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SALT-INDUCED pH CHANGES IN SPINACH CHLOROPLAST SUSPENSION

CHANGES IN SURFACE POTENTIAL AND SURFACE pH OF THYLAKOID MEMBRANES

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Summary

A pH decrease in chloroplast suspension in media of low salt concentration was observed when a salt was added at pH values higher than 4.4, while at lower pH values a pH increase was observed. The salt-induced pH changes depended on the valence and concentration of cations of added salts at neutral pH values (higher than 4.4) and on those of anions at acidic pH values (lower than 4.4). The order of effectiveness was trivalent > divalent > monovalent. The pH value change by salt addition was affected by the presence of ionic detergents depending on the sign of their charges. These characteristics agreed with those expected from the Gouy-Chapman theory on diffuse electrical double layers. The results were interpreted in terms of the changes in surface potential, surface pH and the ionization of surface groups which result in the release (or binding) of H^+ to (or from) the outer medium.

The analysis of the data of KCl-induced pH change suggests that the change in the surface charge density of thylakoid membranes depends mainly on the ionization of carboxyl groups, which is determined by the surface pH. When the carboxyl groups are fully dissociated, the surface charge density reaches $-1.0 \pm 0.1 \cdot 10^{-3}$ elementary charge/square Å.

Dependence of the estimated surface potential on the bulk pH was similar to that of electrophoretic mobility of thylakoid membrane vesicles.

Introduction

Chloroplast thylakoid membranes have ionizable groups due to proteins and lipids on their surfaces [1—4] and are negatively charged at neutral pH

[5—7]. At the charged membrane surface, electrical potential is known to be different from that in the bulk aqueous phase far from the membranes. Due to the potential difference (surface potential), ionic concentrations at the surface are not the same as those in the bulk aqueous phase [8]. At the negatively charged surface, cation concentrations become higher while anion concentrations become lower than those in the bulk phase. The surface potential of chloroplast thylakoid membranes has been pointed out as being an important parameter in determining the level of chlorophyll *a* fluorescence [9], the extent of 515 nm absorbance change [10] and the reactivity of redox reagents with electron transfer components [11,12]. According to the Gouy-Chapman theory, the surface potential can be obtained if the concentrations of ions in the bulk phase and the charge density on the surface are known. Negatively charged membrane surfaces can also attract hydrogen ions. Thus the surface pH of the thylakoid membranes is expected to be different from the bulk pH, depending on the salt concentration and the surface charge density. When the surface charges are screened by adding salts (decrease in the extent of surface potential), the surface pH will approach the bulk pH. The shift in surface pH will alter the number of ionized groups on the thylakoid surface (change in surface charge density).

In this paper salt-induced pH changes of chloroplast suspension were studied under various conditions. It was concluded that the amount of protons released from (or bound to) the membrane surface can be explained by the change in the degree of ionization of the surface carboxyl groups, which was caused by the salt-induced surface potential and surface pH changes.

Materials and Methods

Chloroplasts were prepared by rupturing spinach leaves in a Waring Blendor with a medium of 0.4 M sucrose, 50 mM Tris-HCl (pH 7.4) and 30 mM NaCl. After being passed through eight layers of cheese cloth, the homogenate was centrifuged at 400 × *g* for several seconds to remove cell debris and larger fragments. The supernatant was centrifuged at 2000 × *g* for 10 min. The pellet was washed twice with a medium of 0.1 M sucrose, 1 mM NaCl and 0.01 mM $MgSO_4$ and then suspended in it. Chlorophyll concentration in the reaction mixture was approx. 40 μg chlorophyll/ml which corresponded to 30-fold dilution of the stock suspension.

