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SURFACE POTENTIAL ON THE PERIPLASMIC SIDE OF THE PHOTOSYNTHETIC MEMBRANE OF *RHODOPSEUDOMONAS SPHAEROIDES*

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Summary

Membrane surface potential on the periplasmic side of the photosynthetic membrane was estimated in cells, spheroplasts and chromatophores of *Rhodopseudomonas sphaeroides*. When the membrane potential (potential difference between bulk aqueous phases) was kept constant in the presence of carbonylcyanide *m*-chlorophenylhydrazone, addition of salt to a suspension of cells or spheroplasts induced a red shift in the carotenoid absorption spectrum which indicated a change in the intramembrane electrical field. The spectral shift is explained by a rise in electrical potential at the outside surface of the photosynthetic membrane due to a decrease in extent of the negative surface potential.

The spectral shift occurred in the direction opposite to that in chromatophores, indicating that the sidedness of the membrane of cells or spheroplasts is opposite to that of chromatophores. The dependences of the extent of the potential change on concentration and valence of cations of salts agreed with the Gouy-Chapman relationship on the electrical diffuse double layer. The charge density on the periplasmic surface of the photosynthetic membrane was estimated to be $-2.9 \cdot 10^{-3}$ elementary charge per Å^2, while that on the cytoplasmic side surface was calculated as $-1.9 \cdot 10^{-3}$ elementary charge per Å^2 (Matsuura, K., Masamoto, K., Itoh, S. and Nishimura, M. (1979) Biochim. Biophys. Acta 547, 91—102). Surface potential on the periplasmic side of the photosynthetic membrane was estimated to be about —50 mV at pH 7.8 in the presence of 0.1 M monovalent salt.

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; Mes, morpholinoethanesulfonate; Tricine, *N*-tris[hydroxymethyl]methylglycine.

Introduction

Surface potential, the electrical potential at the membrane surface with a reference in the bulk aqueous phase, has been shown to be pivotal in some energy transducing processes of photosynthetic membranes [1—7]. In a previous paper [6] we showed that a change in the surface potential induced a change in the intramembrane electrical field in chromatophores from *Rhodopseudomonas sphaeroides*. The addition of salts to the chromatophore suspension induced a blue shift of the carotenoid spectrum under the conditions of constant membrane potential in the presence of CCCP. The change in the intramembrane electrical field measured by the spectral shift [8,9] was explained quantitatively in terms of the change in surface potential on the outer side of the chromatophore membrane [6], according to the Gouy-Capman diffuse double layer theory (note the change in electrical potential profile accompanying the salt addition to chromatophores in Fig. 1).

Chromatophores are membrane vesicles which have the sidedness opposite to cells and spheroplasts in photosynthetic bacteria [9,10]. Reflecting this situation, when an inside-positive membrane potential was induced by KCl addition in the presence of valinomycin, a red shift of carotenoid spectrum was observed in chromatophores, while a blue shift was observed in spheroplasts [9]. In spite of the difference in the direction of the shift, the potential-absorbance change relationship was common if the reference was taken on the cytoplasmic side (or the periplasmic side). The sidedness of membrane structure must be taken into account in discussing the carotenoid shift induced by surface potential change (Fig. 1).

