Biochima et Biophysica Acta, 679 (1982) 436–443
Elsevier Biomedical Press

BBA 41046

TRANSIENT KINETICS OF THE REACTION BETWEEN CYTOCHROME *c*-552 OR PLASTOCYANIN AND *P*-700 IN SUBCHLOROPLAST PARTICLES

LARS F. OLSEN and RAYMOND P. COX

Institute of Biochemistry, Odense University, Campusvej 55, 5230 Odense M (Denmark)

(Received September 18th, 1981)

Key words: Cytochrome c-552; Plastocyanin; P-700 reduction; Electron transfer; Transient kinetics; (Subchloroplast particle)

We have investigated the kinetics of reduction of *P*-700 in Photosystem I-enriched subchlroplast particles by cytochrome *c*-552 from *Euglena* and by plastocyanin, and the effects of cations on these reactions. In both cases, the results can be explained in terms of a bimolecular reaction between *P*-700$^+$ and the electron donor. Addition of low concentrations of mono-, di- or polyvalent cations stimulate, whereas addition of high concentrations of cations inhibit these reactions. The effects of cations can be interpreted as effects on the membrane surface potential of the Photosystem I particles, as shown previously (Tamura, N., Yamamoto, Y. and Nishimura, M. (1980) Biochim. Biophys. Acta 592, 536–545). The maximal second-order rate constants obtained for the reduction of *P*-700$^+$ are $9 \cdot 10^7$ M^{-1} · s^{-1} if cytochrome *c*-552 is the electron donor, and $1.5 \cdot 10^8$ M^{-1} · s^{-1} when the donor is plastocyanin. Using the latter rate constant, we are able to demonstrate that the apparently biphasic kinetics of reduction of *P*-700$^+$ observed in intact chloroplasts show a good fit to those expected for a bimolecular reaction between *P*-700$^+$ and plastocyanin.

Introduction

In the chloroplasts of higher plants, electron transfer to *P*-700, the reaction centre chlorophyll of PS I, is known to involve plastocyanin. However, the complicated kinetics of reduction of *P*-700$^+$ following photooxidation by a single turnover flash [1–3] and the relatively small absorbance changes due to plastocyanin in the spectral region between 550 and 600 nm [3,4] have caused some debate about the identity of the primary electron donor to *P*-700 and the mechanism of electron transfer. The rate of reduction of *P*-700$^+$ in chloroplasts following a single turnover flash is extremely rapid, and the kinetics can be fitted to two exponential functions with half-times of 20 and 200 μs at 20°C [1–3]. Haehnel [4] studied the kinetics of the absorbance change at 584 nm, which may be used to follow redox changes of plastocyanin when the contribution from the electrochromic shift at this wavelength has been eliminated. He was able to correlate the 200-μs phase of *P*-700$^+$ reduction with the oxidation of plastocyanin, but the 20-μs phase could not be resolved under these conditions. Bouges-Bocquet and Delosme [3] reported a lag in the oxidation of plastocyanin in *Chlorella* cells and concluded that an as yet unidentified electron donor is situated between *P*-700 and plastocyanin, and is responsible for the 20-μs phase of electron transfer to *P*-700$^+$. However, these authors had to correct for the absorbance changes due to several other components before the time course of plastocyanin oxidation could be established. In a recent publication, Haehnel et al. [5] have argued in favour of complexed plastocyanin acting as the primary electron donor for *P*-700$^+$ in order to explain the apparently biphasic kinetics. Wood and Bendall [6] have studied the photooxidation of exogenously added plastocyanin in

---

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; PS, Photosystem.

005-2728/82/0000–0000/$02.75 © 1982 Elsevier Biomedical Press

continuous light by chloroplasts treated with digitonin to allow plastocyanin access to P-700. They found evidence for a second-order reaction between P-700$^+$ and reduced plastocyanin with a rate constant of $8 \cdot 10^7$ $M^{-1} \cdot s^{-1}$. Haehnel et al. [7] estimated a rate constant of $1.5 \cdot 10^8$ $M^{-1} \cdot s^{-1}$ for the reduction by plastocyanin of P-700$^+$ in PS I-enriched subchloroplast particles following its photooxidation by a flash.

Plastocyanin is replaced by a soluble *c*-type cytochrome (cytochrome *c*-552) in some algal chloroplasts. This cytochrome has a well defined absorption spectrum in the region 540–560 nm, and a considerably higher extinction coefficient at 552 nm than that of plastocyanin at 584 nm, facilitating an investigation of its kinetics of oxidation. We report here measurements of the kinetics of electron transfer from plastocyanin or cytochrome *c*-552 from *Euglena* gracilis to P-700 in subchloroplast particles enriched in PS I, and investigations of the effect of the ionic composition of the medium on the rate of reaction.