For the sonicated preparation, the broken chloroplasts in the medium were treated at 20 kHz with the power of 140 W for 180 s. The sonicated chloroplasts were diluted as above and used for the salt-addition experiments.

pH change in chloroplast suspension on salt addition was measured with a glass electrode in a cuvette containing 2.5 ml of the suspension under continuous stirring at 25°C. Initial pH values were adjusted by adding 0.1 N NaOH or HCl. Salts were added in a small volume of dense solution with a 25-μl syringe. Addition of the same volume of deionized water did not induce any pH change. The number of hydrogen ions liberated from chloroplasts on salt addition was calibrated from the pH change by adding an aliquot of 0.01 N HCl, corrected for the change observed upon the salt addition without chloroplasts. The salt-induced pH change was also measured spectrophotometrically by the absorbance change of bromocresol purple at 590 nm with a reference wavelength at 490 nm using a Hitachi 356 dual wavelength spectrophotometer.

The electrophoretic mobility of broken chloroplasts was measured by microelectrophoresis equipment with a Briggs cell (Mitamura Riken Kogyo Co.) at 25°C in a solution containing 5 mM sodium barbital and 5 mM sodium acetate buffer. The voltage applied was regulated at 200 V. The current was in the range of 0.9—1.4 mA (3.3—3.5 mA with 200 mM NaCl solution).

Electrofocusing electrophoresis was done with the carrier ampholyte of the pH range 3—6 in a LKB 8100 ampholine electrofocusing equipment (110 ml column). Broken chloroplasts (12 μg chlorophyll) were applied to the column at the center. To check the effect of gravity, the electrodes were set either regularly (cathode at the top of the column) or inversely.

Results

Salt-induced pH changes of chloroplast suspension

Addition of salts to the chloroplast suspension induced pH changes (Fig. 1, lower traces) which were significantly larger than the small electrode response in the absence of chloroplasts (Fig. 1, upper traces). The traces were stabilized within a few minutes of the salt addition. Traces after 3 min were extrapolated to the zero time of salt addition to estimate the amount of H^+ change (ΔH^+). The amount of H^+ changes by addition of salt to the chloroplast suspension was corrected for the charge without chloroplasts. All the experiments were done at pH values lower than 6.0 to avoid artifacts due to the apparent electrode response to the salt addition which became more significant at higher pH values. There was a linear relationship between salt-induced ΔH^+ (by $MgCl_2$ addition, final 8 mM) and chlorophyll concentration up to at least 200 μg/ml at pH 5.7. The extent of the salt-induced ΔH^+ was affected neither by sonic disrupture of chloroplasts nor by osmolarity change with sucrose up to 0.6 M. These facts show that the salt-induced ΔH^+ is independent of the higher order organization of thylakoids. Similar pH changes were also observed in the salt-induced absorbance changes of bromocresol purple.

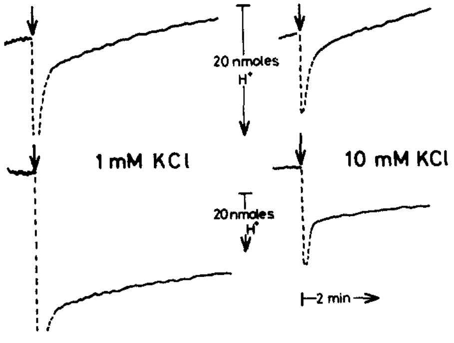


Fig. 1. Traces of pH changes induced by addition of salt. A small volume of concentrated KCl solution (2.5 M, 10 μl) was added at the points indicated by arrows to chloroplast suspension or medium (2.5 ml) at pH 5.60. Initial KCl concentrations were 1 mM (left traces) and 10 mM(right traces). Upper traces were controls without chloroplasts and lower ones contained chloroplasts at 50 μg chlorophyll/ml.