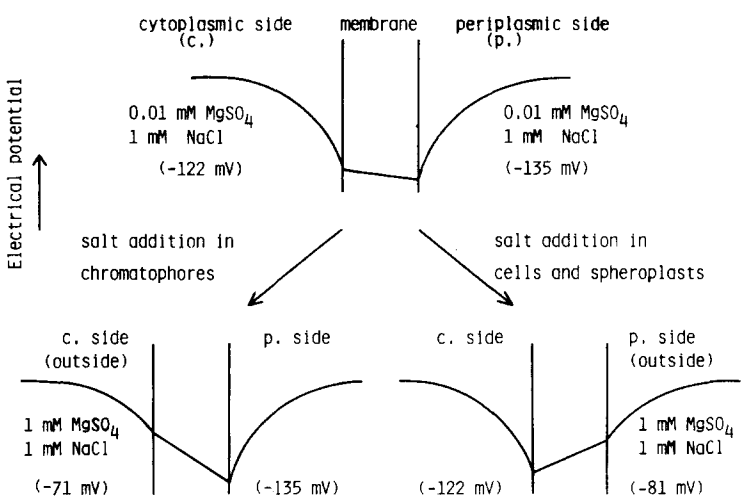

Fig. 1. Schematic diagram showing potential profiles in the vicinity of the photosynthetic membrane of *R. sphaeroides* before and after salt addition to each side of the membrane in the presence of CCCP. The values of surface potential indicated in parentheses were estimated using the Gouy-Chapman equation for the electrolyte concentrations indicated. The values of $-1.9 \cdot 10^{-3}$ elementary charge per $Å^2$ for the cytoplasmic surface [6] and $-2.9 \cdot 10^{-3}$ elementary charge per $Å^2$ for the periplasmic surface (estimated in the present study) were used. Membrane permeability to the salt added (1 mM $MgSO_4$) and the presence of fixed charges (except those on the membrane surfaces) were ignored.

In the present study, we estimated the surface potential on the periplasmic side of the photosynthetic membrane, by means of the spectral shift of carotenoid induced by adding salts to suspensions of cells and spheroplasts of *R. sphaeroides* in the presence of CCCP. The value of surface potential on the inner side of chromatophores, which is equivalent to the periplasmic side of photosynthetic membrane in cells [10], was also estimated with the spectral shift in the presence of Triton X-100 at a low concentration.

Materials and Methods

R. sphaeroides was grown under illumination as described previously [6]. Cells were washed three times with 2 mM Tricine/NaOH (pH 7.8) and 0.01 mM $MgSO_4$ and suspended in the same medium. Spheroplasts were prepared as described previously [9] except that the suspension was diluted with the same volume of distilled water after the lysozyme addition [11]. The spheroplasts were washed once with 0.1 M sucrose, 1 mM $MgSO_4$ and 2 mM Tricine/NaOH (pH 7.8) and suspended in 0.1 M sucrose and 2 mM Tricine/NaOH (pH 7.8). Chromatophores were prepared as described previously [6,12].

Absorbance changes of carotenoid by salt additions were measured as previously described [6,9] in the presence of 10 μM CCCP at 22°C.

Results

Salt-induced carotenoid absorbance changes in cells

When 10 mM NaCl or 0.1 mM $MgSO_4$ was added to cells suspended in 2 mM Tricine/NaOH and 0.01 mM $MgSO_4$ (pH 7.8) in the presence of 10 μM CCCP, the difference absorbance (488 minus 506 nm) was increased (Fig. 2). The changes were smaller after the addition of another salt. The direction of the salt-induced carotenoid absorbance change was opposite to that in chromatophores [6]. The spectral change was of the red-shift type, superimposed on a nonspecific change in absorbance probably due to the light-scattering change. The maxima of the difference spectra were at 459, 491, 525 nm and the minima, at 444, 474, 509 nm (Fig. 3). The wavelengths of these peaks were similar to those of the red shift induced by light or K^+-diffusion potential [8,9].

Fig. 4A shows the pH dependence of the extent of the salt-induced absorbance change of carotenoid. At pH 4.5 the salt addition induced little change. At pH values higher than 4.5, the difference absorbance was increased by salt additions. At the zero-change pH (approx. 4.5), the membrane surface is probably close to electrical neutrality (no net charge). The pH dependences were similar in spite of the difference in salt-species used.

Fig. 5A shows the concentration dependence of carotenoid absorbance change induced by adding various salts of mono- and divalent ions at pH 7.8. The salts of divalent cations were effective at much lower concentrations than monovalent ones. NaCl, Na_2SO_4 and KCl gave an essentially identical concentration dependence curve. $CaCl_2$ induced larger increases than $MgCl_2$ or $MgSO_4$ at the same concentrations. The difference between calcium and magnesium was not seen in the experiments with chromatophores [6]. Binding of Ca^{2+}

