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Vibrational Spectroscopy of Bacteriorhodopsin Mutants: Light-Driven Proton Transport Involves Protonation Changes of Aspartic Acid Residues 85, 96, and 212†

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ABSTRACT: Fourier transform infrared (FTIR) difference spectra have been obtained for the bR → K, bR → L, and bR → M photoreactions in bacteriorhodopsin mutants in which Asp residues 85, 96, 115, and 212 have been replaced by Asn and by Glu. Difference peaks that had previously been attributed to Asp COOH groups on the basis of isotopic labeling were absent or shifted in these mutants. In general, each COOH peak was affected strongly by mutation at only one of the four residues. Thus, it was possible to assign each peak tentatively to a particular Asp. From these assignments, a model for the proton-pumping mechanism of bR is derived, which features proton transfers among Asp-85, -96, and -212, the chromophore Schiff base, and other ionizable groups within the protein. The model can explain the observed COOH peaks in the FTIR difference spectra of bR photointermediates and could also account for other recent results on site-directed mutants of bR.

Proton transport by membrane proteins is of great interest because it is involved in energy coupling in numerous biological systems. The purple membrane (PM)[1] from *Halobacterium halobium* is one of the simplest and most intensively studied proton transport systems (Stoeckenius & Bogomolni, 1982). PM contains a single 26 000-Da protein, bacteriorhodopsin (bR), which utilizes the energy of visible light to pump protons against an electrochemical gradient across the membrane. Elucidating the mechanism of bR will almost certainly require determining the details of the structural changes that occur during its photocycle.

Vibrational spectroscopy is a useful tool for examining the role of individual groups in protein mechanisms. For example, resonance Raman spectra of bR photointermediates have provided detailed information about structural changes of the retinylidene Schiff base chromophore [for a review, see Smith et al. (1985)]. Unraveling the bR mechanism will also require elucidation of structural changes in the protein moiety, and for this FTIR spectroscopy is especially well suited [for a recent review, see Braiman and Rothschild (1988)]. Techniques for obtaining high-quality FTIR difference spectra from bR and its K, L, and M photointermediates have been developed (Rothschild et al., 1981; Rothschild & Marrero, 1982; Bagley et al., 1982; Siebert & Mäntele, 1983). It is possible to assign some of the bands in these difference spectra to specific residues in the primary sequence by using a combination of isotope labeling and site-directed mutagenesis. For example, selective isotope labeling of tyrosines in bR permitted the assignment of a set of bands in the FTIR difference spectra to tyrosine and tyrosinate (Rothschild et al., 1986; Dollinger et al., 1986; Roepe et al., 1987). Site-directed mutagenesis of each of the 11 Tyr residues (Mogi et al., 1987) made it possible to assign some of these bands specifically to Tyr-185 (Braiman et al., 1988). These assignments led to the conclusion that tyrosinate-185 protonates and then deprotonates during the photocycle.

Protonation changes in a carboxyl residue also occur during the bR photocycle, as could be inferred from the first FTIR difference measurements on bR (Rothschild et al., 1981). Subsequent studies indicated that more than one residue was involved (Siebert et al., 1982; Engelhard et al., 1985; Roepe et al., 1987), and selective isotope labeling demonstrated that all of the residues involved are Asp rather than Glu (Eisenstein et al., 1987). Of the nine Asp residues in bR, only four (Asp-85, -96, -115, and -212) are thought to be buried in the bilayer region of the membrane (Engelman et al., 1985; Huang et al., 1982). Recently all of the Asp residues were substituted by Asn and/or Glu in order to determine their role in proton pumping (Mogi et al., 1988). Evidence was obtained for the first time that Asp-85, -96, and -212 are involved in a proton-conductance mechanism. Mutations at Asp-115 were also found to affect proton-pumping activity. Therefore, in the present FTIR measurements we have focused on these four residues. On the basis of spectra of bR mutants, we can make preliminary assignments for each of the COOH (1720–1760

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[1] Abbreviations: PM, purple membrane; bR, bacteriorhodopsin; FTIR, Fourier transform infrared; au, absorbance units; sh, shoulder.

