Biochimica et Biophysica Acta, 504 (1978) 215—225

BBA 47542

SURFACE CHARGES ON CHLOROPLAST MEMBRANES AS STUDIED BY PARTICLE ELECTROPHORESIS

H.Y.NAKATANI [a], J. BARBER [a], and J.A. FORRESTER [b]

[a] *Department of Botany, Imperial College, London S.W.7, and* [b] *Chester Beatty Research Institute, London, S.W.3 (U.K.)*

(Received December 5th, 1977)

Summary

1. Particle microelectrophoresis mobility studies have been conducted with chloroplast thylakoid membranes and with isolated intact chloroplasts.

2. The pH dependence of the electrophoretic mobility indicated that at pH values above 4.3 both membrane systems carry a net negative charge.

3. Chemical treatment of thylakoids has shown that neither the sugar residues of the galactolipids in the membrane nor the basic groups of the membrane proteins having pK values between 6 and 10 are exposed at the surface.

4. However, treatment with 1-ethyl-3(3-dimethylaminopropyl)carbodiimide, together with glycine methyl ester, neutralized the negative charges on the thylakoid membrane surface indicating the involvement of carboxyl groups which, because of their pH sensitivity, are likely to be the carboxyl groups of aspartic and glutamic acid residues.

5. The nature of the protein giving rise to the negative surface charges on the thylakoids is not known but is shown not to involve the coupling factor or the light harvesting chlorophyll *a*/chlorophyll *b* pigment · protein complex.

6. No significant effect of light was observed on the electrophoretic mobility of either thylakoids or intact chloroplasts.

7. The striking difference in the ability of divalent and monovalent cations to screen the surface charges was demonstrated and explained in terms of the Gouy-Chapman theory.

8. Calculations of the ζ-potentials for thylakoid membranes gave values for the charge density at the plane of shear to be in the region of one electronic charge per 1500—2000 Å^2.

9. The significance of the results is discussed in terms of cation distribution in chloroplasts and the effect of cations on photosynthetic phenomena.

Abbreviations: DNFB, dinitrofluorobenzene; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EDC, 1-ethyl-3(3-dimethylaminopropyl) carbodiimide; chl, chlorophyll.

Introduction

A striking feature of chloroplast structure is the extent of the thylakoid membrane surface area in relation to the volume of the intrathylakoid and stromal compartments. Although these surface area to volume ratios will vary, simple estimates show that they are in the region of 10^6 cm^{-1} [1]. Therefore it is not surprising that membrane surface phenomena can influence photosynthetic processes. Over the years there have been many reports demonstrating that the thylakoid membrane, at neutral pH, carries excess negative charges [2—5]. A consequence of this will be an associated diffuse electrical layer containing sufficient cations to neutralize the negative charges on the surface. As explained in recent publications by Barber et al. [6,7] changes in the nature of the diffuse layer can control changes in chlorophyll fluorescence [8], the rate of electron transport [9], light scattering [10] and membrane stacking [11]. Rumberg [12] also recently pointed out that changes in surface charge can effect the extent of the 515 nm shift while changes in the ionic composition of the diffuse electrical layer, adjacent to the thylakoid membrane surface, can affect the action of various optical probes such as 9-amino acridine [13,14] and pH indicators [15].

Clearly there is a need to gain a better understanding of the density and nature of the surface charges which exist on the thylakoid membrane. To this end we have conducted a microelectrophoretic study of chloroplast membranes and the results are reported in this paper.

Materials and Methods

Intact chloroplasts were isolated from peas (Feltham first) and from market spinach, by a method recently described by Nakatani and Barber [16]. Thylakoid membrane preparations were obtained either by centrifugation of the supernatant after removal of the intact chloroplasts or by osmotic treatment of intact chloroplasts. In the case of barley chloroplasts the thylakoids were obtained by scraping the leaf surface with a razor blade with the leaves submerged in 0.33 M sorbitol/20 mM Tris at pH 7.6. The final suspension medium for all preparation was 0.11 M sorbitol brought to pH 7.6 with Tris. In order to remove residual divalent cations on the membranes the chloroplast thylakoids were washed with 0.1 M KCl in 0.11 M sorbitol, pH 7.6 with Tris, and washed twice with the sorbitol-Tris buffer to remove excess KCl. This washing procedure would also be expected to remove any loosely associated extrinsic membrane proteins.

