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EFFECT OF SURFACE POTENTIAL ON THE INTRAMEMBRANE ELECTRICAL FIELD MEASURED WITH CAROTENOID SPECTRAL SHIFT IN CHROMATOPHORES FROM *RHODOPSEUDOMONAS SPHAEROIDES*

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Summary

Changes in the surface potential, the electrical potential difference between the membrane surface and the bulk aqueous phase were measured with the carotenoid spectral shift which indicates the change of electrical field in the membrane. Chromatophores were prepared from a non-sulfur purple bacterium, *Rhodopseudomonas sphaeroides*, in a low-salt buffer. Surface potential was changed by addition of salt or by pH jump as predicted by the Gouy-Chapman diffuse double layer theory.

When a salt was added at neutral pH, the shift of carotenoid spectrum to shorter wavelength, corresponding to an increase in electrical potential at the outside surface, was observed. The salts of divalent cations ($MgSO_4$, $MgCl_2$, $CaCl_2$) were effective at concentrations lower than those of monovalent cation salts (NaCl, KCl, Na_2SO_4) by a factor of about 50. Among the salts of mono- or divalent cation used, little ionic species-dependent difference was observed in the low-concentration range except that due to the valence of cations. The pH dependence of the salt-induced carotenoid change was explained in terms of the change in surface charge density, which was about 0 at pH 5—5.5 and had negative values at higher pH values. The dependence of the pH jump-induced absorbance change on the salt concentration was also consistent with the change in the charge density. The surface potential change by the salt addition, which was calibrated by H^+ diffusion potential, was about 90 mV at the maximum. From the difference between the effective concentrations with salts

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Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; MES, morpholinoethanesulfonate.

of mono- and divalent cations at pH 7.8, the surface charge density of $(-1.9 \pm 0.5) \cdot 10^{-3}$ elementary charge per $Å^2$, and the surface potential of about −100 mV in the presence of about 0.1 mM divalent cation or 5 mM monovalent cation were calculated.

Introduction

Electrical properties of photosynthetic membranes are important in the energy transduction of the membrane because the electrochemical potential of H^+ across the membrane plays a key role in the process of phosphorylation [1] and because many ions, including charged substrates, interact with the membrane. Much attention has been paid to the transmembrane potential difference [2—6]. In certain photosynthetic bacteria, the spectral shift of carotenoid has been shown to indicate intramembrane electrical field [7—9] generated by light or concentration gradient of ions [3,6,10]. In those studies, generally, the changes in membrane potential (between bulk aqueous phases) have been assumed to be linear to the changes in electrical field in the membrane.

The surface of biological membranes has charges which are generally net negative at neutral pH [11]. According to the Gouy-Chapman diffuse double layer theory, these changes cause the electrical potential difference between the surface and the bulk aqueous phase, i.e., the surface potential [11,12]. The theory has been applied to artificial lipid bilayer membranes [11,13—15] and excitable membranes of nerves [16—18]. Recently Rumberg [19], Barber et al. [20,21] and Itoh [22,23] have applied the theory to the chloroplast membrane.

When the surface potential is taken into account, the change in the intra-

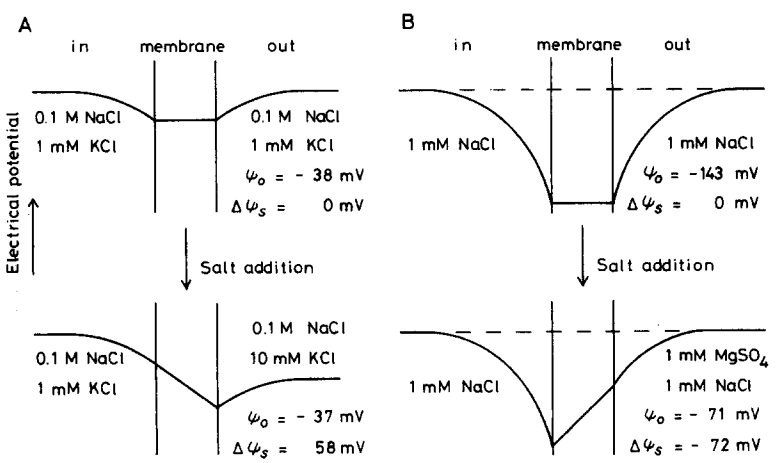

Fig. 1. Schematic diagram of the potential profiles in the vicinity of a charged membrane before and after salt addition. The surface potential (ψ_0) was estimated by the Gouy-Chapman theory for the charge density of $-1.9 \cdot 10^{-3}$ elementary charge per $Å^2$ and the electrolyte concentrations indicated. $\Delta\psi_s$ is the potential difference between two surfaces of the membrane. A. In the presence of valinomycin, 9 mM KCl is added. B. In the presence of CCCP, 1 mM $MgSO_4$ is added.

