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Bacteriorhodopsin's M_{412} and BR_{605} protein conformations are similar

Significance for proton transport

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Cation removal or acidification induces a transition of bacteriorhodopsin (BR_{568}) to a blue species (BR_{605}). Fourier transform infrared spectroscopy now reveals that the protein conformational changes accompanying this transition resemble those which occur during the BR_{568} to M_{412} transition and associated light-driven proton transport. This and other results suggest that BR_{605} and M_{412} both have similar voltage-dependent protein conformations in which a secondary structure is stabilized by a change in the local electric potential sensed by internal charged groups involved in proton transport.

Purple membrane; Infrared spectroscopy; Cation binding; Membrane potential; Conformational change

1. INTRODUCTION

The membrane protein, bacteriorhodopsin (BR), found in the purple membrane of *Halobacterium halobium* functions as a light-driven proton pump [1]. Absorption of a photon by the light-adapted state, BR_{568}, results in a photocycle with intermediates exhibiting well defined absorption maxima and lifetimes. The formation of the M_{412} intermediate is of special interest since it is correlated with the release of at least one proton at the extracellular surface of purple membrane.

Both lowering of the pH and removal of divalent cations induce a reversible transition of bacteriorhodopsin to a blue state denoted BR_{605} [2–6]. In order to study this transition, we have utilized FTIR difference spectroscopy which is extremely sensitive to structural alterations in the protein, chromophore and lipid components of the membrane [7]. Instead of the conventional transmission method, IR absorption was measured by the attenuated total reflection (ATR) of a thin layer of purple membrane coated on a Ge internal reflection crystal [8]. This method allowed us to control the pH and ionic composition of the solution bathing the purple membrane film.

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2. MATERIALS AND METHODS

FTIR-ATR pH difference spectra were recorded for light-adapted purple membrane films deposited on the surface of a Ge internal reflection crystal following the method given in [8]. The bathing solution consisted of 35 mM $CaCl_2$ and the pH was adjusted by HCl and $Ca(OH)_2$ titration. In order to insure full light adaptation, measurements were made with continuous illumination. Fourier self-deconvolution [9] was performed using a half-width of 15 cm^{-1} and a resolution enhancement

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factor of 2.5 chosen to avoid the appearance of side lobes in the individual absorbance spectra. The absence of visible light in the dark-adapted pH difference measurements precludes the possibility that steady-state M_{412} is present.

3. RESULTS AND DISCUSSION

Fig.1 shows difference spectra measured for various pH shifts including the range 2.5→2.2 which is near the p*K*a for the purple to blue transition as determined by visible absorption measurements. Figs 2A and 3A show deconvolved difference spectra over this same pH range. Deconvolution has the effect of enhancing peaks which arise from single molecular groups [9]. Several of the vibrational modes which are characteristic of the BR_{568} chromophore appear as negative peaks including those at 1527 cm^{-1} (C=C stretch); 1254, 1214, 1200 and 1167 cm^{-1} (C−C stretches); and 1640 cm^{-1} (C=N stretch) [10]. Positive peaks characteristic of the BR_{605} chromophore are also found in the fingerprint region at 1181 and 1173 cm^{-1} (C−C stretch), 1520 cm^{-1} (C=C stretch) and 1624 cm^{-1} (C=N stretch) [11–13] (cf. fig.2A). Overall, these spectral features reflect the isomerization of the chromophore from a 13-*trans* to 13-*cis*/13-*trans* mixture during the conversion of BR_{568} to BR_{605} [3]. When a pH difference spectrum (figs 1B and 2B) is measured for dark-adapted purple membrane which consists of a mixture of BR_{568} and BR_{548} [14], negative peaks are also detected which are characteristic of the BR_{548} chromophore at 1636 cm^{-1} (C=N stretch) and 1536 (C=C stretch). A partial cancellation of the 1182 cm^{-1} peak in BR_{605} by a peak at slightly lower frequency in BR_{548} is also observed.

A remarkable feature of the deconvolved pH 2.5→2.2 difference spectra (figs 2A and 3A) is the high degree of similarity to the deconvolved BR_{568}→M_{412} difference spectrum (figs 2C and 3C). The agreement holds particularly well for those peaks assigned to protein bands. For example, the peaks at 1516 (−) and 1511 (+) cm^{-1} which arise from two tyrosine groups, one which deprotonates during the formation of M_{412} (negative 1516 cm^{-1}) [16] and a second group which protonates earlier during the formation of K_{630} (positive 1511 cm^{-1}) [17,18]. The appearance of these bands as well as