The following chemical treatments were carried out to attempt to modify the surface charges on the thylakoid membranes:

(i) Treatment with dinitrofluorobenzene (DNFB). Thylakoid membranes (chl ~ 100 μg/ml) were incubated with 5 mM DNFB at pH 8.0 for 1 h at room temperature and washed twice with 0.11 M sorbitol-Tris, pH 7.6.

(ii) Treatment with 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) plus glycine methyl ester. Thylakoids (chl ~ 100 μg/ml) were initially treated with 0.1 M KCl to remove residual divalent cations and then washed. They were then incubated with 0.1 M EDC plus 0.2 M glycine methyl

ester at pH 4—4.5 (HCl was added to maintain pH). Control thylakoids were also incubated with glycine methyl ester. After incubation for 1 h in the dark at room temperature the membranes were washed twice with 0.11 M sorbitol at pH 6.0 with Tris-maleate.

The electrophoretic mobility measurements were conducted in a 6% (0.33 M) sorbitol support medium generally with 20 mM KCl and a chlorophyll concentration of 50 μg/ml. The instrument used was a Zeiss Cytopherometer, thermostated at 20 ± 0.5°C with a Churchill Chiller Thermoregulator, Model 02 CTCV. Constant applied currents were varied from 1 to 10 mA corresponding to voltage gradients of about 8—15 $V \cdot cm^{-1}$. Conductance measurements were done on a LKB Conductolyzer Model 5300B with a cell constant of 5.487 cm^{-1}. The pH was held constant by either one, or a combination of the following buffers: Tris, maleate, citrate and carbonate. For pH values less than 3, small amounts of HCl were added. None of these additions significantly altered the conductance of the solution.

Electrophoretic mobility (u) was calculated using the expression: $u = (d/t)/E$, where d = distance traversed by particle, t = time to travel distance d, E = electrical field strength, determined from the measured current and conductance of the suspending medium and cross-sectional area of the electrophoresis chamber.

For measurements made there was no need to correct for the particle shape or size since the thickness of the double layer (100 Å or less for ionic conditions used) is much less than the radius of curvature for the particles being studied. That is the κa value was large ($\kappa a > 300$) where $1/\kappa$, the Debye length, is the distance from the surface to a point where the value of the potential (ψ) is $1/e$ of the surface potential (ψ_0) (i.e., the double layer thickness) and a is the radius of the curvature of the surface. Under these conditions the diffuse layer is essentially flat such that the Smoluchowski equation (Eqn. 1) is applicable, assuming that the particle is non-conducting (ref. 7). Light intensity measurements were done with a YS1 model 65A Radiometer.

Results

1. Effect of pH

When isolated thylakoid membranes are placed in a conducting solution with a pH above 4.3 and subjected to an electric field they move towards the positive electrode indicating that they carry an excess of negative charges on their outer surface. As Fig. 1 shows, when the pH is dropped below 4.3 the chloroplasts change their direction of electrophoretic migration indicating that under these acidic conditions the negative charges are neutralized and that the membranes now carry an excess of positive charge. Thus this shows, in agreement with other work [2—4], that at physiological pH the thylakoid membrane surface is negatively charged. The independence of the mobility to pH from 6 to 10 suggests that the ionogenic groups involved are solely acidic, a result not expected for typical proteins. Similar results were obtained for Nitella chloroplasts by Mercer et al. [2] up to approx. pH 7.5.

Qualitatively the pH mobility relationship resembles that for erythrocytes [18] where the surface charge is principally conferred by sialic acid derivatives.