Materials and Methods

PS I-enriched subchloroplast particles were obtained from lettuce chloroplasts by treatment with digitonin as described by Anderson and Boardman [8] with the modification that the homogenisation medium for chloroplast preparation also contained 5 mM ascorbate. The resulting D-144 * fraction was resuspended in 20 mM Hepes-KOH, pH 7.5, and either used immediately or stored frozen in liquid N_2 until required.

Plastocyanin was prepared from spinach. Frozen leaves were minced and allowed to thaw. The green liquid obtained after passage through cheesecloth was treated with 3 vol. of cold acetone (–20°C). The precipitate was collected and resuspended at 0°C in buffer containing 35% (v/v) acetone. The material which did not redissolve was discarded and the acetone concentration was increased to 75% (v/v) by the further addition of cold acetone (–20°C). The final precipitate was collected, redissolved in buffer and further purified to obtain plastocyanin essentially as described by Plesničar and Bendall [9]. Cytochrome *c*-552 was obtained from *E. gracilis* (Strain 1224/5z from the Culture Collection of Algae and Protozoa, Cambridge, U.K.), essentially as described by Perini et al. [10].

Horse heart cytochrome *c* was Type VI from Sigma and was used withour further purification. Polybrene and polylysine (Type V, mol. wt. 20000) were also purchased from Sigma.

Flash-induced redox changes of P-700 and cytochrome *c*-552 were measured in a single-beam spectrophotometer constructed by Applied Photophysics Ltd., London, and modified in our workshop. The signal from the photomultiplier was passed through a differential amplifier and an interface built in our workshop into a Nova 1200 mini-computer. The interface also contained logic outputs enabling control of the actinic flash (a Braun 410C photographic flash) operated at minimum energy output, corresponding to a flash width of 20 μs at half-height. This was saturating under the experimental conditions used and produced only a single turnover as shown by comparing the amount of cytochrome *c*-552 oxidised following a flash with the amount of P-700 present in the PS I particles. The response time of the apparatus was limited by the data sampling rate of the computer (0.3 ms per address).

Redox changes due to P-700 were measured at 703 nm. The signal-to-noise ratio was improved by accumulating 20 single sweeps (flash frequency 0.2 Hz) and the contribution from fluorescence was minimized by subtraction of the accumulated signal in the absence of the monitoring light. The flash was filtered through a combination of a Wratten 47B filter plus 30 mm of a 2% $CuSO_4$ solution, and the photomultiplier tube was protected by a 3 mm Schott RG 715 filter. Cytochrome *c*-552 was measured at 552 minus 542 nm by accumulating 20 single sweeps at the measuring wavelength and subtracting the same number of sweeps at the reference wavelength. A Wratten 70 filter was placed in front of the flash and the photomultiplier tube was protected by a 3 mm Schott BG 18 filter.

The basic suspension medium consisted of 10 mM Hepes/NaOH, pH 7.5, unless otherwise indicated. Further additions were 1 mM sodium ascorbate, 20–40 μM methyl viologen, 10 μM diaminodurene, PS I particles corresponding to 15 μM chlorophyll (50 nM P-700) and 0.24 μM cytochrome *c*-552 or 0.3 μM plastocyanin, unless otherwise stated. The basic me-

---

* D-144, PS I-enriched subchloroplast particles prepared by treatment with digitonin and centrifugation at 144 000 × *g*.

dium thus contained 6 mM monovalent cations plus 20–40 μM divalent cations. Kinetic measurements were made at room temperature (20°C).

Results

Illumination of the PS I particles with a 20 μs flash resulted in photooxidation of *P*-700 to an extent corresponding to about 1 molecule per 300 chlorophylls. Nine-tenths of this could be reduced by plastocyanin or cytochrome *c*-552, whereas all of it could be reduced by *N*-methylphenazonium methosulphate and ascorbate. This demonstrates that almost all of the *P*-700 in our preparation is accessible to externally added proteins. 10% of the *P*-700 appears to be protected from the external solution, possibly because some of the particles form vesicular structures with *P*-700 inside.