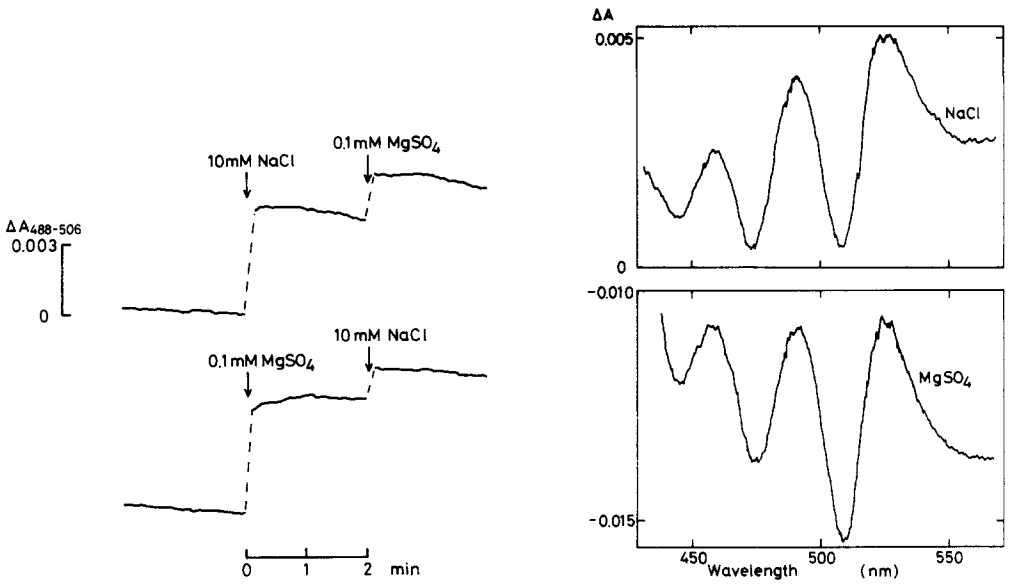

Fig. 2. Absorbance changes of carotenoid in cells induced by NaCl and $MgSO_4$ additions. Cells were suspended in 2 mM Tricine/NaOH (pH 7.8) and 0.01 mM $MgSO_4$ at the concentration of 10 μM bacteriochlorophyll. 10 μM CCCP was present. Traces of absorbance change on the dual-wavelength mode (488 minus 506 nm) are shown. An aliquot of 3 M NaCl or 0.15 M $MgSO_4$ solution was added at the times indicated by arrows to make the final concentration indicated.

Fig. 3. Absorption spectrum changes in cells upon additions of salts. The difference spectra ('salt-added' minus 'no addition') were recorded in the split-beam mode with a scanning speed of 10 nm/s about 30 s after the addition of 10 mM NaCl or 0.1 mM $MgSO_4$ (final concentrations). Other conditions were the same as those in Fig. 2.

on the periplasmic surface might have reduced the negative surface-charge density and induced the larger surface-potential change than that found with magnesium [13]. Pre-existence of other salts made the salt-induced changes smaller, as expected from the Gouy-Chapman theory.

Salt-induced carotenoid absorbance changes in spheroplasts

Intact cells have cell walls and contain protein molecules in the periplasmic space. Charges on these may influence the electrical potential on the outer surface of the inner photosynthetic membrane. To evaluate this effect we used spheroplasts in comparison with intact cells. Fig. 4B shows the pH dependence of the carotenoid change induced by the addition of 1 mM $MgSO_4$ in spheroplasts. The curve was similar to that in cells. Fig. 5B shows the dependence on salt concentration. Additions of salts of monovalent cations caused almost no effect (data not shown). The extent of the change caused by the divalent cation salt was smaller than that in cells. The presence of 10 mM NaCl in the suspending medium decreased the change by $CaCl_2$ or $MgSO_4$ only slightly, as contrasted to the case with cells. The smaller effect (divalent cations) and the diminished effect (monovalent cations) in spheroplasts are probably due to the situation that some salts used during the preparation of spheroplasts were introduced into the reaction mixture, as the washing was rather mild to prevent