membrane electrical field does not always parallel the change of the membrane potential. The Gouy-Chapman theory determines the surface potential of diffuse double layer depending on the surface charge density or the salt concentration in the aqueous phase. The schematic relationship between membrane potential, surface potential and intramembrane electrical field is depicted in Fig. 1. Fig. 1A shows the effect of KCl addition on the potential profile in the presence of valinomycin, due to the effect of diffusion potential of potassium. In this case, the change in surface potential is expected to be negligible because of the presence of higher concentrations of coexisting ions. On the other hand, when the salt concentration in the aqueous phase is low, an addition of nonpermeant ions reduces the extent of the surface potential on the outer side. Under the situation of constant membrane potential, the surface potential change induces a change in the intramembrane electrical field (Fig. 1B). Intramembrane electrical field changes induced by additions of salts can be classified into three cases, depending on ionic concentration and pH of the medium, and permeability to ions of the membrane: (a) surface potential change negligible (Fig. 1A); (b) diffusion potential (membrane potential) change negligible (Fig. 1B); (c) both of them significant and additive.

We analyzed the intramembrane electrical field changes in the chromatophore membrane of *Rhodopseudomonas sphaeroides* mostly under conditions of negligible diffusion potential changes (case b). The carotenoid spectral shift was used to indicate the intramembrane electrical field. Membrane potential between bulk aqueous phases was kept practically constant in the presence of CCCP.

Materials and Methods

R. sphaeroides was grown under illumination in a synthetic medium similar to that described by Cohen-Bazire et al. [24]. The differences of the medium from the original formula were the following: 20 mM sodium succinate used as the carbon source, 7.5 mM $(NH_4)_2SO_4$ as the nitrogen source, and 0.1 mM EDTA as the chelating agent. The concentrations of potassium phosphate (pH 6.8) and $MgSO_4$ were changed to 10 mM and 1 mM, respectively. The cells were harvested and washed with 5 mM MES/NaOH, pH 6.5. Chromatophores were prepared as described previously [25] by use of the French pressure cell, washed with the MES buffer, and suspended in distilled water.

Absorbance changes of carotenoid by salt or alkali additions were measured as described previously [10,25]. 1—100 μl of 0.25 M $MgSO_4$, 0.25 M $MgCl_2$, 0.25 M $CaCl_2$, 3.5 M NaCl, 1 M Na_2SO_4, 3 M KCl, or 0.1 M KOH was added to chromatophores suspended in 3.5 ml of distilled water or a salt solution. 1 μM CCCP was present except in the measurements of KCl-induced absorbance change in the presence of 130 nM valinomycin. The pH of the reaction mixture was measured with a glass electrode before and after the absorbance measurement. 1 mM NaCl was added for the pH measurement of low salt solution after the absorbance measurement to stabilize electrode response. The initial pH of the reaction mixture was adjusted by adding a small amount of H_2SO_4 or KOH.

The measurements were carried out at 22°C.

Results

Time courses of carotenoid absorbance changes induced by $MgSO_4$ and KOH additions in the presence of CCCP are shown in Fig. 2. The difference absorbance of 488-minus-506 nm was used for the measurement of time courses because at these two wavelengths the chromatophore suspension had the same absorbance. At pH 5.8, $MgSO_4$ induced a slight decrease of the difference absorbance (trace a), but at pH 7.8 the same addition induced a large decrease (trace b). On the other hand, addition of KOH in the absence of $MgSO_4$ (trace b) decreased the difference absorbance less than that in the presence of $MgSO_4$ (trace a). Regardless of the order of the additions, the total extent of the absorbance changes by the additions of both $MgSO_4$ and KOH was almost equal.