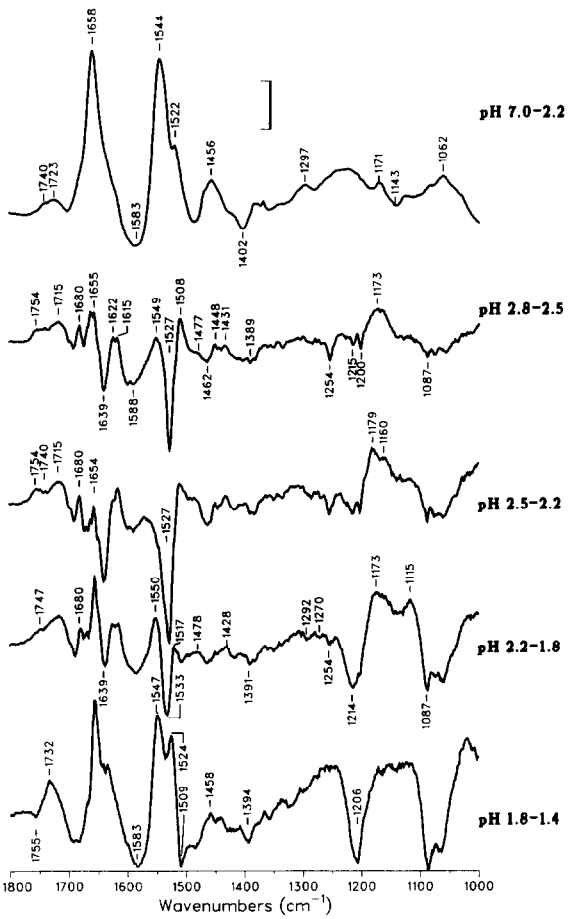

Fig.1. FTIR-ATR difference spectra of light-adapted purple membrane films. The scale bar size from top to bottom spectrum equals 0.0253, 0.0060, 0.0047, 0.0038, 0.0024 A.U.

other characteristic tyrosinate marker peaks such as at 1276 and 1271 cm^{-1} in the pH difference spectrum indicates that similar tyrosine deprotonation/protonation reactions occur for the conversion of BR_{568} to BR_{605}.

Close agreement is also evident in other regions, including peaks found above 1700 cm^{-1} which arise from protonation changes in Asp or Glu carboxyl groups [7,16–19]. One exception is the 1762 cm^{-1} band which may originate from the protonation of a carboxylate group which acts as the acceptor for the Schiff base proton during formation of M_{412}. Since BR_{605} has a protonated Schiff base [11], the appearance of this peak would not be expected.

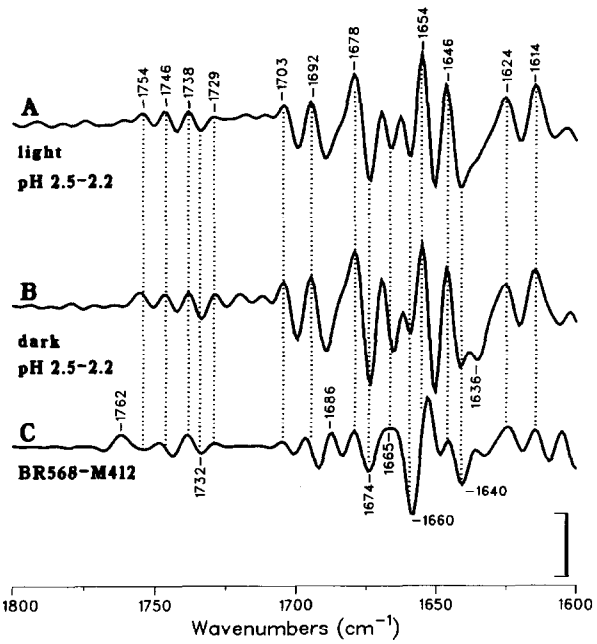

Fig.2. Deconvolution of light-adapted (A), dark-adapted (B) pH difference spectra (2.5→2.2) with BR_{568} to M_{412} difference spectrum (C) measured at 250 K [16]. Scale bar is 0.0270, 0.0258 and 0.1169 A.U. (A–C).

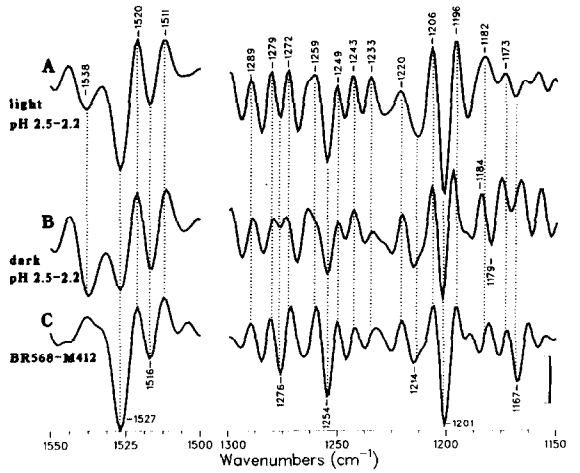

Fig.3. Same as fig.2 over wavenumber ranges as indicated. Scale bar in the 1550–1500 cm^{-1} range is 0.0281, 0.0337 and 0.1014 A.U. (A–C) and in the 1300–1150 cm^{-1} range is 0.0113, 0.0107 and 0.0884 A.U. (A–C).