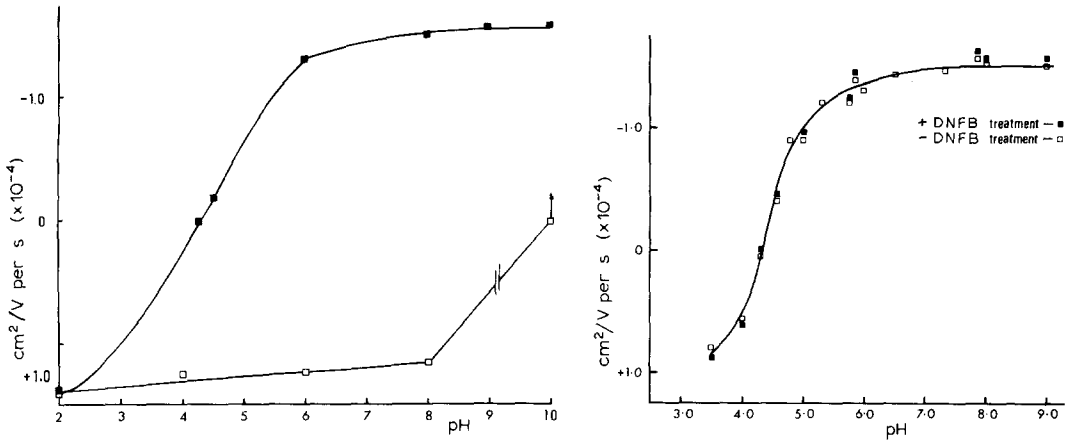

Fig. 1. pH electrophoretic mobility profile for pea chloroplast thylakoids (~50 μg chl/ml) plus (□) and minus (■) carbodiimide treatment (see Methods). The thylakoids were suspended in 0.33 M sorbitol and 20 mM KCl and pH was adjusted with minimal amounts of the following buffers: Tris base, maleate, citrate and carbonate. All measurements were conducted in a Zeiss cytopherometer at 20°C with voltages adjusted to maintain measurement times between 7—10 s per particle. The mean of 10—20 particles is represented by each point. Arrow indicates changing mobility suggesting hydrolysis of amide.

Fig. 2. pH electrophoretic mobility profile for pea chloroplast thylakoids plus and minus DNFB treatment. Conditions of measurements as in Fig. 1.

However, the pK of the ionization, which is the principal feature of the curve in Fig. 1, has a value of about 4.3, nearer to those for acidic amino acids (glutamic acid, γ-carboxyl pK = 4.25 and aspartic acid, β-carboxyl pK = 3.85) than that for sialic acids (pK ≈ 2.6). The isoelectric point would of course be the summation of all ionisable groups at the surface. However it seems that the basic groups of the membrane polypeptides are sterically unavailable for ionisation at the surface of shear or alternatively environmental constraints could alter their pK values and shift them to much higher values. A significant contribution of phosphate groups of phospholipids to the membrane charge seems unlikely since the majority of lipids found in the thylakoids are neutral with only about 8% being charged (3% phosphatidylcholine and 5% phosphatidylglycerol; pK values about 3, ref. 19).

The majority of the lipids in thylakoid membranes are galactolipids, 65% monogalactosyl diglyceride and 26% digalactosyl diglyceride [19]. To try and determine if these sugar residues are available at the membrane surface we studied the agglutinability of thylakoid preparations by a number of purified lectins at final lectin concentrations of up to 2 mg/ml. None of the lectins used (concanavalin A, wheat germ, soybean and peanut) brought about agglutination suggesting that sugar residues are not exposed at the surface.

2. Action of chemical modifiers

Thylakoid membranes were pretreated with a number of chemical modifiers in an attempt to clarify the nature of the species which give rise to the surface charges.

(a) DNFB. In addition to interacting with NH_2 groups this compound will also react with sulfhydryl, phenolic, imidazole and guanidino groups. As Fig. 2

TABLE I

ELECTROPHORETIC MOBILITIES OF PEA CHLOROPLAST THYLAKOID MEMBRANES AFTER VARIOUS TREATMENTS

EDTA treatment consisted of incubating the washed thylakoids (100 μg chl/ml) in 0.11 M sorbitol-Tris, pH 8.0 plus 1 mM EDTA for 20 min in ice and washed with 0.11 M sorbitol-Tris, pH 7.6. All measurements at pH 7.6 in 20 mM KCl plus 6% sorbitol. S.D. for minimum of 20 measurements. GME, glycine methyl ester.

	Electrophoretic mobility ± S.D. (cm^2/V per s)	
	Control	EDC + GME
Experiment 1	-1.50×10^{-4} (±0.09)	$+1.37 \times 10^{-4}$ (±0.09)
	Control	EDTA
Experiment 2	-1.67×10^{-4} (±0.11)	-1.54×10^{-4} (±0.05)

shows treatment with this compound at pH 8.0 did not alter the electrophoretic mobility of the thylakoid membrane. It therefore seems that basic groups with p*K* values below 10 do not contribute to the surface charge.