Fig. 3 shows the spectral changes induced by $MgSO_4$ and KOH additions. Addition of $MgSO_4$ at pH 5.8 (a) induced an absorbance increase without distinct peak between 420 and 560 nm, while at pH 7.8 (b) the same addition induced a spectral change which corresponded to the shift of carotenoid spectrum to shorter wavelengths superposed on the non-specific increase of absorbance. When medium pH was changed from 5.8 to 7.8 by the addition of KOH (c, d), the spectral shift to shorter wavelengths as well as a nonspecific decrease of absorbance was observed. The extent of the spectral shift in the presence of 0.36 mM $MgSO_4$ (d) was larger than that in the absence of $MgSO_4$ (c) as shown in the time courses in Fig. 2. The blue shift of the carotenoid spectrum by the alkalinization can be explained by the effect of diffusion potential of H^+, but the difference in the extent between the media with two different salt concentrations cannot be explained in terms of the diffusion potential. The spectra of the blue shift (b, c, d) had the maxima at 440, 471,

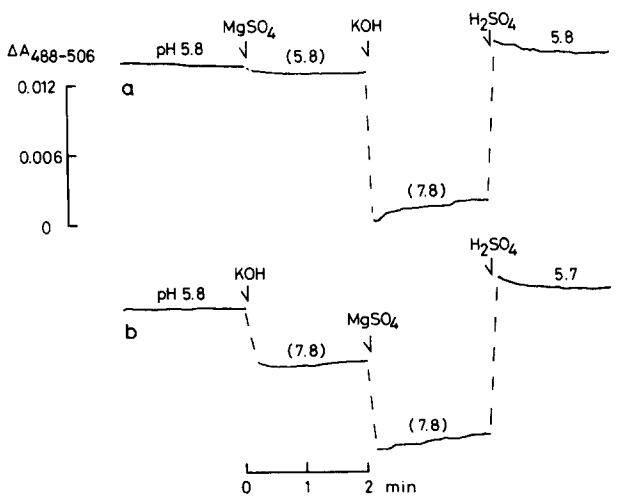

Fig. 2. Absorbance changes of carotenoid induced by $MgSO_4$ addition or by pH jump in the presence of CCCP. Chromatophores were prepared as described in Materials and Methods and suspended in distilled water (equivalent to 10 μM bacteriochlorophyll). Traces of absorbance change on the dual-wavelength mode (488-minus-506 nm) are shown. 5 μl of 0.25 M $MgSO_4$ (final 0.36 mM), 0.1 M KOH or 0.05 M H_2SO_4 was added as indicated by arrows. 1 μM CCCP was present. The numbers above the traces indicate the pH of the sample or of another sample with the same additions (in the parentheses).

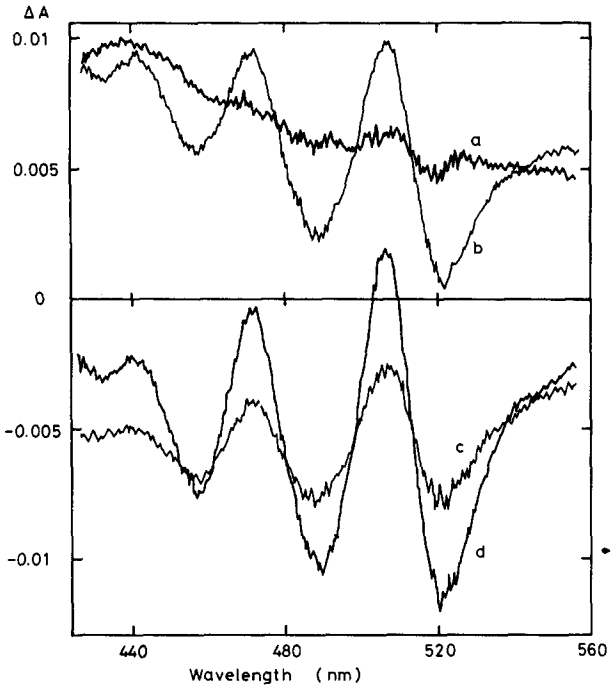

Fig. 3. Absorption spectrum changes upon $MgSO_4$ addition (a, b) and pH jump (c, d). The difference spectra ($MgSO_4$ or KOH added minus no addition) were recorded on the split-beam mode with a scanning speed of 10 nm/s about 30 s after the addition of 0.36 mM $MgSO_4$ at pH 5.8 (a) or pH 7.8 (b). Medium pH was changed from 5.8 to 7.8 by adding KOH in the presence (d) or absence (c) of 0.36 mM $MgSO_4$. The conditions and procedures were the same as those in Fig. 2.