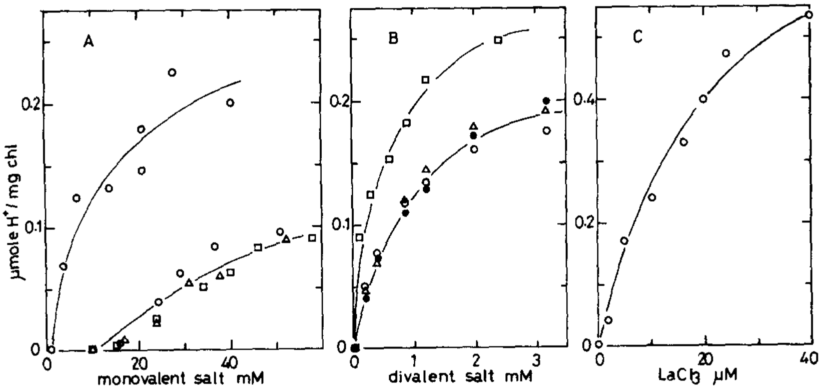
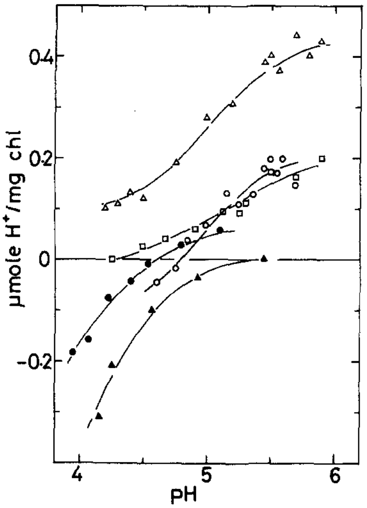


Fig. 2. Effect of mono-, di- and trivalent cations on salt-induced pH decrease in chloroplast suspension. Chloroplasts were suspended in 10 mM KCl except in one of the KCl titrations in which chloroplasts were suspended in 1 mM KCl. Final concentrations of added salt are indicated on the abscissa. Other conditions were the same as in Fig. 1. A. Salts of monovalent cations. ○, KCl (upper curve, chloroplast suspension in 1 mM KCl; lower curve, in 10 mM KCl); △, NaCl; □, choline chloride. B. Salts of divalent cations. ○, $MgCl_2$; ●, $MgSO_4$; △, $CaCl_2$; □, $MnSO_4$. C. $LaCl_3$.

Effectiveness of salts on pH change of chloroplast suspension

Salt-induced ΔH^+ depended on the valence of cations of salts at pH 5.7 (Fig. 2). The concentrations of salts required to induce release of 0.1 µmol H^+/mg chlorophyll from chloroplasts suspended in 10 mM KCl were 40—50 mM, 0.6 mM and 3 µM for salts of monovalent ions (K^+, Na^+, choline$^+$), divalent ions (Mg^{2+} and Ca^{2+} except for Mn^{2+}) and trivalent ion (La^{3+}), respectively. These correspond to an almost 80-fold difference between monovalent and divalent salts and a 200-fold difference between divalent and trivalent (La^{3+}) salt in effectiveness. No specificity among the monovalent cations (Na^+, K^+, choline$^+$) and divalent cations (Ca^{2+}, Mg^{2+} except for Mn^{2+}), respectively, was observed in inducing pH decrease at neutral pH. The salt-induced pH decrease was also independent of the anion species.

pH dependence of the salt-induced ΔH^+ in the cases of salts of divalent and trivalent ions is shown in Fig. 3. At pH values higher than approx. 4.4, salt-induced H^+ release was generally observed. The cases with $LaCl_3$ and potassium ferricyanide, which were somewhat different from the general pattern, will be discussed later. The effectiveness of the salts depended on the valence of constituting cations. The sets of Na^+ and Ca^{2+} gave results similar to those with salts of K^+ and Mg^{2+}, respectively (data not shown). The higher the valence of the cation, the more the extent of H^+ release irrespective of the anion constituents. At pH values lower than approx. 4.4 the converse held true. However, near the isoelectric point the relationship was less clear than that at the pH values far from p*I*. Near p*I*, i.e., with low charge densities, the specificity of ions may become more prominent in the interaction with membranes. In the acidic pH range, H^+ binding was observed on addition of salts. Effectiveness of the salts in inducing the H^+ binding depended on the valence of anions of the salts. The higher the negative valence, the more the H^+ binding. These data indicate that the salt-induced pH changes are due to the electrical effect rather than the chemically specific process [11,13], especially at pH values far from p*I*.