(b) Carbodiimides. The lack of affect of DNFB together with the insensitivity of the electrophoretic mobility to changes in pH between 6 and 10 suggests that the negative charges on thylakoid membranes are mainly due to acidic acids. We have attempted to modify the carboxyl groups of these acids using the carbodiimide (EDC) in the presence of glycine methyl ester as the nucleophile. Prochaska and Gross [20] have been successful in using EDC with chloroplasts and found optimal modifications with pH values below 6.0. We therefore decided to use this water-soluble carbodiimide in the presence of glycine methyl ester and to incubate at a low pH. The effect of this treatment on electrophoretic mobility is shown in Table I and Fig. 1. As can be seen charge reversal occurred over a range extending to pH 8.0 indicating that this treatment had brought about complete neutralization of the carboxyl groups. Above this pH the amide bond is subject to hydrolysis which probably accounts for the decrease in mobility at pH 10 shown in Fig. 1. The degree of charge reversal is comparable with that seen when the carboxyl groups are fully protonated. The origin of the positive charge which exists on the surface after treatment with EDC and glycine methyl ester or at low pH is not clear, but could arise from lysine and arginine, a possibility which is at present under study.

3. Origin of surface charges

The above results suggest that the negative charges on the thylakoid membrane surface are due to carboxyl groups and that the most likely candidates are glutamic and aspartic acid residues. Each of these amino acids makes up about 10% of the total thylakoid membrane protein [22] and could be in several different membrane bound proteins. As Table I shows treatment of the chloroplasts with 1 mM disodium EDTA for 20 min to remove the coupling factor did not significantly alter their electrophoretic mobility. Another possible origin for the charges would be the pigment · protein complexes which

TABLE II

ELECTROPHORETIC MOBILITIES OF WILD TYPE AND CHLOROPHYLL *b*-LESS MUTANT OF BARLEY

S.D. for minimum of 20 measurements in 20 mM KCl plus 6% sorbitol.

Condition	Electrophoretic mobility ± S.D. (cm^2/V per s)		
	Wild type	Mutant	Change (%)
pH 7.7	$-1.48 \cdot 10^{-4}$ (±0.07)	$-1.43 \cdot 10^{-4}$ (±0.09)	3.4
pH 6.6	$-1.34 \cdot 10^{-4}$ (±0.05)	$-1.29 \cdot 10^{-4}$ (±0.04)	3.7

serve as light harvesting systems for reaction centres. In particular in higher plants, 50% of the membrane protein is associated with the light harvesting chlorophyll *a*/chlorophyll *b* pigment · protein complex [21]. Moreover this major protein is often implicated with cation induced conformational changes in the membrane which affect the yield of chlorophyll fluorescence [22]. For this reason we have compared the electrophoretic mobility of normal thylakoids with those not having the light harvesting chl *a*/chl *b* · protein complex. The membranes were obtained from the wild type and chlorophyll *b* deficient mutant barley (chlorina-f2 mutant of *Hordeum vulgare* L, ref. 24). The mutant not only fails to make the pigments but also does not produce the associated polypeptides [24–26]. Even so, as Table II shows, the membranes from the mutant were only slightly less mobile (~4%) than those from the wild type which does not correlate with the considerable reduction of the chl *a*/chl *b* pigment · protein and suggests a highly hydrophobic environment for this complex.

4. Effect of light and the electrophoretic mobility of intact chloroplasts

Nobel and Mel [3] reported that a 15% increase in electrophoretic mobility was observed when chloroplasts were illuminated. However, we carried out intensive efforts to repeat their observations using isolated thylakoids but found little or no difference in changing from a dim green illumination (40 J/m^2 per s, taken as dark) to a bright red illumination (2000 J/m^2 per s), see Table III. We observed no effect of DCMU. Nobel and Mel stated that they

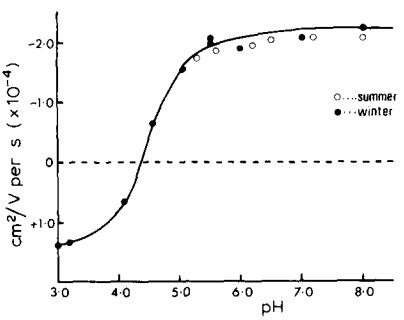

Fig. 3. pH electrophoretic mobility profile for intact pea chloroplasts, summer and winter material. Conditions of measurements as in Fig. 1.

