig. 7 may not necessarily give the exact value corresponding to the salt concentration, due to the change in surface pH as one of the possible factors [25]. There was little difference in the ψ_0-dependence between NaCl and $MgSO_4$. This supports the assumption that the rate of *P*-700 reduction is dependent on the surface potential and not on the ionic species per se. Furthermore, the agreement between the data shown in Figs. 2 and 3 is rather remarkable. This suggests that the nature of interaction between the added plastocyanin and the plastocyanin-depleted thylakoid membranes was similar to that between the endogenous plastocyanin and thylakoid membranes, in spite of the possible difference in the localization of plastocyanin.

If the screening of the fixed negative charges of thylakoid membranes by added salts causes the increase of accessibility of negatively charged plastocyanin to thylakoid membranes to accelerate the rate of *P*-700 reduction, the half-maximal concentration of added plastocyanin will become smaller with the increasing NaCl concentration. Indeed, the result in Fig. 4 indicates the possibility that the rate of *P*-700 reduction is regulated by the accessibility of added plastocyanin to thylakoid membranes. The ψ_0-dependence of the rate of *P*-700 reduction in sonicated chloroplasts was in fair agreement with that in broken chloroplasts (Fig. 7). This suggests that the rate of *P*-700 reduction in broken chloroplasts is limited by the local concentration at or by the accessibility to the site of *P*-700 reduction of plastocyanin which can be dissociated from the membrane rather easily. A possible explanation for the appearance of the 'maximum' in Fig. 7 is that the decrease in the thermodynamic activity of plastocyanin at higher salt concentrations, as suggested by Itoh [14,15]. This is also suggested from the salt concentration dependence of the rate of the reaction between added high-molecular-weight electron carriers and thylakoid membranes (data not shown).

In this study, the effect of salts on the rate of *P*-700 reduction is mainly interpreted in terms of electrostatic interactions between charged electron transfer components and membranes (i.e., 'electrostatic screening' proposed by Barber et al. [9,10] and the activity of plastocyanin at membrane surfaces [14,15]). However, there is an additional effect of salts probably inducing a change of conformation of reactant molecules or that of microenvironment at the reaction site. As shown in Fig. 4, the curves of dependence of the reduction rate on plastocyanin concentration were not of the Michaelis-Menten type. Instead, they were sigmoidal. Below 10 mM NaCl, the saturation level of the reduction rate was higher at higher salt concentrations and the half-maximal plastocyanin concentration became lower at higher NaCl concentrations. The change of the molecular environment and/or molecular conformation, in addition to the screening of surface charges, is possible. The fact that in glutaraldehyde-treated chloroplasts the rate of *P*-700 reduction was not affected by added salts (Fig. 5) suggested that the surface potential-dependent change in the distribution of plastocyanin does not take place after the fixation of plastocyanin on thylakoid membranes. The fixation will also suppress the salt-induced conformation change which may be involved in the salt effect. As thylakoid membranes have negative charges at the pH in our experiments, they may attract positive ions such as H^+. The addition of salts will decrease the magnitude of the negative surface potential, hence inducing the rise of surface pH [25]. This change of surface pH may also affect the rate of *P*-700 reduction.

Acknowledgment

We thank Dr. S. Itoh for stimulating discussions and suggestions and also thank Drs. K. Masamoto and K. Matsuura for helpful discussions.

References

1 Shavit, N. and Avron, M. (1967) Biochim. Biophys. Acta 131, 516—525
2 Gross, E.L., Dilley, R.A. and San Pietro, A. (1969) Arch. Biochem. Biophys. 134, 450—462

3 Marsho, T.V. and Kok, B. (1974) Biochim. Biophys. Acta 333, 353—365
4 Murata, N. (1969) Biochim. Biophys. Acta 189, 171—181
5 Davis, D.J., Janovitz, E.B. and Gross, E.L. (1977) Arch. Biochem. Biophys. 184, 197—203
6 Izawa, S. and Good, N.E. (1966) Plant Physiol. 41, 544—552
7 Argyroudi-Akoyunoglou, J.H. and Tsakiris, S. (1977) Arch. Biochem. Biophys. 184, 307—315
8 Murakami, S. and Packer, L. (1971) Arch. Biochem. Biophys. 146, 337—347
9 Barber, J., Mills, J. and Love, A. (1977) FEBS Lett. 74, 174—181
10 Barber, J. and Chow, W.S. (1979) FEBS Lett. 105, 5—10
11 Nelson, N. and Racker, E. (1972) J. Biol. Chem. 247, 3848—3853
12 Wood, P.M. and Bendall, D.S. (1975) Biochim. Biophys. Acta 387, 115—128
13 Searle, G.F.W., Barber, J. and Mills, J.D. (1977) Biochim. Biophys. Acta 461, 413—425
14 Itoh, S. (1979) Biochim. Biophys. Acta 548, 579—595
15 Itoh, S. (1979) Biochim. Biophys. Acta 548, 596—607
16 Takabe, T., Niwa, S., Ishikawa, H. and Miyakawa, M. (1980) J. Biochem. 87, 111—115
17 Haehnel, W. (1977) Biochim. Biophys. Acta 459, 418—441
18 Mackinney, G. (1941) J. Biol. Chem. 140, 315—322
19 Anderson, J.M. and Boardman, N.K. (1966) Biochim. Biophys. Acta 112, 403—421
20 Katoh, S. (1971) Methods Enzymol. 23, 408—413
21 Lockau, W. (1979) Eur. J. Biochem. 94, 365—373
22 Hardt, H. and Kok, B. (1977) Plant Physiol. 60, 225—229
23 Stark, G.R. (1970) in Advances in Protein Chemistry (Anfinsen, C.B., Edsall, J.T. and Richards, F.M., eds.), Vol. 24, pp. 261—308, Academic Press, New York
24 Mercer, F.V., Hodge, A.J., Hope, A.B. and McLean, J.D. (1955) Aust. J. Biol. Sci. 8, 1—18
25 Masamoto, K., Itoh, S. and Nishimura, M. (1980) Biochim. Biophys. Acta 591, 142—152