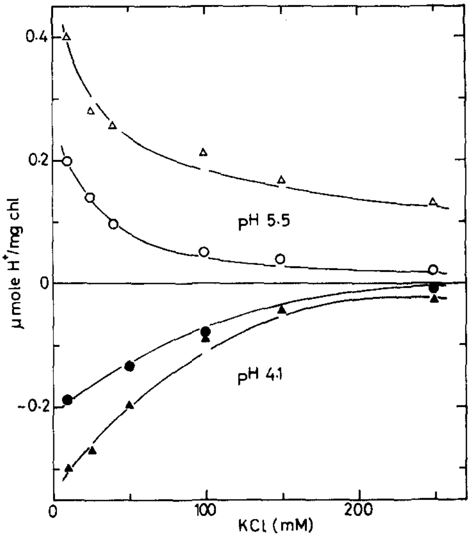


Fig. 3. Bulk pH dependence on salt-induced ΔH^+. Chloroplasts (40 μg chlorophyll/ml) were suspended in 10 mM KCl. pH values were adjusted by 0.1 N HCl or NaOH. Added salts were as follows, with their final concentrations in parentheses: □, $MgCl_2$ (2 mM); ○, $MgSO_4$ (2 mM); ●, K_2SO_4 (2 mM); △, $LaCl_3$ (20 μM); ▲, potassium ferricyanide (0.24 mM).

Fig. 4. Dependence of salt-induced pH changes in chloroplast suspension on concentration of KCl in the medium. Conditions and symbols are the same as those in Fig. 3. pH values were 5.5 for the upper part of the figure (pH decrease by cations) and 4.1 for the lower part of the figure (pH increase by anions).

In the presence of Mn^{2+} and La^{3+} some binding seemed to occur, since chloroplasts suspended in $MnCl_2$ (1.2 mM) or $LaCl_3$ (50 μM) with 10 mM KCl at pH 5.60 showed a pH increase on addition of K_2SO_4. This implies that Mn^{2+} or La^{3+} can make the chloroplasts, at pH 5.60, as if they were suspended at a lower pH (less than 4.4) (see Fig. 3), i.e., these cations can make chloroplasts positively charged, even at higher pH values by binding to the surface. Similar binding to the surface also seems to occur in the case of trivalent anion ferricyanide since even at pH 5.0, where the membrane surface is expected to be net negative and where an addition of divalent cation induced an H^+ release, an H^+ binding was observed on addition of ferricyanide.

According to the Gouy-Chapman theory, ions screen the oppositely charged surface and decrease the surface potential. The theory predicts that the increasing initial electrolyte concentration of the suspension would decrease the effect of the added salt on pH. Fig. 4 shows the suppressive effect of KCl on the salt-induced pH changes. The divalent or trivalent cation-induced H^+ release at pH 5.5 was decreased and the divalent or trivalent anion-induced H^+ binding at pH 4.1 was decreased with increasing pre-existing KCl. This shows that the negatively charged surface (at pH 5.5) was screened by K^+ and the positively charged one (at pH 4.1) by Cl^-, respectively. The incomplete suppression by KCl of the La^{3+}-induced pH decrease may be explained by partial binding of La^{3+} to the thylakoid surface. The binding of ferricyanide seems to be weak.

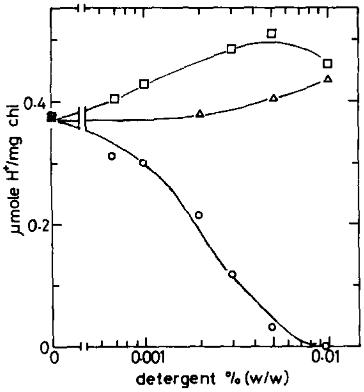


Fig. 5. Effect of detergents on LaCl$_3$-induced pH decrease in chloroplast suspension. LaCl$_3$ was added to make a final concentration of 20 µM at pH 5.50. Other conditions were the same as those in Fig. 3. ○, cetyltrimethylammonium chloride; △, Triton X-100; □, sodium dodecyl sulfate.