TABLE III

ELECTROPHORETIC MOBILITIES OF THYLAKOID AND INTACT CHLOROPLASTS UPON ILLUMINATION

Particles suspended in 0.33 M sorbitol, 20 mM KCl, pH 8.0 (20 mM Tris added where indicated) and measured at about 25 μg chl/ml. "Dark" was green light illumination of about 40 J/m^2 per s and "Light" was red light of approximately 2000 J/m^2 per s or white light 2500 J/m^2 per s. S.D. for minimum of 20 measurements. MV, methyl viologen.

Condition	Type of chloroplasts	Electrophoretic mobility ± S.D. (cm^2/V per s)
Dark	Thylakoid (peas)	$-1.62 \cdot 10^{-4}$ (±0.12)
Red light + 50 μM MV		$-1.61 \cdot 10^{-4}$ (±0.07)
Dark	Intact pea chloroplasts	$-1.95 \cdot 10^{-4}$ (±0.03)
Red light		$-1.98 \cdot 10^{-4}$ (±0.03)
Dark	Intact spinach chloroplasts	$-2.20 \cdot 10^{-4}$ (±0.11)
White light		$-2.21 \cdot 10^{-4}$ (±0.10)
Dark (+20 mM Tris · HCl)		$-2.06 \cdot 10^{-4}$ (±0.10)
White light (+20 mM Tris · HCl)		$-2.03 \cdot 10^{-4}$ (±0.10)

had conducted their experiments using intact chloroplasts [3]. We have, for this reason, checked the electrophoretic mobility of isolated intact pea chloroplasts retaining their outer membranes. Fig. 3 shows the pH profile for this type of preparation and as can be seen it is remarkably similar to the results obtained for thylakoids. Like the thylakoid membranes intact spinach and pea chloroplasts showed no significant light sensitivity (see Table III).

5. Effect of monovalent and divalent cations

Cation effects have been observed for many photosynthetic phenomena and their action seems to be via the diffuse electrical layer resulting from fixed negative surface charges [7]. The ability of divalent cations to screen the surface charges (reduce the surface potential) in preference to monovalent cations, as demonstrated by a decrease in electrophoretic mobility, is shown in

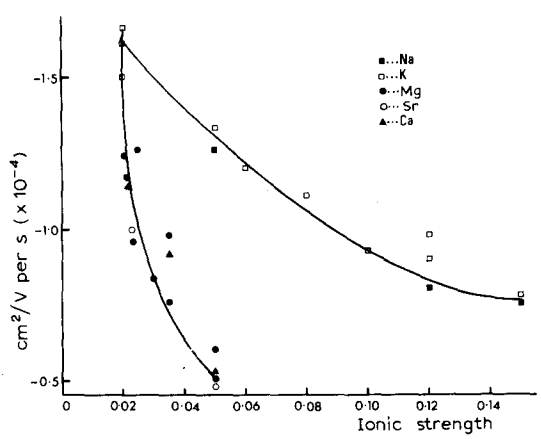

Fig. 4. Effect of monovalent and divalent cations on electrophoretic mobility of pea chloroplast thylakoids. Thylakoids (~50 μg chl/ml) suspended in 0.33 M sorbitol-Tris, pH 7.6. Divalent cation concentration added upon background monovalent cation concentration of 20 mM.

Fig. 4. Clearly this result shows that the decrease in mobility due to screening is not dependent on the chemical nature of the cations used nor is it totally dependent on the changes in ionic strength. The effect is more closely linked to the charge carried by the cation.

Discussion

It should be emphasized that microelectrophoresis studies only give information about the summation of the charges at the plane of shear. Moreover this type of measurement does not take into account the inevitable heterogenic nature of the membrane surface with regard to charge distribution. Nevertheless the results demonstrate that the outer surface of the thylakoid membrane is negatively charged at physiological pH and that the negative charge is probably due to the carboxyl groups of aspartic and glutamic acid. Since the thylakoid membrane contains very few charged lipids it is unlikely that phosphate groups of phospholipids play any significant role in the overall electrical property of the surface. This is supported by the fact that modification of the carboxyl groups, by carbodiimide-mediated amide bond formation, can neutralize the surface negative charges. Although detailed chemical modification studies have not been conducted with intact chloroplasts it does seem that the outer membrane of the envelope shares characteristics similar to those of the thylakoid membrane, an observation which needs further investigation. The origin of the surface charges on the thylakoid membrane do not seem to involve either the coupling factor protein or the light harvesting chlorophyll *a/b* pigment · protein. The lack of involvement of the latter is particularly striking since it can represent 50% of the membrane protein and does contain the acidic amino acids at a comparable level with the other thylakoid membrane proteins [21]. It seems that this pigment complex is not extensively exposed at the membrane surface which is consistent with the hydrophobic nature of the isolated protein complex [24] and with freeze-fracture studies [22].