In Fig. 1A we have plotted the rate of reduction of *P*-700$^+$ for different concentrations of cytochrome *c*-552 in the presence and absence of 5 mM $MgCl_2$. In both cases the results are those expected for a second-order reaction with rate constants of $7.7 \cdot 10^7$ $M^{-1} \cdot s^{-1-}$ and $2.0 \cdot 10^7$ $M^{-1} \cdot s^{-1}$, respectively. Fig. 1B shows the effect of addition of mono- or divalent cations on the reaction of reduced cytochrome *c*-552 with *P*-700$^+$. Increasing concentrations lead to an increase in the rate until a maximum is obtained at 20–40 mM monovalent cations or 3–4 mM divalent cations. Above these concentrations, the rate declines with increasing salt concentration. No specificity within a valency group is observed indicating that the effect of cations is electrostatic.

Fig. 2. shows experiments similar to those in Fig. 1, but with plastocyanin as the electron donor. Again the kinetics of *P*-700$^+$ reduction fit a second-order reaction between *P*-700$^+$ and plastocyanin (Fig. 2A) with rate constants of $1.6 \cdot 10^7$ and $1.15 \cdot 10^8$ $M^{-1} \cdot s^{-1}$ in the absence and presence of 32 mM NaCl, respectively. The effects of cations are similar to those on the reaction between *P*-700 and cytochrome *c*-552.

Cytochrome *c*-552 and plastocyanin both carry a net negative charge at pH 7.5. The isoelectric point of the cytochrome is 5.5 [10] below which it carries a net positive charge. The isoelectric point of plastocyanin is 4.0 or less [11] which is close to the isoelectric point of thylakoid membranes [12,13], and hence presumably of the D-144 particles. Fig. 3A shows the effects of salt concentrations on the reduction of *P*-700$^+$ by cytochrome *c*-552 below the isoelectric point. Under these conditions we observe no stimulation following addition of cations, but only the inhibition at higher concentrations. As shown in

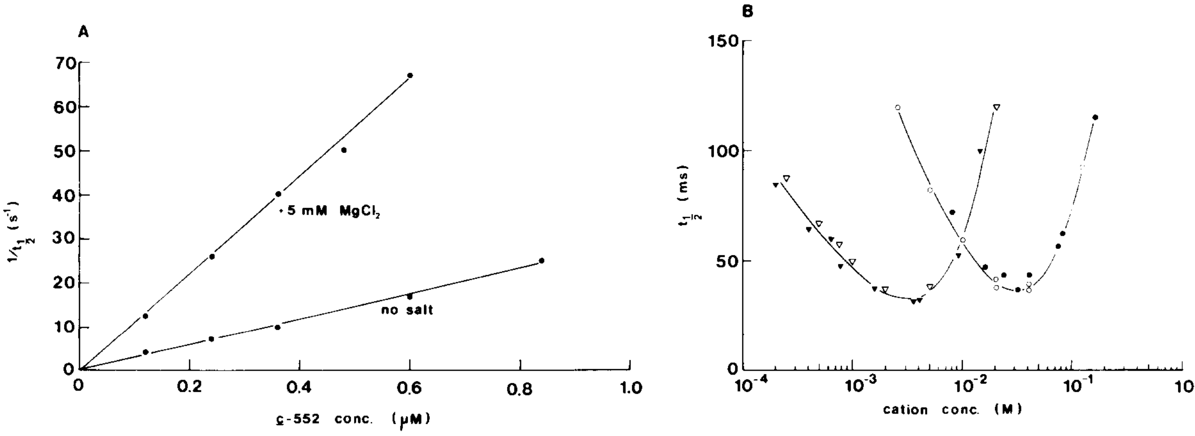

Fig. 1. Reduction of *P*-700$^+$ in D-144 particles by cytochrome *c*-552 from *Euglena*. (A) The reciprocal half-time of *P*-700$^+$ reduction plotted against the concentration of cytochrome *c*-552 (*c*-552) in the presence and absence of 5 mM $MgCl_2$. (B) The half-time of *P*-700$^+$ reduction is plotted against the concentration of salt added to the basic suspension medium in the presence of 0.24 μM cytochrome *c*-552. ○, KCl; ●, NaCl; ▽, $MgCl_2$; ▼, $CaCl_2$. Other conditions were as described in Materials and Methods.