Effects of detergents on the salt-induced pH change in chloroplast suspension

If the salt-induced pH changes are due to the changes in surface charge density with simultaneous surface potential and surface pH changes, the detergent which has charges opposite to the membrane surface will decrease the salt-induced pH change while the detergent with a like charge will stimulate the salt-induced pH change. The presence of an anionic detergent, sodium dodecyl sulfate, in the suspension increased the La^{3+}-induced H$^+$ release (at pH 5.5), while a cationic detergent, cetyltrimethylammonium chloride, suppressed it (Fig. 5). Triton X-100, which carries no charges, had little effect on it up to 0.005%. The opposite effects of charged detergents in the acidic pH range (e.g., sodium dodecyl sulfate-induced decrease) were observed in the SO$_4^{2-}$- or ferricyanide^{3-}-induced H$^+$ binding (data not shown). These effects were consistent with those on the reactivity of the redox reagents to the electron transfer components in the membrane [12].

Discussion

The salt-induced pH change in the chloroplast suspension depended on the valence of cations of the salts irrespective of their anion constituents at pH values higher than 4.4, while the converse held true at pH values lower than 4.4. There was almost no specificity among cations with the same valence. These characteristics can be understood by applying the diffuse electrical double layer Gouy-Chapman theory [8] to chloroplast thylakoid membranes which have fixed charges on their surfaces [1—7,9—13]. In the vicinity of a charged membrane the concentration of ions differs from those in the bulk aqueous phase depending on the surface potential. Using the Boltzmann equation, the relationship between the concentration of ionic species i (c_{is}) at the surface and the concentration in the bulk solution (c_{ib}) is expressed as follows:

$$c_{is} = c_{ib}\exp(-z_i F\psi_0/RT) \tag{1}$$

where ψ_0 is the electrical potential at the surface, z_i is the valence of ionic species i, and the other symbols have their usual meanings. For H^+ at 25°C Eqn. 1 is rewritten:

$$pH_s = pH_b + \psi_0/59 \tag{2}$$

where pH_s and pH_b are the pH values at the surface of thylakoids and in the bulk phase, respectively, and ψ_0 is the surface potential in mV.

Two possible reservoirs for H^+ on the membrane surface are considered to explain the salt-induced pH change in chloroplast suspension. One is the H^+ in the diffuse double layer adjacent to the membrane surface. The other is the presence of surface ionizable groups which change the degree of ionization depending on the surface pH.

Firstly, the amount of H^+ in the double layer is considered. The thickness of the double layer can be approximated by $1/\kappa$ where κ is the Debye parameter. By applying Eqn. 1, the total excess amount of H^+ in the double layer is given by Eqn 3.

$$I_{H^+} = S(1/\kappa)[H^+_b](\exp(-F\psi_0/RT) - 1) \tag{3}$$

where S is the surface area of thylakoid membranes present in the suspension. The Gouy-Chapman theory gives an equation for symmetrical salts,

$$\sigma = (1/136)c_{ib}^{1/2}\sinh(zF\psi_0/2\,RT) \tag{4}$$

where σ is the surface charge density in the elementary charge per square Ångstrom. Using the surface charge density of thylakoids obtained by electrophoresis [5], the amount of the excess H^+ in the double layer and its pH dependence were calculated at 10 mM KCl as c_{ib} with Eqns. 3 and 4. The excess amount was much smaller than the H^+ change observed when the salt was added. The pH-dependence curve calculated was also different from that observed when salt was added (Fig. 3). For example, the value of I_{H^+} was calculated to be 0.04 nmol H^+/mg chlorophyll at pH 5.5, when we used the area/chlorophyll ratio of 220 Å^2 per molecule [14]. Even when I_{H^+} is completely released by the salt addition, this cannot account for the observed H^+ change, as the amount of I_{H^+} is smaller than the experimental value by approx. four orders of magnitude. The accuracy of the value for S, which may be argued, will not affect the situation for comparison. Therefore, the salt-induced change in H^+ concentration in the double layer has little, if any, contribution to the salt-induced pH change in the suspension.