506 nm and the minima at 457, 488, 521 nm. These peaks were 2—3 nm shorter than those of the red shift induced by light illumination or K^+ diffusion potential [3,8,10]. The small difference of the peak wavelengths between the blue and red shifts may be explained by the difference of the direction of the shift [8,9]. The nonspecific changes of absorbance, which are probably caused by light-scattering changes, were not negligible in the wavelength range studied. However, the nonspecific changes of absorbance were rather wavelength independent. To make the light-scattering effect small, the difference absorbance, 488-minus-506 nm, was used for quantitative analysis.

Fig. 4 shows the pH dependence of the extent of the salt-induced carotenoid shift. In the pH range 5—5.5, additions of $MgSO_4$ up to 0.36 mM (Fig. 4A) or NaCl up to 5 mM (Fig. 4B) induced scarcely any change in the difference absorbance. At high pH values the additions of low-concentration salts decreased the difference absorbance ($\Delta A_{488-506}$). When salts were added at higher concentrations (greater than 0.5 mM for $MgSO_4$, greater than 10 mM for NaCl), the null-change pH (pH where the salt addition induced no absorbance change) shifted to a pH higher than 5—5.5. Time courses of the change by the salt addition at higher concentrations showed a slow increase in absorbance after a rapid decrease.

The salt-induced absorbance change was dependent on the valence of cation as well as the concentration of salt added (Fig. 5). Divalent cation salts were as

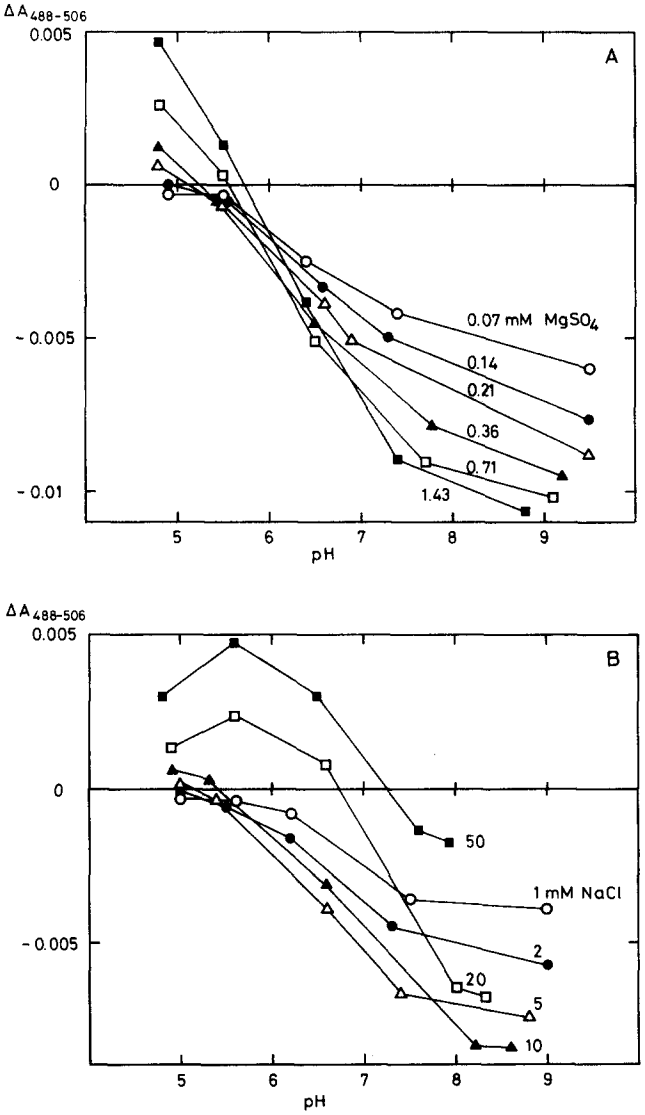

Fig. 4. pH dependence of carotenoid absorbance change induced by addition of $MgSO_4$ (A) or NaCl (B). Various volumes of 0.25 M $MgSO_4$ or 3.5 M NaCl solution were added in a series of experiments similar to those in Fig. 2. Absorbance changes 30 s after the additions were plotted against pH. Medium pH was varied by adding H_2SO_4 or KOH more than 5 min before the addition of the salts. Final concentrations of the salts added are indicated in the figure.