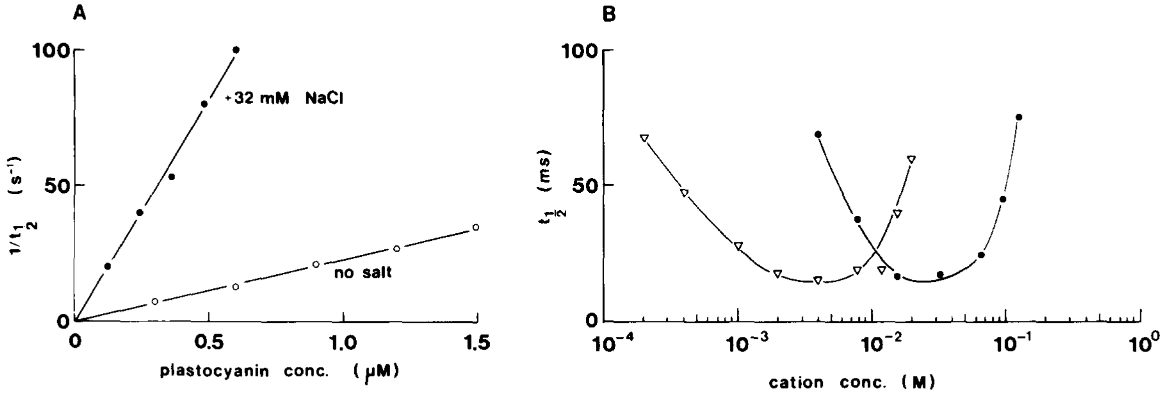

Fig. 2. Reduction of P-700$^+$ in D-144 particles by plastocyanin. (A) The reciprocal half-time of P-700$^+$ reduction plotted against the concentration of plastocyanin in the presence and absence of 32 mM NaCl. (B) The half-time of P-700$^+$ reduction is plotted against the concentrations of salt added to the basic suspension medium in the presence of 0.3 μM plastocyanin. ●, NaCl; ▽, $MgCl_2$. Other conditions were as described in Materials and Methods.

Fig. 3B, similar results are observed for mammalian cytochrome *c* at neutral pH where it carries a net positive charge [14].

Fig. 4 shows the effects of polybrene (a polycationic heparin antagonist) and polylysine on the reduction of P-700$^+$ by plastocyanin. The effects of these are very similar to the effects of mono- and divalent cations, since at low concentrations they stimulate the reaction whereas at high concentrations they inhibit it.

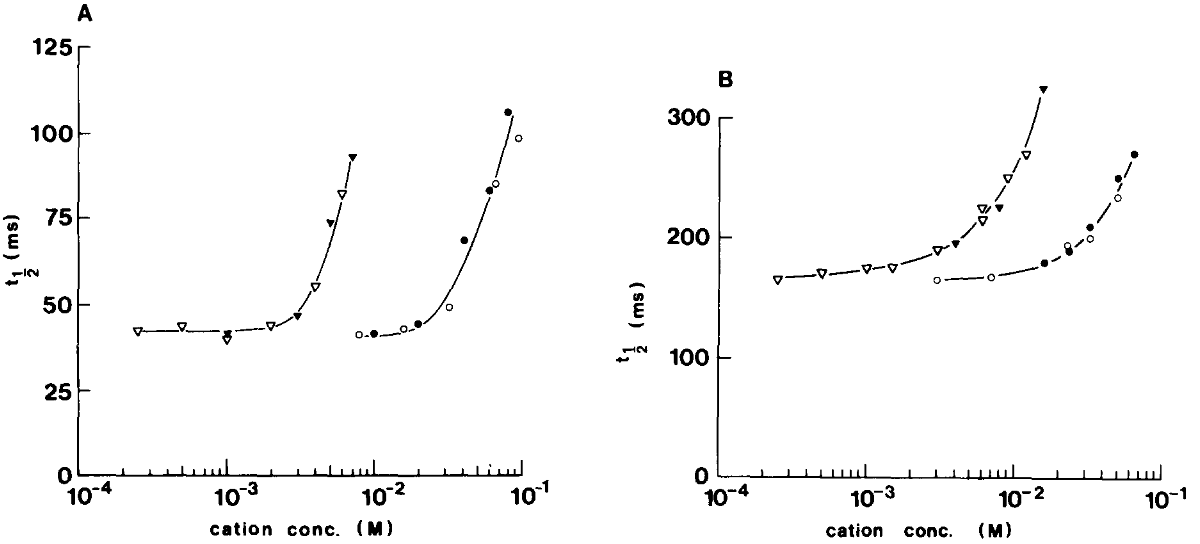

Fig. 3. Effect of mono- and divalent cations on the reduction of P-700$^+$ in D-144 particles by positively charged reductants. (A) Half-time of reduction of P-700$^+$ by *Euglena* cytochrome *c*-552 at pH 5.0 plotted against the concentration of salt added to the basic suspension medium. Other conditions were as given in the legend to Fig. 1B, except that 10 mM acetate-NaOH was present instead of Hepes, and the cytochrome *c*-552 concentration was 0.36 μM. (B) half-time of reduction of P-700$^+$ by horse heart cytochrome *c* at pH 7.5 plotted against the concentration of salt. Conditions as in Fig. 1B, except that 10 μM cytochrome *c* was present and tetrachlorobenzoquinone (20 μM) replaced diaminodurene. ○, KCl; ●, NaCl; ▽, $MgCl_2$; ▼, $CaCl_2$.