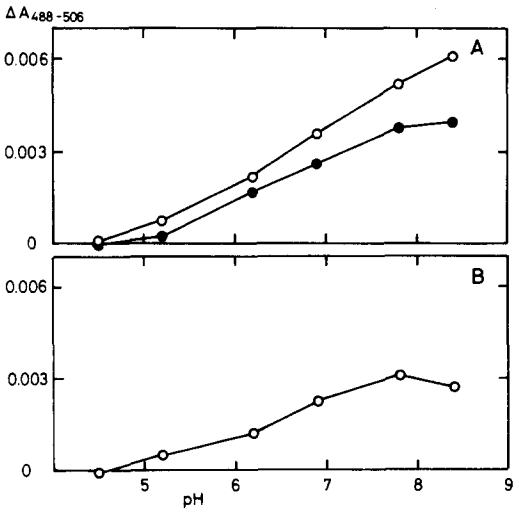

Fig. 4. pH dependence of carotenoid absorbance in cells (A) and in spheroplasts (B) induced by addition of $MgSO_4$ or NaCl. Cells or spheroplasts were suspended in Mes (pH < 7) or Tricine (pH > 7) buffer adjusted at various pH values with NaOH in which Na^+ concentration was kept at 1 mM by changing the buffer concentration or by adding NaCl. 0.01 mM $MgSO_4$ was present. Absorbance changes 30 s after the additions in a series of experiments similar to those in Fig. 2 were plotted. ○, 0.5 mM $MgSO_4$ in cells (A) and 1 mM $MgSO_4$ in spheroplasts (B); ●, 5 mM NaCl.

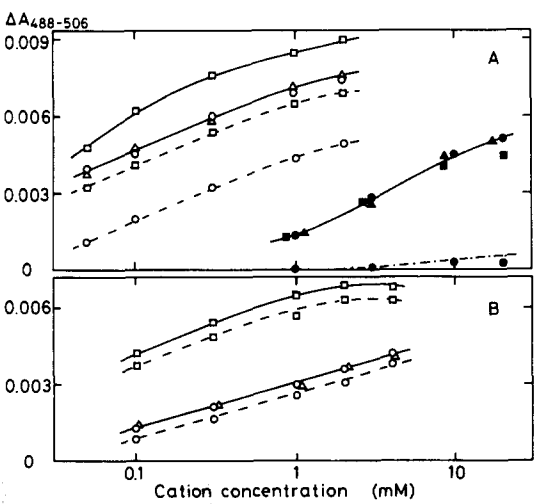

Fig. 5. Dependence of carotenoid absorbance change in cells (A) and spheroplasts (B) on concentration of salts added. Cells or spheroplasts were suspended in 2 mM Tricine/NaOH (pH 7.8) and 0.01 mM $MgSO_4$ (———), in 2 mM Tricine/NaOH (pH 7.8), 0.01 mM $MgSO_4$ and 10 mM NaCl (- - - - -), and in 2 mM Tricine/NaOH (pH 7.8) and 10 mM $MgSO_4$ (· — · — ·). Absorbance changes 30 s after the salt additions were plotted against the final concentrations of the salts (on logarithmic scale). Salts added were $CaCl_2$ (□), $MgSO_4$ (○), $MgCl_2$ (△), NaCl (●), Na_2SO_4 (▲), KCl (■). Other conditions were the same as those in Fig. 2.

the disruption of spheroplast structure. $CaCl_2$ was more effective than $MgSO_4$ in spheroplasts, too.

Salt-induced carotenoid absorbance changes in chromatophores in the presence of detergent

Photosynthetic membrane of *R. sphaeroides* has low permeability to the ionic species used in this study. Therefore, only the outside surface potential is expected to change upon salt addition. The inner surface of chromatophores is known to correspond to the outer surface of photosynthetic membrane of cells or spheroplasts, i.e., the surface on the periplasmic side. To change the surface potential of inner side of chromatophores, salts were added in the presence of a neutral detergent, Triton X-100, at a low concentration. In the presence of 0.01% Triton X-100, chromatophores maintained the closed vesicle structure yet had high permeability to ions, as judged from the time courses of light-induced carotenoid absorption change and of pH change detected by electrode. In thylakoid membranes, 0.01% Triton X-100 is known to cause little effect on the surface charge density of the membrane [5,7].