From the value of the mobility it is possible to calculate from the Smoluchowski equation (Eqn. 1) the zeta-potential and then estimate the surface charge density. The zeta-potential is the potential difference at the surface of shear relative to the bulk medium and can be related to the electrophoretic mobility (u) in the following way [27]:

$$\zeta = \frac{4\pi\eta u}{\epsilon} \tag{1}$$

where η is the viscosity of the suspending medium and ϵ is the permittivity of water.

The charge density at the membrane surface (σ) can be estimated if it is assumed that surface potential (ψ_0) is equal to ζ. That is assuming that the plane of shear is at the membrane surface. The appropriate expression is derived from the Gouy-Chapman theory [26—28]:

$$\sigma = \pm\left[\frac{RT\epsilon}{2\pi}\sum C_{i\infty}\left(\exp\left(\frac{-Z_i F\psi_0}{RT}\right)-1\right)\right]^{1/2} \tag{2}$$

where R is gas constant, T absolute temperature and $C_{i\alpha}$ concentration of ion in bulk solution, F the Faraday and Z is the charge of the electrolyte.

Using a medium containing 20 mM KCl at pH 7.0, values of u were in the region of -1.5 to $-2.0 \cdot 10^{-4}$ cm$^2 \cdot$ V$^{-1} \cdot$ s^{-1} which gives calculated ζ-potentials of -25 to -33 mV. These values correspond to surface charge densities of 0.82–1.13 μC/cm^2 which corresponds to approx. one electronic charge per 2000–1500 Å^2 respectively in agreement with the earlier measurements of Mercer et al. [2]. As pointed out by Haydon [31] this method almost certainly underestimates the surface charge density since it assumes that $\zeta = \psi_0$ thus ignoring the existence of counterions which are adsorbed on the surface constituting the Stern layer and the portion of the Gouy-Chapman layer within the plane of shear. From other types of studies a value of σ has been estimated to be 2.5 μC/cm^2 corresponding to one electronic charge per 625 Å^2 (see refs. 6–8, 13).

When chloroplasts are illuminated charge separation occurs at the reaction centres of Photosystems I and II. This charge separation is thought to occur across the thylakoid membrane with the electron acceptors on the outer surface. That is, assuming that the negative charge is exposed at the surface, two additional electronic charges would appear on illumination over the area corresponding to one complete electron transfer chain. Witt has stated that one electron transport chain corresponds to an area of 10^5 Å^2 (ref. 32). Therefore the light-induced increase of surface charge would only represent about 1% and account for our inability to detect a significant light-induced change in electrophoretic mobility of thylakoid membranes. However, more sensitive techniques can detect light-induced changes in surface charge densities [33,34].

Applying the Gouy-Chapman theory to a complex surface such as thylakoid membrane involves making many assumptions (see ref. 7). Nevertheless the theory can qualitatively predict the differential screening effects observed with monovalent and divalent cations. Using Eqn. 2 it is possible to calculate changes in ψ_0 as a function of concentration. When symmetrical monovalent salts are used the expression is reduced to:

$$\sigma = 2A\, C'^{1/2}_{\alpha} \sinh\left(\frac{F\psi_0}{2RT}\right) \tag{3}$$

where C'_{α} is the concentration of the monovalent salt (Z^+—Z^-) in the bulk solution, and $A = (RT\epsilon/2\pi)^{1/2}$.

When a symmetrical divalent salt is used, remembering that the background solution contained 20 mM KCl, it is necessary to expand Eqn. 2 to take account of the mixed electrolytes.

$$\sigma = A\left[2C'_{\alpha}\cosh\left(\frac{F\psi_0}{RT}\right) - 2C'_{\alpha} + 4C''_{\alpha}\cosh^2\left(\frac{F\psi_0}{RT}\right) - 4C''_{\alpha}\right]^{1/2} \tag{4}$$

where C''_{α} is the bulk concentration of the divalent salt. Fig. 5 compares the calculated changes in ψ_0 as a funtion of C'_{α} and C''_{α} using $\sigma = 2.5$ μC/cm^2 with the experimental results on electrophoretic mobility as a function of cation concentration. Qualitatively the theory predicts the observed screening