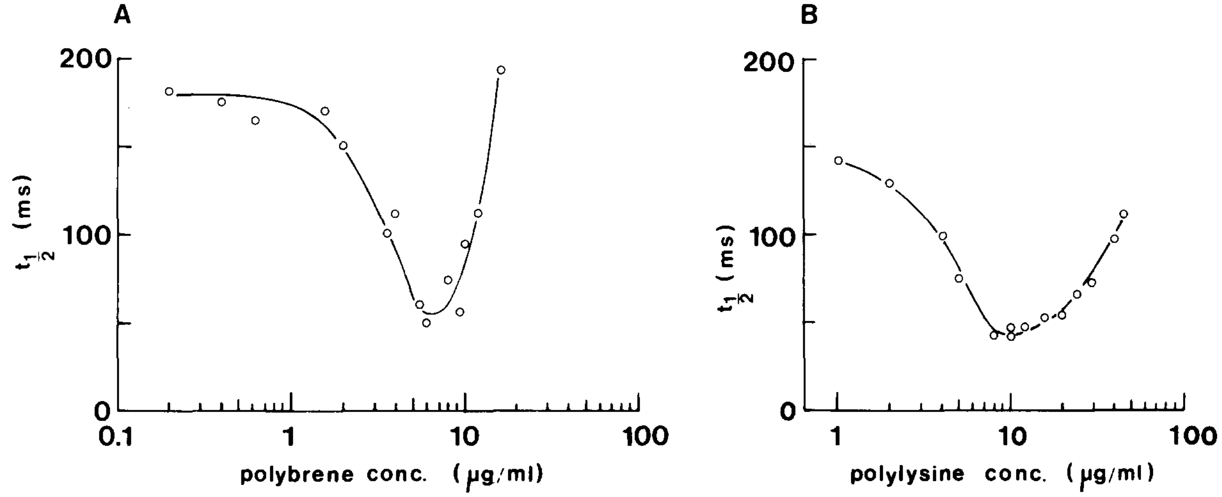

Fig. 4. Effect of polycations on the reduction of P-700$^+$ in D-144 particles by plastocyanin. Half-time of reduction of P-700$^+$ plotted against the concentrations of polybrene (A) or polylysine (B) added to the basic suspension medium. Other conditions were as given in Materials and Methods.

Cytochrome c-552 is easier to measure spectrophotometrically than plastocyanin, and it is therefore possible to compare its kinetics of oxidation with the kinetics of reduction of P-700$^+$. The results showed that the kinetics and the effects of cations on the reduction of P-700 and the oxidation of c-552 are identical (data not shown). The spectrum of the light-induced absorbance changes in the region 540–560 nm was in excellent agreement with the chemical difference spectrum of c-552 (oxidized minus reduced).

Discussion

The experiments in Figs. 1A and 2A demonstrate that P-700$^+$ reacts with cytochrome c-552 or plastocyanin in a second-order reaction. Both reactions are stimulated by low concentrations of cations, whereas high concentrations of cations inhibit. The maximal second-order rate constants that could be estimated for these reactions from the data in Figs. 1B and 2B are $9 \cdot 10^7$ M^{-1} · s^{-1} for cytochrome c-552 and $1.5 \cdot 10^8$ M^{-1} · s^{-1} for plastocyanin. The latter value agrees with those estimated by others [6,7]. The close agreement between the two rate constants supports the idea that cytochrome c-552 serves a similar function in *Euglena* chloroplasts to that of plastocyanin in chloroplasts from higher plants and demonstrates that the site of interaction between P-700 and its primary electron donor is not absolutely specific for plastocyanin.

It has been suggested that plastocyanin forms a complex with P-700 [5]. One of the arguments for such a complex has been that in intact chloroplasts, P-700$^+$ is reduced with kinetics that can be fitted to a sum of exponentials. However, we have previously suggested that the apparent biphasic kinetics of reduction of P-700$^+$ in broken chloroplast result from a second-order reaction between P-700$^+$ and plastocyanin [15]. The present results provide further support for this view, as can be demonstrated by the following calculation based on the evidence that plastocyanin is located in the intrathylakoid space [16].