Fig. 6 shows time courses of carotenoid absorbance changes by salt additions in chromatophores in the presence and absence of 0.01% Triton X-100. In the absence of the detergent, the salt addition decreased the difference absorbance, which corresponds to the blue shift of carotenoid spectrum as reported previously [6]. In the presence of 0.01% Triton X-100, the salt addition induced a small transient decrease and a new stationary level in a few minutes. The level finally attained probably reflects the changes of surface potential on both sides of the chromatophore membrane by the salt addition. On the other hand, the level attained after the salt addition in the absence of the detergent represented the change only in the outside surface potential [6]. Therefore,

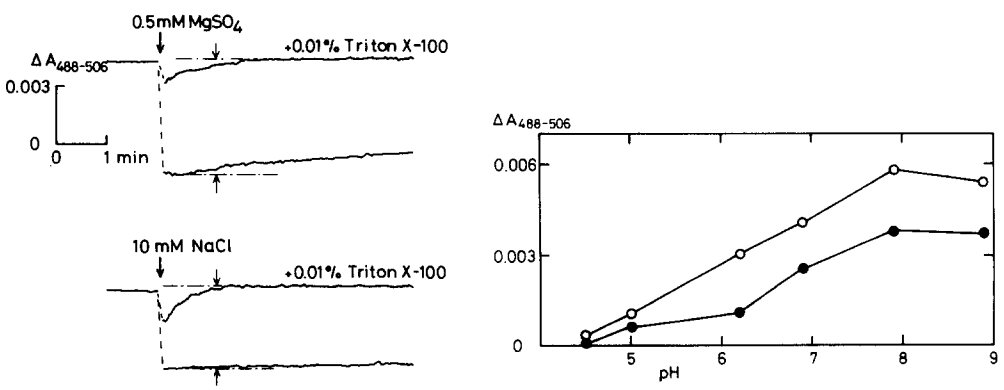

Fig. 6. Absorbance changes of carotenoid in chromatophores induced by $MgSO_4$ and NaCl additions. Chromatophores (10 μM bacteriochlorophyll) were suspended in 2 mM Tricine/NaOH (pH 7.8) and 0.01 mM $MgSO_4$ with and without 0.01% Triton X-100. The small arrows are explained in the text. Other conditions were the same as those in Fig. 2.

Fig. 7. pH dependence of the salt-induced carotenoid absorbance change in chromatophores related to the inside surface potential change. The difference between the absorbance with and without 0.01% Triton X-100 as indicated with small arrows in Fig. 6 was plotted. ○, 0.5 mM $MgSO_4$; ●, 5 mM NaCl. The conditions and procedures were similar to those given in Figs. 4 and 6.

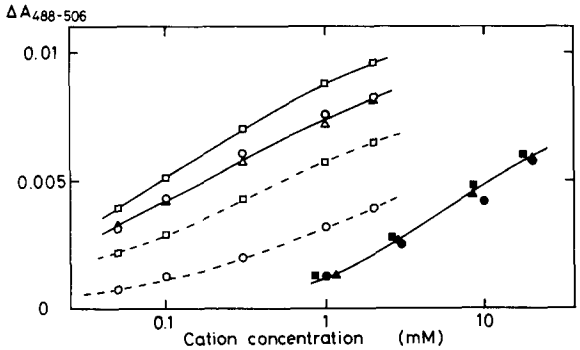

Fig. 8. Concentration dependence of salt-induced carotenoid absorbance change in chromatophores related to the inside surface potential change. The difference between the absorbance changes with and without 0.01% Triton X-100 was plotted from a series of experiments as given in Fig. 6. ———, 2 mM Tricine/NaOH (pH 7.8) and 0.01 mM $MgSO_4$; - - - - -, supplemented with 10 mM NaCl. Salts added were $CaCl_2$ (□), $MgSO_4$ (○), $MgCl_2$ (△), NaCl (●), Na_2SO_4 (▲), and KCl (■).

the difference between the two levels, which is shown by small arrows in Fig. 6, should indicate the surface potential change of the inner side.

Figs. 7 and 8 show the pH and salt concentration dependences of the carotenoid change which is ascribed to the change in the inside surface potential. The data in both figures are similar to those obtained in the case of cells (Figs. 4A and 5A). However, they are different from the results for chromatophores in the absence of detergent [6] in two respects, besides the direction of the change. One is the values of null-change pH, about 4.5 on the inner surface of chromatophores and about 5.2 on the outer surface [6]. The other is the effect of Ca^{2+}, which was stronger than that of magnesium on the inner surface of chromatophores but not on the outer surface of chromatophores [6].