effective as salts of monovalent cations at much lower concentrations. Among the salts of monovalent and divalent cations used, up to 9 mM and 0.7 mM, respectively, no specificity in the ionic species was observed. At higher concentrations of salts, the extent of the absorbance change became dependent on the cation species and became smaller with increasing concentration in some cases. Time courses of the absorbance change at higher concentrations were composed of a rapid decrease and a slow increase. The reason for the slow increase

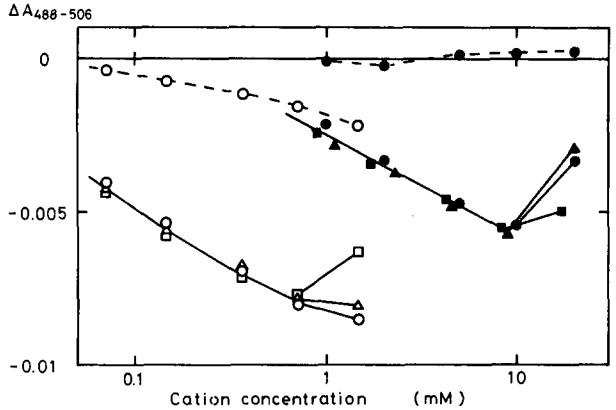

Fig. 5. Dependence of carotenoid absorbance change on concentration of salts added. Chromatophores were suspended in 0.6 mM Tricine-NaOH buffer, pH 7.8. Absorbance changes 30 s after the additions were plotted. ○——○, $MgSO_4$; △——△, $MgCl_2$; □——□, $CaCl_2$; ○- - - - -○, $MgSO_4$ in the presence of 10 mM Na_2SO_4; ●——●, NaCl; ▲——▲, Na_2SO_4; ■——■, KCl; ●- - - - -●, NaCl in the presence of 5 mM $MgCl_2$. Other conditions were the same as those in Fig. 2.

is not clear. The diffusion potential of cations of the added salts, which is consistent with the direction of the slow absorbance change, seems to be a minor factor because the absorbance change induced by choline chloride showed a concentration dependence and kinetics similar to those with NaCl or KCl. The addition of $MgSO_4$ in the presence of 10 mM Na_2SO_4 induced only a small decrease of the difference absorbance. The addition of NaCl in the presence of 5 mM $MgCl_2$ induced almost no change.

Fig. 6 shows the carotenoid absorbance changes as a function of the final

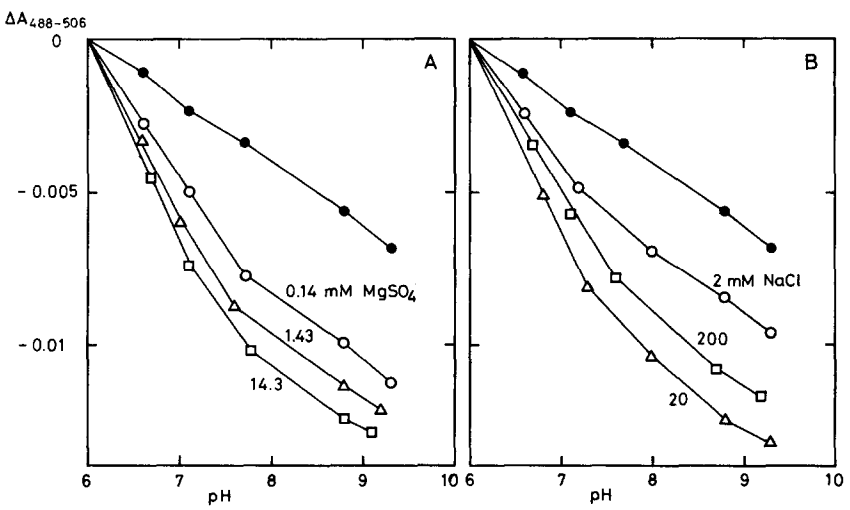

Fig. 6. Dependence of pH-induced carotenoid absorbance change on the final pH at various concentrations of $MgSO_4$ (A) or NaCl (B). Chromatophores were suspended in distilled water (closed circles) or in the salt solution of various concentration as indicated in the figure. Initial pH was 6.0. Up to 8 μl of 0.1 M KOH was added. Absorbance changes 1 min after the additions were plotted against the pH after the additions. Other conditions were the same as those in Fig. 2.