Rottenberg et al. [17] have estimated the intrathylakoid volume of chloroplasts to be 8–10 l/mol chlorophyll for chloroplasts suspended in an isosmolar medium. If we assume stoichiometries of 600 chlorophylls per P-700, and a ratio of plastocyanin to P-700 of 1 : 1, we get an internal plastocyanin concentration of 170–200 µM. Using the second-order rate constant estimated here ($1.5 \cdot 10^8$ M^{-1} · s^{-1}), we can calculate that P-700$^+$ will be reduced by plastocyanin with a half-time of approx. 30 µs. The simulated time course for the bimolecular reaction under these conditions is shown in Fig. 5. The simulated kinetics show a close fit to the experimental results

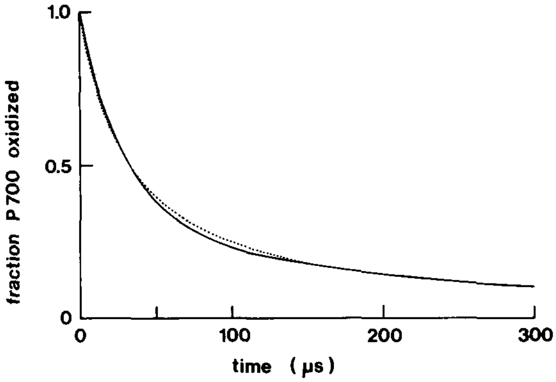

Fig. 5. Time course for the reduction of P-700$^+$ by plastocyanin in chloroplasts; (......) predicted time course for a bimolecular reaction between P-700$^+$ and plastocyanin using the rate constant, $1.5 \cdot 10^8$ $M^{-1} \cdot s^{-1}$, estimated from Fig. 2B. (———) sum of three exponentials with half-times of 20 µs, 140 µs and at least 5 ms and with the relative contributions of 67, 30 and 3%, respectively (data taken from Ref. 5, Fig. 2 and Table I).

reported by Haehnel et al. [5] for the reaction in chloroplasts. The good agreement between the observed rates in intact thylakoids and those predicted from a second-order mechanism supports the idea that the reaction mechanism is similar in our system with subchloroplast particles and in chloroplasts where the intrathylakoid plastocyanin concentrations are much higher.

Haehnel et al. [5] also investigated the effects of the inhibitors KCN and $HgCl_2$ and reported that these affected the 20-µs phase of reduction of P-700$^+$ by decreasing the amplitude without affecting the half-time. The effects of these inhibitors on the 200-µs phase were reported to be more complex as the amplitude of this phase was only slightly decreased wheras the half-time was increased. Haehnel et al. [5] concluded that these inhibitor effects could not be accounted for by a bimolecular reaction. However, if the effects of KCN and $HgCl_2$ are to decrease the amount of active plastocyanin [18] without affecting P-700, simulations of the expected time courses demonstrate that second-order kinetics of reduction of P-700$^+$ can still be fitted with good agreement to a sum of two exponentials. The expected effects of decreasing the amount of plastocyanin relative to P-700 are exactly as described by Haehnel et al. [5] for the effects of KCN and $HgCl_2$ when the second-order curves are deconvoluted to a sum of exponentials. We therefore conclude that there is no need to postulate the formation of a complex to explain the experimental results.

The results also confirm and extend previous observations that the interaction between plastocyanin and P-700 is affected by the cation composition of the suspension medium [6,19–22]. We find a similar effect of cations on the reaction of P-700$^+$ with cytochrome c-552 from *Euglena*. The lack of specificity within a valency group and the order of efficiency, $C^{n+} > ... > C^{2+} > C^+$, suggest that that the effect is electrostatic.

It has previously been shown [23–26] that a variety of salt-induced changes in chloroplast functions and the salt effect on the electron transfer reactions between electrically charged redox components and the components embedded in the thylakoid membrane may be explained in terms of the classical Gouy-Chapman theory for the electrical diffuse layer adjacent to the membrane surface (see Ref. 27 for a review on this subject). According to this theory the membrane surface potential, ψ_0, can be calculated for a solution containing a mixture of mono- and divalent cations from the equation [27]:

$$2C_b'' \cdot \cosh^2\left(\frac{F\psi_0}{RT}\right) + C_b' \cdot \cosh\left(\frac{F\psi_0}{RT}\right) - \left(2C_b'' + C_b' + 1/2\left(\frac{\sigma}{A}\right)^2\right) = 0 \qquad (1)$$

where C_b' and C_b'' are the concentrations of the mono- and divalent cations in the bulk solution, respectively, σ is the surface charge density (in $\mu C/cm^2$), A is equal to $(2RT\epsilon_r\epsilon_0)^{1/2}$ where ϵ_r is the dielectric constant of water and ϵ_0 is the permittivity of vacuum, and the other symbols have their usual meanings.