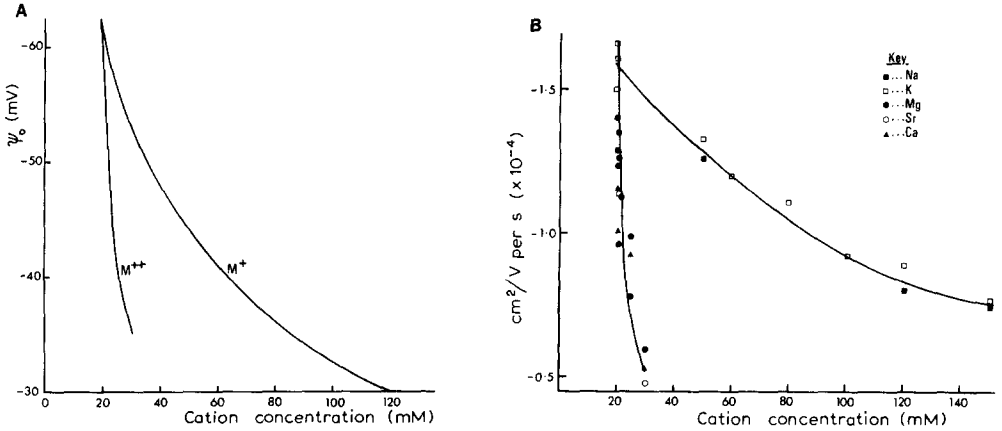

Fig. 5. (A) Theoretical curves for surface potential as function of monovalent and divalent salt concentration obtained from Eqns. 3 and 4 with $\sigma = 2.5$ $\mu C/cm^2$ (see text). (B) Electrophoretic data of Fig. 4 plotted as a function of monovalent and divalent cation concentration for comparison with the theoretical curve in (A).

effect although the monovalent cations seem less effective than expected. Similar calculations using the observed ζ-potential as the surface potential gave essentially identical results (not shown). These results emphasise that the electrophoretic mobility observed was mainly controlled by the surface potential and not by the shape or size of the particles.

The above experiments have focused attention on the existence and properties of the negative charges on the outer surface of the thylakoid membrane. Although estimates of the surface charge density have been made above, there is every reason to believe that the value of this parameter will not be constant under certain conditions since changing the ionic composition of the medium may also alter the H^+ concentration at the membrane surface sufficiently to affect the protonation of some of the charges. Moreover, as demonstrated by the calculations using Eqns. 3 and 4 and emphasised previously [6,7,13,14], divalent cations are drawn to the surface in preference to monovalent cations. This is of special interest since in the intact chloroplasts of peas the levels of the major cations K^+ and Mg^{2+} are about the same [1,35] indicating that Mg^{2+} would be the preferred cation in the diffuse layer adjacent to the membrane [35]. The ability of divalent cations to screen the surface negative charges at much lower concentrations than monovalent cations can explain properties of the cation induced thylakoid stacking phenomena and membrane conformational changes leading to changes in the yield of chlorophyll fluorescence (see ref. 7). A slight seasonal effect on electrophoretic mobility of thylakoids was observed and is thought to be due to the difference in the ability of the 0.1 M KCl washing procedure (see Methods) to remove all the Mg^{2+} from the membrane surface. Variations in the ability of monovalent cations to displace residual Mg^{2+} from thylakoids have been detected directly by neutron activation analyses (Nakatani, H.Y. and Barber, J., unpublished) as well as being deduced from indirect methods [36,37]. Finally it is worth mentioning that there is every reason to believe that the inner side of the thylakoid membrane also has an excess of negative charges at neutral pH [38].

Unlike the outer surface these charges are likely to become protonated as the intrathylakoid pH drops during illumination [37,38]. The effect would be to alter the difference between the inner and outer surface potentials and induce a change in the electric field across the membrane itself, a point already noted by Rumberg [12].

Acknowledgements

The work was supported by the Science Research Council and by the EEC Solar Energy Research and Development Program. We thank Philip Thornber for the mutant and wild type barley seeds and for valuable discussion.