pH, when KOH was added in the presence of CCCP and of various concentrations of $MgSO_4$ or NaCl. The initial pH was adjusted to 6.0 in all mixtures, and KOH added was 0.23 mM in the final concentration at the maximum. The addition of K^+ in this concentration range in the absence of valinomycin produced no effect due to the diffusion potential of K^+. The extent of absorbance decrease became larger with an increase in the final pH in each salt solution. In the range of the extent less than about —0.007 of absorbance, the extent was almost linear to the rise in pH. The salt concentration in the reaction mixture affected the extent of the carotenoid absorbance changes induced by the pH jump. The decrease in the absorbance became larger when the salt concentration increased except in the presence of 200 mM NaCl. The largest $\Delta A/\Delta pH$ value was —0.0066 observed in 14.3 mM $MgSO_4$ for the pH jump from 6 to 7. The value, —0.00011 absorbance/mV, was used in calculation of potential change as in the Discussion section.

Fig. 7 shows the changes in the extent of the difference absorbance as a function of concentration of KCl added in the presence of valinomycin. At pH 5.8 and up to 10 mM KCl, the concentration-absorbance relationship in the presence of 5 mM $MgSO_4$ was similar to that in its absence. On the other hand, at pH 7.8, absorbance changes in the presence of 5 mM $MgSO_4$ were larger than

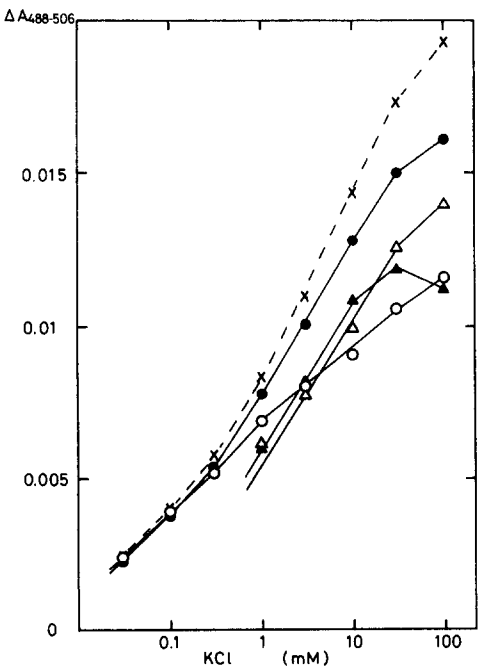

Fig. 7. Absorbance changes of carotenoid induced by KCl addition in the presence of valinomycin. Chromatophores were suspended in the following media: △———△, distilled water, pH 5.8 (final); ▲———▲, 5 mM $MgSO_4$, pH 5.8; ○———○, 0.6 mM Tricine-NaOH, pH 7.8; ●———●, 0.6 mM Tricine-NaOH and 5 mM $MgSO_4$, pH 7.8. Changes in the difference absorbance ($\Delta A_{488-506}$) extraporated to the time of the KCl addition were plotted against the final concentration of KCl added. Valinomycin concentration was 130 nM. Bacteriochlorophyll concentration was 10 μM. Dashed line represents the data ($\Delta A_{490-508}$) in 0.6 mM Tricine-NaOH and 5 mM $MgSO_4$, pH 7.8, taken with the wavelength pair at the peak positions of the K^+-induced red shift (see Discussion).

that in its absence in the range above 1 mM KCl. In the range of KCl concentration from 0.03 to 0.3 mM, no difference was observed between the difference absorbance in the presence and in the absence of $MgSO_4$. Linear relationships between the absorbance change and the logarithm of KCl concentration gave a value of 0.0050 as $\Delta A/\Delta\log[KCl]$ under the three conditions, pH 5.8 with and without $MgSO_4$ and pH 7.8 with $MgSO_4$. The wavelengths used to measure the difference absorbance, 488 and 506 nm, do not correspond to the wavelengths that give the maximal change in the case of red shift by the KCl addition [3,10]. When the pair of peak positions, 490, 508 nm, was used (Fig. 7, dashed line), $\Delta A/\Delta\log[KCl]$ was 0.0060.