The change in the degree of ionization of surface ionizable groups is considered next. The calculation mentioned above gives pH_s at a given pH_b. At pH values higher than 4.4, the addition of salt will raise pH_s by reducing the extent of negative ψ_0. At the pH_s, thus increased, surface groups will dissociate, resulting in H^+ release to the outer medium. On the contrary, when pH_b is lower than 4.4, the salt addition decreases pH_s by decreasing positive ψ_0. The protonation of dissociated groups, increased at low pH_s, will result in H^+ uptake from the outer medium (Fig. 3). A calculation with Eqns. 2 and 4 with fixed values of σ, gives smaller values of ΔpH ($pH_s - pH_b$) at higher bulk KCl concentrations. Such ΔpH will give a rough estimation of ΔH^+ induced by salt addition. The extent of ΔH^+ on addition of higher-valence cation (anion) salt will become smaller with the increase of pre-existing KCl concentration. This situation is seen in Fig. 4. Thus, the salt-induced pH changes probably reflect the change in the degree of ionization of dissociable groups on the thylakoid membrane surface.

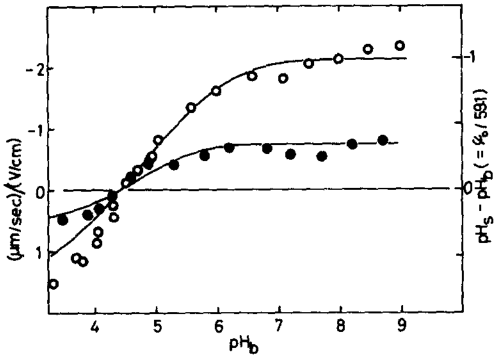
Thylakoid membranes have ionizable groups due to lipids and proteins. Among these, carboxyl groups of proteins most suitably explain the salt-induced pH changes observed within the pH range tested, judging from their pK and amount. Carboxyl groups, in fact, determine the pH dependence of electrophoretic mobility of chloroplasts [7] and the higher-order structure of thylakoids [2]. Assuming a single species of carboxyl groups with a dissociation constant of K, the number of dissociated carboxyl groups per unit area n, on the surface is expressed as follows,

$$n = NK/([\mathrm{H}^+]_s + K) \tag{5}$$

where N is the number of carboxyl groups per unit surface area (Å^2) and $[\mathrm{H}^+]_s$ the surface H$^+$ concentration. Then the surface charge density of thylakoids (elementary charges/Å^2) is expressed as follows,

$$\sigma = N(K/([\mathrm{H}^+]_I + K) - K/([\mathrm{H}^+]_s + K)) \tag{6}$$

where $[\mathrm{H}^+]_I$ is the H$^+$ concentration at the isoelectric pH of thylakoids. Proteins have carboxyl groups of side chains of aspartate and glutamate, in addition to the terminal carboxyl groups which are generally much less in amount than the side-chain carboxyl groups. Although the γ-carboxyl pK of glutamic acid and β-carboxyl pK of aspartic acid, as free amino acids, are 4.25 and 3.85, respectively, pK values of these groups in peptides are the same (4.6) [15]. Isoelectric