References

1 Barber, J. (1977) in Bioenergetics of Membranes (Packer, L., Papageorgiou, G.C. and Trebst, A., eds.), pp. 459—469, Elsevier, Amsterdam
2 Mercer, F.V., Hodge, A.J., Hope, A.B. and McLean, J.D. (1955) Aus. J. Biol. Sci. 8, 1—18
3 Nobel, P.S. and Mel, H.C. (1966) Arch. Biochem. Biophys. 113, 695—702
4 Berg, S., Dodge, S., Krogmann, D.W. and Dilley, R.A. (1974) Plant Physiol. 53, 619—627
5 Gross, E.L. and Hess, S. (1974) Biochim. Biophys. Acta 339, 334—346
6 Barber, J. and Mills, J. (1976) FEBS Lett. 68, 288—292
7 Barber, J., Mills, J. and Love, A. (1977) FEBS Lett. 74, 174—181
8 Mills, J. and Barber, J. (1978) Biophys. J. 21, 257—272
9 Walz, D., Schuldiner, S. and Avron, M. (1971) Eur. J. Biochem. 22, 439—444
10 Vandermeulen, D.L. and Govindjee (1974) Biochim. Biophys. Acta 368, 61—70
11 Gross, E.L. and Prasher, S.H. (1974) Arch. Biochem. Biophys. 164, 460—468
12 Rumberg, B. (1977) In Encyc. Plant Physiol., (Trebst, A. and Avron, M., eds.), Vol. 5, pp. 405—415, Springer-Verlag, Berlin
13 Searle, G.F.W., Barber, J. and Mills, J. (1977) Biochim. Biophys. Acta 461, 413—425
14 Searle, G.F.W. and Barber, J. (1978) Biochim. Biophys. Acta, in press
15 Junge, W. (1977) Ann. Rev. Plant Physiol. 28, 503—536
16 Nakatani, H.Y. and Barber, J. (1977) Biochim. Biophys. Acta 461, 510—512
17 Shaw, D.J. (1966) Introduction to Colloid and Surface Chemistry p. 117—136, Butterworth, London
18 Seaman, G.V.E. and Cook, G.M.W. (1965) in Cell Electrophoresis (Ambrose, E.J., ed.), pp. 48—65, J. and A. Churchill, London
19 Leech, R.M. and Murphy, D.J. (1976) in The Intact Chloroplast Vol. 1 Topics in Photosynthesis (Barber, J., ed.), pp. 365—401, Elsevier, Amsterdam
20 Prochaska, L.J. and Gross, E.L. (1975) Biochim. Biophys. Acta 376, 126—135
21 Thornber, J.P. (1975) Ann. Rev. Plant Physiol. 26, 127—158
22 Arntzen, C.J., Armond, P.A., Briantais, J.M., Burke, J.J. and Novitzky, W.P. (1976) Brookhaven Symp. Biology 28, 316—336
23 Highkin, H.R. (1950) Plant Physiol. 25, 294—306
24 Thornber, J.P. and Highkin, H.R. (1974) Eur. J. Biochem. 41, 109—116
25 Anderson, J.M. and Levine, R.P. (1974) Biochim. Biophys. Acta 333, 378—387
26 Machold, O., Meister, A., Sagromsky, H., Hoyer-Hansen, G. and von Wettstein, D. (1977) Photosynthetica 11, 200—206
27 James, A.M. (1957) in Progress in Biophysics and Biophysical Chemistry (Butler, J.A.V. and Katz, B., eds.), Vol. 8, pp. 96—142, Pergamon, London
28 Gouy, G. (1910) Ann. Phys. (Paris) Ser. (4) 9, 457—468
29 Chapman, D.L. (1913) Phil. Mag. 25, 475—481
30 Delahay, P. (1965) Double Layer and Electrode Kinetics, Wiley, New York
31 Haydon, D.A. (1961) Biochim. Biophys. Acta 50, 450—457
32 Witt, H.T. (1972) Quart. Rev. Biophys. 4, 365—477
33 Barber, J. (1977) in Fertilizer use and Production of Carbohydrates and Lipids, 13th Colloq. Int. Potash Inst., pp. 67—77
34 Telfer, A., Barber, J. and Nicolson, J. (1975) Biochim. Biophys. Acta 396, 301—309
35 Mills, J., Telfer, A. and Barber, J. (1976) Biochim. Biophys. Acta 440, 495—505
36 Barber, J. (1976) in The Intact Chloroplast Vol. 1, Topics in Photosynthesis (Barber, J., ed.), pp. 89—134, Elsevier, Amsterdam
37 Witt, H.T. and Zickler, A. (1973) FEBS Lett. 37, 307
38 Fowler, C.F. and Kok, B. (1974) Biochim. Biophys. Acta 357, 308—318