Discussion

The blue shift of the carotenoid spectrum by the addition of salts in the presence of CCCP (Figs. 2—5) can be explained satisfactorily in terms of the change of the surface potential. As shown in Fig. 1B, when the membrane potential is kept constant, a change in the surface potential on one side gives a change of potential difference between membrane surfaces in a magnitude equal to the surface potential change. In the presence of CCCP, not only is the electrochemical potential difference (between outside and inside aqueous phases) of H^+ kept close to zero but also the membrane potential remains constant, because the pH change of the outer solution by the salt addition is less than 0.1 and because the change in intravesicular pH is also estimated to be small, taking the small capacitance of the membrane into consideration [26]. The blue shift corresponds to the outside-positive potential change in the chromatophore membrane as judged from the effects of diffusion potential of H^+ (Fig. 6) or K^+ (Fig. 7) [3,6,10]. When the salt addition decreases the magnitude of the negative outside-surface potential, the potential at the outside surface becomes more positive. The following observations support the idea that the blue shift was caused mainly by the surface potential change in the diffuse double layer. (1) The salt-induced blue shift was highly dependent on pH of the medium. Similar pH dependence curves were observed with NaCl and $MgSO_4$ additions. The dependence is interpreted in the change in surface charge density by protonation and deprotonation of the surface groups. The small or absent change at about pH 5.3 indicates the electrical neutrality on the surface of the membrane at this pH. (2) Except for the valence of cations, no ionic species specificity was observed in the concentration-extent relationship in the lower concentration range. (3) Salts of divalent cations were effective at much lower concentrations than were monovalent ones. (4) The salt-induced change became small in the presence of coexisting salts.

According to the Gouy-Chapman diffuse electrical double layer theory, surface charge density σ, surface potential ψ_o, and concentration in the bulk aqueous phase C and valence Z of a symmetrical salt have a relation [11,20],

$$\sigma = 0.00733\sqrt{C}\sinh(Z\psi_o/50.9) \text{ (at 22°C)} \quad (1)$$

where σ is expressed in elementary charges/Å^2, C in M, ψ_o in mV. When mono- and divalent salts at concentrations C' and C'', respectively, give the same surface potential, the following two equations give the surface charge density

and the surface potential,

$$\sigma = \pm 0.00733 \sqrt{\frac{C'^2 - 4C'C''}{4C''}} \quad (2)$$

$$\psi_o = \pm 25.5 \cosh^{-1}\left(\frac{C'}{2C''} - 1\right) \quad (3)$$

In the data of Fig. 5 (pH 7.8), 0.07 mM $MgSO_4$ had the same effect as 3.3 mM NaCl. These values will give a surface potential of −97 mV and a net surface charge density of $-1.4 \cdot 10^{-3}$ elementary charge per Å^2. Another set of concentrations, which gave the same carotenoid change, is 0.14 mM $MgSO_4$ and 8.2 mM NaCl, giving the values of −103 mV and $-2.4 \cdot 10^{-3}$ elementary charge per Å^2. These sets of salt concentrations were selected to give the two extreme values of absorbance change (near maximum and minimum) in the range of linear responses in parallel titrations with salts of divalent and monovalent cations (Fig. 5). These values of surface potential and charge density, incorporated into the Gouy-Chapman theory, explain well the dependence of the potential change on the concentration of added divalent cations in the presence and absence of monovalent ions at a higher concentration. When a value of $-1.9 \cdot 10^{-3}$ elementary charge per Å^2, which is the average of the above two calculations, is used, surface potentials in the presence of 0.07 mM and 0.7 mM $MgSO_4$, respectively, are calculated to be −105 mV and −76 mV from Eqn. 1. The difference, 29 mV, is in good agreement with the corresponding experimental value which was 33 mV (Fig. 5, open circles, solid line) as calibrated by the H^+ diffusion potential. This means that the relationship between the potential change and the carotenoid absorbance change is common regardless of the cause, diffusion potential or surface potential (see Fig. 1). But the agreement does not mean the validity of the charge density calculated, because in the linear part of the concentration dependence as in Fig. 5, values of charge density in a wide range give essentially the same slope [20]. On the other hand, in the case of $MgSO_4$ addition in the presence of Na_2SO_4 at a higher concentration, the slope is highly dependent on the charge density. Using the Gouy-Chapman equation for the situation with both mono- and divalent salts [27], the calculated charge density, $-1.9 \cdot 10^{-3}$ elementary charge per Å^2, gives the following values of surface potential, −68 mV in the presence of 10 mM Na_2SO_4 and 0.07 mM $MgSO_4$ and −61 mV in the presence of 10 mM Na_2SO_4 and 0.7 mM $MgSO_4$. The difference, 7 mV, is in rough agreement with the experimental value, 10 mV (Fig. 5, open circles, dashed line). The dependence of absorbance change on concentration increment of monovalent cations (Fig. 5) was less steep than that theoretically expected. The addition of monovalent salt at a rather high concentration, to observe the surface potential-dependent absorbance change, may also induce other effects on the carotenoid shift. But, up to 10 mM, the surface potential change probably is the major factor for the absorbance change, judged from the effect of the preexisting salts and the pH dependence.