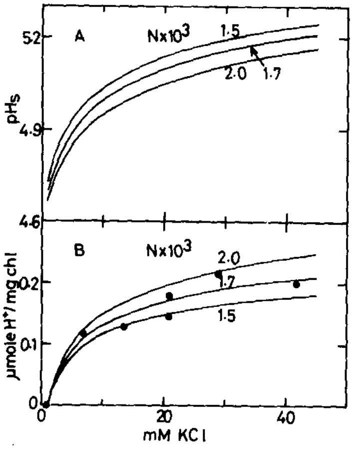


Fig. 6. Relationship between pH$_b$ and electrophoretic mobility of chloroplasts and between pH$_b$ and the calculated pH$_s$ — pH$_b$. Open and closed circles are the electrophoretic mobilities in 10 mM buffer (5 mM sodium barbital and 5 mM sodium acetate buffer) and in 10 mM buffer plus 100 mM KCl, respectively. Solid lines are pH$_s$ — pH$_b$ calculated for 10 mM and 110 mM monovalent salt as in Fig. 8.

Fig. 7. Theoretical curves for dependence of pH$_s$ and ΔH$^+$ of thylakoid membranes on KCl concentration in the bulk aqueous phase. A. Dependence of pH$_s$ on KCl concentration at various levels of N (in number of carboxyl groups/Å^2). Calculated for pH$_b$ = 5.6. B. Amount of H$^+$ released by increasing KCl concentration from 1 mM KCl. Calculated for pH$_b$ = 5.6. Values of ΔH$^+$/mg chlorophyll are plotted against the final level of KCl. ●, experimentally determined values.

pH in the microelectrophoresis of spinach chloroplasts was 4.4 (Fig. 6) which was the same as the value of pI of mechanically broken thylakoids [16]. Electrofocusing electrophoresis gave a single band at 3.8 (with the cathode at the top of the column, electrophoresis in the direction of gravity) or a major band at 4.3 and a minor one at 4.0 (with the electrodes placed at the reverse positions). These values are close to those measured by microelectrophoresis of *Nitella* chloroplasts (4.2) [5], and of pea chloroplasts (4.4) [7]. Using the value of 4.6 as the pK of protein carboxyl groups and of 4.4 as the pI of spinach thylakoid membanes, σ can be obtained from Eqn. 6 when the value of N is given. Then from Eqn. 4 we can get the pairs of c_{ib} and ψ_0. The relationship between $\mathrm{pH_s}$ and bulk KCl concentration at a given $\mathrm{pH_b}$ (5.6) can be obtained from the pair of c_{ib} and ψ_0 by using Eqn. 2 (Fig. 7A). This shows that the increase in bulk KCl concentration raises $\mathrm{pH_s}$. It will result in the increase in the dissociation of carboxyl groups. Using the pair of c_{ib} and ψ_0 and the surface area of 220 $\mathrm{Å}^2$ per chlorophyll molecule [14] which corresponds to 440 $\mathrm{Å}^2$/chlorophyll for the double-faced thylakoid membranes considered in our study, the relationship between the amount of $\mathrm{H^+}$ released and the change in bulk KCl concentration can be obtained (Fig. 7B). It was assumed, for simplicity, that the inner and outer surfaces of thylakoids have the same charge density. The changes in dissociation of carboxyl groups on the inner and the outer surfaces probably contribute to the observed salt-induced $\Delta\mathrm{H^+}$, since the KCl-induced $\Delta\mathrm{H^+}$ was neither affected by the presence of Triton X-100 (0.01%) nor by that of gamicidin. Fig. 7B shows that the experimental data points fall within the group of curves with a set of N values in the range of 1.5—$2.0 \cdot 10^{-3}$ carboxyl group/$\mathrm{Å}^2$. This range of N will give the surface charge density of $-1.0 \pm 0.1 \cdot 10^{-3}$ elementary charge/$\mathrm{Å}^2$ in the fully dissociated state. The value of N depends on the estimation of surface area per chlorophyll molecule. The N value also depends on the value of pK used. With pK values of 4.3 and 4.9, the best fits for N were $2.0 \cdot 10^{-3}$ and $1.6 \cdot 10^{-3}$ carboxyl group/$\mathrm{Å}^2$, respectively. The obtained surface charge density is a little smaller than the value postulated by Barber et al. [9] and a little larger than that estimated by the analysis of the reactivity of the system II acceptor (which may reflect the physical parameters in the local domain of the system II acceptor) [11]. A similar calculation for divalent salt ($\mathrm{MgSO_4}$) using the same values of pK, pI and N did not agree with the data of Fig. 2B. The observed value of $\mathrm{H^+}$ released was larger than that calculated with the same set of pK, pI and N. The deviation of experimental data from the calculation (in which the binding effect was neglected) may indicate the binding of divalent cations [4]. To analyze such a binding a more complicated calculation with additional assumptions is required.