If the surface potential is calculated from this equation, taking the value for the surface charge density as -2.5 $\mu C/cm^2$, the data on the rates of P-700$^+$ reduction for monovalent and divalent cations from Figs. 1B and 2B are found to lie on the same curve, as shown in Fig. 6.

Tamura et al. [22] obtained a similar plot to that in Fig. 6 for the interaction of plastocyanin and P-700 in broken chloroplasts and sonicated chloroplasts supplemented with exogenous plastocyanin.

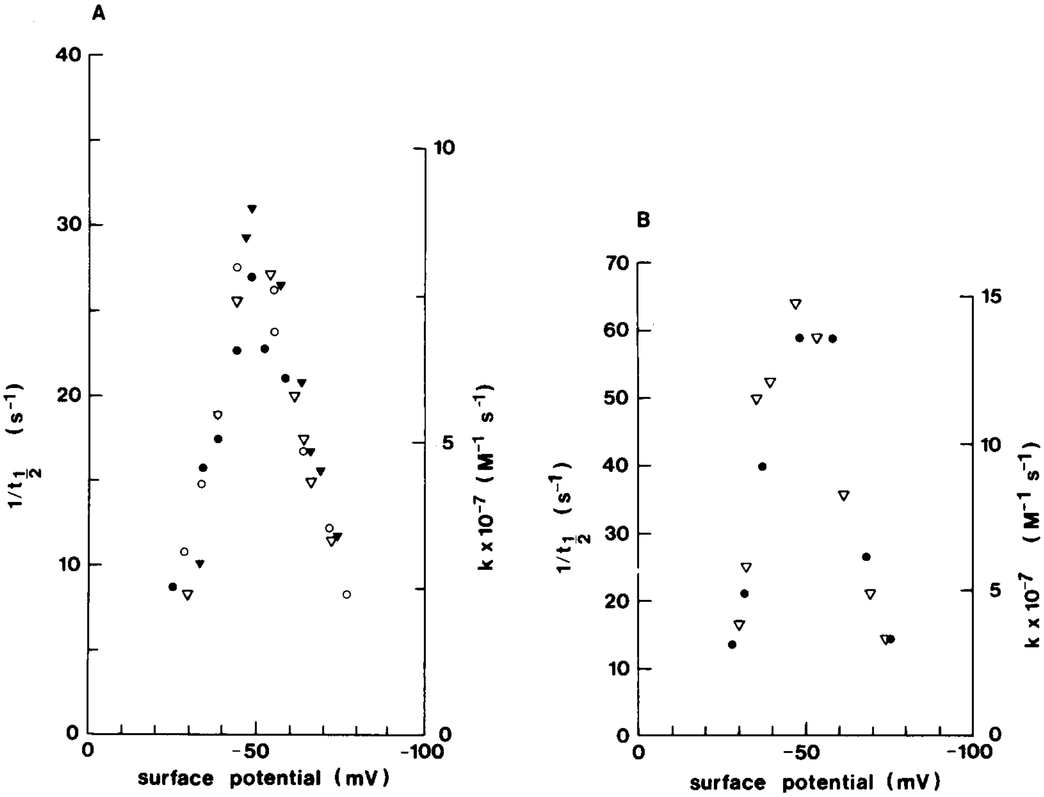

Fig. 6. Effect of the membrane surface potential on the reduction of P-700$^+$ by cytochrome c-552 (A) or by plastocyanin (B). The surface potential was calculated from Eq. 1 by using a surface charge density of -2.5 μC/cm^2 and the data from Figs. 1B and 2B.

However, the rates obtained by these authors, for the reaction between plastocyanin and P-700 in sonicated chloroiplasts, are an order of magnitude lower than those observed here (Fig. 6B). Tamura et al. [22] also observed a sigmoidal dependence of rate on plastocyanin concentration for this reaction in contrast to what is observed here. It is possible that the measuring light used by these authors was sufficiently high to have an actinic effect and hence that plastocyanin was partially oxidised when added at low concentrations. This and the fact that a suspension of sonicated chloroplasts constitutes a system where plastocyanin and P-700 may be in several environments may explain the very low rates and the nonlinear concentration dependence.