Discussion

The red shift of the carotenoid spectrum induced by the addition of salts in uncoupled cells and spheroplasts is explained in terms of the change of surface potential of the photosynthetic membrane on the periplasmic side. As discussed in detail in a previous paper [6], the addition of salts will decrease extent of the negative surface potential, and the potential at the outside surface will become more positive. The outside-positive potential change induces a blue shift in chromatophores, and a red shift in cells and spheroplasts, because of the opposite sidedness of the membrane structures [9,10].

The profile of electrical potential on the outer side of the photosynthetic membrane in cells is affected by fixed charges on cell wall and in the periplasmic space. However, the effect of charges on peptidoglycan, outer membrane and capsule on the surface potential of photosynthetic membrane should be negligible, because the distance of electrostatic interaction in water is short even at relatively low salt concentrations and because the major part of the photosynthetic membrane is invaginated in *R. sphaeroides*. This was verified by the similar pH and salt concentration dependences in cells and in chro-

matophores with detergent (Figs. 4A and 7, Figs. 5A and 8). However, the Donnan potential in the periplasmic space cannot be neglected altogether [14]. Many protein molecules with fixed charges are trapped in the periplasmic space. But, when the charge density on the photosynthetic membrane is large, the potential difference between the membrane surface and external aqueous phase is little affected by the Donnan potential of the periplasmic space, as a simple calculation with the Gouy-Chapman and Donnan equations show. This situation is supported by experiments on spheroplasts (Figs. 4, 5).

The absorbance change of carotenoid was calibrated by H^+-diffusion potential in cells and by K^+-diffusion potential in chromatophores [8,9]. When the difference absorbance, 488 minus 506 nm, was used, ΔA/mV was 0.0001 per 1 cm in the sample with 10 μM bacteriochlorophyll. The surface potential change reached a maximum of about 90 mV in this study. In the data shown in Fig. 8, 0.1 mM $MgSO_4$ had the same effect as 8 mM NaCl. Using the Gouy-Chapman equation [6], the surface charge density was calculated to be $-2.9 \cdot 10^{-3}$ elementary charge per Å^2 from those values. It was somewhat larger than the charge density on the cytoplasmic side of the membrane ($-1.9 \cdot 10^{-3}$ elementary charges per Å^2) [6]. The calculated charge density explains satisfactorily the salt concentration dependence in cells (Fig. 5A) and chromatophores in the presence of detergent (Fig. 8) according to the Gouy-Chapman theory. Although high salt concentrations cannot be used in the method described in this study, a surface potential of -53 mV was calculated for the periplasmic surface of membranes in 100 mM monovalent salt at a neutral pH from the charge density estimated.

Rumberg and Muhle [1] have discussed the intramembrane electrical field change in terms of the surface potential change of the inner surface of the thylakoid vesicles induced by the pH decrease under illumination (see also Ref. 15). In chromatophores, too, the intravesicular pH decreases by illumination [16], and extent of the negative surface potential on the inner surface must decrease. Because of the surface potential change, the light-induced potential change measured with carotenoid shift is an overestimation as the change of membrane potential (potential difference between bulk aqueous phases). Values of electrochemical potential of H^+ estimated by the carotenoid shift and by an indicator of transmembrane pH difference, such as 9-aminoacridine [16], may also become overestimated. In the extreme cases when the surface potential on the inner side becomes zero due to internal acidification, the error may reach 50 mV in 0.1 M monovalent salt at pH 8.0. In many cases the error may be smaller due to the higher internal pH than to the zero-charge-density pH under illumination [17].

The periplasmic side of the photosynthetic membrane in living cells is exposed to medium containing various ionic species. Variation of the surface pH [7] may be smaller than that of the bulk pH. At the neutral-to-alkaline pH values, the surface potential has negative values and the surface pH should become lower than the bulk pH. A rise (or drop) in the bulk pH will induce a drop (or rise) in the surface potential, which will partially counteract the rise (or drop) of pH at the surface. Surface concentrations of ionic substrates must also differ from those in the external medium [5], and the surface concentrations may be more important than the bulk-phase values in many biological processes involving ionic reactants.

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