The ionization of protonated carboxyl groups might expand the surface area per lipid molecule [17]. Since the fraction of charged lipids in the thylakoid membranes is small [1], we assume that the change of surface area with ionization of carboxyl groups is also small. We assumed also that the fixed charges were evenly distributed in the plane of the membrane surface. This may not, however, be precisely true. If some of the fixed charges are distributed above the exact plane of lipid bilayer surface, the effective potential becomes smaller than that calculated from the Gouy-Chapman theory because of the presence

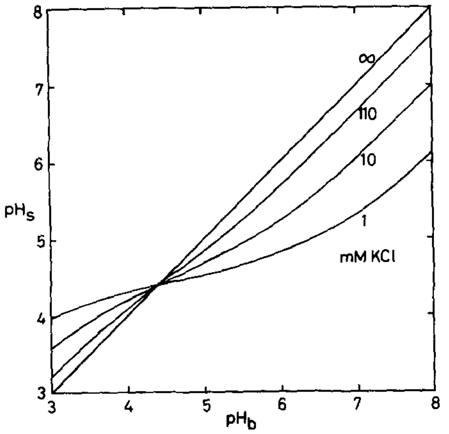


Fig. 8. Calculated relationship between pH$_s$ and pH$_b$ at various KCl concentrations. For explanation see text.

of diffusible ions in the fixed charge region. Such a situation leads to an underestimation of N for carboxyl groups, which may explain the discrepancy between our data and the value obtained by titration [3]. The difference between the ζ-potential and the calculated surface potential may be involved in some part of this difference (Fig. 6 and see below).

With the N value of $1.7 \cdot 10^{-3}$ carboxyl group/Å^2, pK = 4.6 and pI = 4.4, the pH$_s$ dependence of the surface charge density of thylakoids is obtained. From this, the relationship between pH$_b$ and pH$_s$ at a given salt concentrations is calculated (Fig. 8) by using Eqns. 2 and 4. As ΔpH_{s-b} ($pH_s - pH_b$) depends on ψ_0 (Eqn. 2), the pH$_b$-dependence of ψ_0 at a given bulk salt concentration can be also obtained. The ζ-potential is proportional to the electrophoretic mobility. Therefore, ψ_0 should be related to the electrophoretic mobility of thylakoids if the proportionality of these potentials hold. Indeed, a linearity between the values of ψ_0 and ζ-potential was observed in the chloroplast suspension (Fig. 6). pH-dependence of ψ_0 also showed good agreement with that of the electrophoretic mobility (Fig. 6). The deviation of the pattern of the calculated ψ_0 from that of the electrophoretic mobility in the acidic pH region (pH$_b$ lower than 4.4), where the membranes are positively charged (Fig. 6), suggests that the effective pK of the carboxyl groups is lower than 4.6, because the use of a lower pK (e.g., 4.3) makes the ψ_0 pattern in this pH region closer to that of electrophoretic mobility without any significant change of the pattern in the negatively charged pH region.

From these results it can be concluded that the change in the charge density of the thylakoid surface mainly depends on the degree of ionization of protein carboxyl groups between pH values of pH 3.4 and 9.0 and that the the salt-induced pH change in the suspension of chloroplasts is caused by the H$^+$ release (or uptake) by the change of ionization of carboxyl groups induced by the surface pH change.

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