Not all of the peaks in the pH 2.5→2.2 difference spectrum or spectra in other pH ranges agree with the BR_{568}→M_{412} difference spectrum, particularly near 1720, 1655, 1585, 1545, 1400, 1210, 1087 and 1062 cm^{-1}. These discrepancies are evident in spectra over the pH range 7→2.2 as well as in the smaller pH ranges shown in fig.1 and can be attributed to either variation in the film thickness or to pH titration of ionized carboxylate (Asp and Glu) or phosphoryl groups from lipids.

The changes associated with the titration of carboxylate groups appear at 1720 cm^{-1} (C=O stretch of COOH), 1400 and 1583 cm^{-1} (symmetric and antisymmetric stretch, respectively, of COO^-) and occur over the entire range 7 to 1.4. In contrast features associated with phosphoryl groups including at 1210 and 1087 cm^{-1} (antisymmetric and symmetric PO_2^-, respectively) and 1062 cm^{-1} (P–O–C stretch) occur mainly below pH 2.5. Both sets of changes are expected on the basis of the titration of solvent accessible carboxylate and phosphoryl groups which have p*K*a values of 4 and 1.5, respectively. Similar features were also observed recently by FTIR difference spectroscopy using the transmittance method upon exposure of a deionized PM film to NH_4 vapor [20]. This study concluded that over 14 water-exposed carboxyl groups are protonated during the formation of blue purple membrane (BR_{605}). However, our present results indicate that the actual BR_{568}→BR_{605} transition involves a more limited set of protonation changes which occur mostly in the pH range 3 to 2.

The large pH induced absorbance changes at 1655 and 1545 cm^{-1} can be attributed to a decrease of negative charge at the membrane surface and subsequent reduction in the purple membrane film thickness. In the case of ATR, a reduction in film thickness is expected to increase the sample absorbance including the strongly absorbing amide I and II bands at 1655 and 1545 cm^{-1}, respectively. Interestingly, these bands decrease slightly in the range between pH 2.5 and 2.2 and then begin to increase again at lower pH (fig.1). We believe that this reflects the buildup of predominantly positive charge on the membrane surface in this pH range which causes increasing repulsion between the layers. Below pH 2 anion shielding of the positive charge reduces the repulsion and allows the interlayer separation distance to decrease.

The question remains why the BR_{605} and M_{412} intermediates exhibit similar conformations. A

possible explanation is based on the existence of a positive charge displacement towards the extracellular side of the membrane during the formation of M_{412} which most likely reflects proton translocation [21]. While M_{412} formation and the accompanying charge polarization normally require photon energy, a local electrostatic field sufficiently large within BR in the reverse direction of the normal field could favor this charge movement and thereby stabilize an M_{412}-like conformation of BR (i.e. BR_{605}).

One mechanism by which the internal potential [22] of BR could be significantly altered is by changes in net charge on the cytoplasmic and extracellular surfaces. On the basis of the predicted 2-dimensional topology of BR [23], the net charge at pH 7 is expected to be -3 on the cytoplasmic side and -2 on the extracellular side, thus producing an electrostatic field in the same direction as the normal transmembrane field (i.e. opposing proton transport). However, as the pH is lowered below the p*K*a for Asp and Glu carboxyl groups (4.0), the positively charged lysine and arginine groups begin to dominate, producing a net charge of $+7$ on the cytoplasmic surface and $+3$ on the external surface. This would tend to favor positive charge movement towards the extramembrane surface, and could explain why purple membrane binds to polylysine-coated glass with its cytoplasmic side at pH 7 and extracellular side at pH 3 [24]. The predominance of phosphoryl groups from lipids on the cytoplasmic side which have a p*K*a near 1.4 [25] may also play a role in this transition and lower the effective pH at which the local electrostatic field favors rather than opposes the positive charge movement. At even lower pH, the positive charge is expected to be shielded by the high concentration of anions, in this case Cl^- from HCl, thus offering an explanation for the formation of acid BR_{568}.

Several previous studies suggest that the major effect of cation removal is to lower the effective surface pH below the p*K*a for carboxyl groups by increasing the negative surface charge density due to membrane lipids [3–5]. Consistent with this picture, acid induced formation of BR_{605} is independent of cation concentration in delipidated purple membrane [26]. It is not clear, however, from these studies whether the transition to BR_{605} requires the titration of one or more key carboxyl groups or can occur as long as the local internal potential favors the movement of charges associated with this transition.

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