The overlapping effects of surface potential and diffusion potential appear in the data shown in Figs. 6 and 7. When KOH is added in the presence of CCCP, outside-positive diffusion potential (blue shift) is induced and by the

deprotonation of ionizable groups, outside surface potential is expected to become negative (red shift). The surface potential change is more significant in the medium of a lower salt concentration. In the experiments of KCl addition in the presence of valinomycin, as well as the outside-negative diffusion potential, an outside-positive potential change by a decrease of the surface potential may take place at low salt concentrations at pH 7.8. These opposing effects, though qualitatively apparent in these data, must be quantitatively analyzed to elucidate the effects of electrolyte additions on the chemical and electrical potential profiles across the membrane.

The carotenoid shift is usually calibrated by the K^+ diffusion potential in the presence of valinomycin [3,6,10], but as mentioned above, calibration by the H^+ diffusion potential in the presence of CCCP was used. The value obtained by the former method was 76% of that by the latter. One of the causes for the difference must be the direction of the shift [8,9]. In the blue shift by KOH addition the wavelengths used for the difference absorbance (488-minus-506 nm) were almost at the positions of maximal changes. But in the red shift, the wavelengths used for measuring the difference absorbance were about 2 nm off the peaks. In the K^+-induced red shift, $\Delta A_{488-506}$ was 83% of $\Delta A_{490-508}$, which is the difference absorbance with measuring and reference wavelengths at peak positions of the change. If we use the calibration by the K^+ diffusion potential at the peak wavelengths, the potential change calculated becomes 9% higher. The 2 nm difference of the peaks in the blue- and red-shifted spectra may be too large to be quantitatively explained by the shift-type mechanism as suggested by De Grooth and Amesz [8] and Symons et al. [9]. Further investigation is needed to clarify the phenomena with a special reference to the mechanisms of the spectral shift.

In the absence of CCCP the addition of $MgSO_4$ at pH 7.8 also induced a blue shift of the carotenoid spectrum but the extent was about a half of that in the presence of CCCP after 30 s. This probably means that the membrane potential change does not relax quickly enough without CCCP in the time range used. CCCP has a negative charge in its deprotonated form, but the presence of CCCP at concentrations from 0.5 to 25 μM in the medium did not change the extent of carotenoid change induced by the salt addition and apparently did not affect the surface charge density effectively.

Existence of the surface potential of several tens of millivolts as estimated here must be considered in investigations of energy transduction in the chromatophore membrane. The concentration of ions at the surface (C_s) is different from that in the bulk aqueous phase (C_b) as given by the Boltzmann equation,

$$C_s = C_b \exp(-Ze\psi_o/kT)$$

where Z is valence of the ion and ψ_o is the surface potential. Many reactions on the membrane are known to be pH dependent. They are probably related to pH at the surface rather than that in the bulk phase. The reaction between ionic substrates in the aqueous phase and the membrane component must also be affected by the surface potential as shown in the chloroplast membrane [22,23]. On the other hand, if surface potentials on two sides of the membrane are different, the electrical potential difference between surfaces is different from that between bulk aqueous phases. The former, which determines the

intramembrane electrical field, can be considered as a factor to regulate the fluxes of ions and electrons across the membrane and the microenvironment of the coupling factor, while the latter, the membrane potential, may be one of the factors to determine the extent of energy-transducing processes and free energy available for them.

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