The stimulation of the rates at low concentrations of cations ($\psi_0 < -50$ mV) is that expected for the interaction of a negatively charged molecule with a negatively charged surface. However, the inhibition at high concentrations of cations ($\psi_0 > -50$ mV) is less readily understood. It is clear that the latter effect is observed with both negatively and positively charged reductants. It is interesting that the effects of mono- and divalent cations are also found with polycations. Polycations have long been known to inhibit chloroplast electron transport, when added to a suspension of broken chloroplasts [28], and the site of inhibition has been identified as that of the reactions involving plastocyanin [29,30]. Since polycations are unlikely to penetrate the membrane, it is possible that the inhibitory action of polycations in chloroplasts is an effect on the surface potential of the outer membrane surface, possibly inducing a conformational change in the PS I reaction centre complex. A similar phenomenon could possibly explain the results observed here with mono- and divalent cations. It is noteworthy in this context that addition of polylysine or high concentrations of mono- or divalent cations also inhibit the rapid reduction of P-700$^+$ by plastocyanin in broken chloroplasts suspended in a fluid medium at sub-zero temperatures (Olsen, L.F., unpublished data).

Acknowledgements

We thank Bodil Kristensen, Bodil Møller, Morten Nielsen and Jørgen Bang Nielsen for skilled technical assistance and Aase Reffstrup and Ann Lønholdt for typing the manuscript. L.F.O. is supported by a grant from the Danish Natural Science Research Council (J. No. 11-2107).

References

1 Haehnel, W., Döring, G. and Witt, H.T. (1971) Z. Naturforsch. 26b, 1171–1174
2 Haehnel, W. and Witt, H.T. (1972) in Proceedings of the 2nd Int. Congress on Photosynthesis (Forti, G., Avron, M. and Melandri, A., eds.), pp. 469–476, Junk, The Hague
3 Bouges-Bocquet, B. and Delosme, R. (1978) FEBS Lett. 94, 100–104
4 Haehnel, W., Biochim. Biophys. Acta 459, 418–441
5 Haehnel, W., Pröpper, A. and Krause, H. (1980) Biochim. Biophys. Acta 593, 384–399
6 Wood, P.M. and Bendall, D.S. (1975) Biochim. Biophys. Acta 387, 115–128
7 Haehnel, W., Hesse, V. and Pröpper, A. (1980) FEBS Lett. 111, 79–82
8 Anderson, J.M. and Boardman, N.K. (1966) Biochim. Biophys. Acta 112, 403–421
9 Plesničar, M. and Bendall, D.S. (1970) Biochim. Biophys. Acta 216, 192–199
10 Perini, F., Kamen, M.D. and Schiff, J.A. (1964) Biochim. Biophys. Acta 88, 74–90
11 Katoh, S. (1971) Methods Enzymol. 23, 408–413
12 Mercer, F.V., Hodge, A.J., Hope, A.B. and McLean, J.D. (1955) Aust. J. Biol. Sci. 8, 1–18
13 Nakatani, H.Y., Barber, J. and Forrester, J.A. (1978) Biochim. Biophys. Acta 504, 215–225
14 Barlow, G.H. and Margoliash, E. (1966) J. Biol. Chem Chem. 241, 1473–1477
15 Olsen, L.F., Cox, R.P. and Barber, J. (1980) FEBS Lett. 122, 13–16
16 Haehnel, W., Berzborn, R.J. and Andersson, B. (1981) Biochim. Biophys. Acta 637, 389–399
17 Rottenberg, H., Grunwald, T. and Avron, M. (1972) Eur. J. Biochem. 25, 54–63
18 Selman, B.R., Johnson, G.L., Giaquinta, R.T. and Dilley, R.A. (1975) J. Bioenerg. 6, 221–231
19 Gross, E.L. (1979) Arch. Biochem. Biophys. 195, 198–204
20 Lien, S. and San Pietro, A. (1979) Arch. Biochem. Biophys. 194, 128–137
21 Davis, D.J., Krogmann, D.W. and San Pietro, A. (1980) Plant Physiol. 65, 697–702
22 Tamura, N., Yamamoto, Y and Nishimura, M. (1980) Biochim. Biophys. Acta 592, 536–545
23 Barber, J., Mills, J.D. and Love, A. (1977) FEBS Lett. 74, 174–181
24 Itoh, S. (1979) Biochim. Biophys. Acta 548, 579–595
25 Itoh, S. (1979) Biochim. Biophys. Acta 548, 596–607
26 Olsen, L.F. and Cox, R.P. (1980) Photobiochem. Photobiophys. 1, 147–153
27 Barber, J. (1980) Biochim. Biophys. Acta 594, 253–308
28 Brand, J., Baszynski, T., Crane, F.L. and Krogmann, D.W. (1972) J. Biol. Chem. 247, 2814–2819
29 Brand, J., San Pietro, A. and Mayne, B.C. (1972) Arch. Biochem. Biophys. 152, 426–428
30 Olsen, L.F. and Cox, R.P. (1979) Eur. J. Biochem. 102, 139–145