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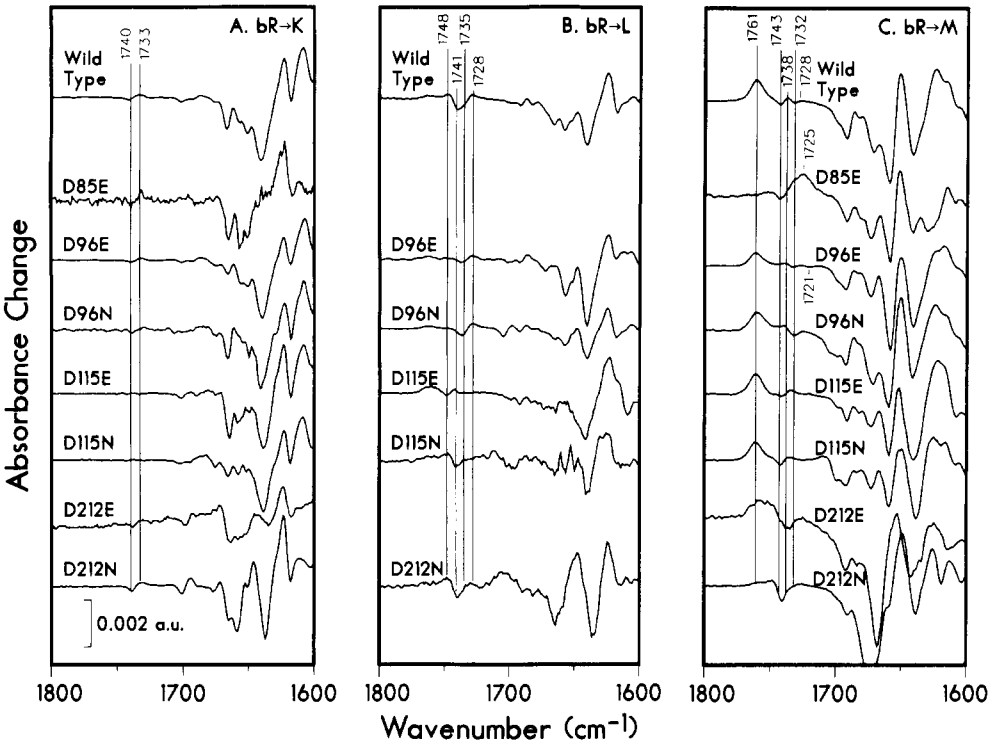

FIGURE 1: FTIR difference spectra of bacteriorhodopsins with the indicated point mutations. The bar in panel A indicates approximately the scale of all the spectra. (A) bR → K difference spectra, obtained at 77 K according to the procedure described in Braiman et al. (1988). (B) bR → L difference spectra, obtained as follows. After light adaptation at 295 K, the sample was cooled in the dark to 170 K. An absorbance difference spectrum was calculated from scans obtained for 20 min in the dark followed by scans obtained for 20 min under 500-nm illumination. The sample was then warmed to 250 K, held at that temperature for 20 min, and recooled to 170 K (all in the dark). When the temperature was stable, another light–dark difference spectrum was obtained. The cycle was repeated 15–25 times, and the difference spectra were averaged together. (C) bR → M difference spectra, obtained according to the procedure described in Braiman et al. (1988).

cm^{-1}) peaks to one of these four buried Asp groups. These assignments allow us to specify the protonation states of Asp-85, -96, -115, and -212 at the bR, K, L, and M states of the photocycle. We propose a model for the mechanism of proton transport by bR that is consistent with these results.

Methods

The construction of genes coding for the Asp → Asn and Asp → Glu mutants was described previously (Mogi et al., 1988). Mutant bacterioopsin apoproteins were expressed, purified, and reconstituted as with the Tyr → Phe mutants (Braiman et al., 1988). FTIR difference spectra of the bR → K, bR → L, and bR → M transitions were measured as described previously (Rothschild et al., 1984; Roepe et al., 1987; Braiman et al., 1988), with a spectral resolution of 4 cm^{-1}. See the caption to Figure 1 for additional details.

Results

FTIR difference spectra of the bR → K, bR → L, and bR → M photoreactions of the wild type and mutants are shown in Figure 1. Each of the four Asp residues could be replaced with either Asn or Glu and still bind retinal to give a protonated Schiff base chromophore (Mogi et al., 1988). However, not all of the mutants exhibited normal photochemical behavior. For example, the mutants D212E[2] and D212N, which showed abnormal light/dark adaptation (Mogi et al., 1988), also gave "bR → K" difference spectra with significant contributions due to the primary photoreaction of the 13-cis component of dark-adapted bR (bR_{548}).

Attempts to obtain difference spectra of the L and M photoproducts of some of the mutants also gave perturbed results. For example, the D212N mutant exhibited a difference spectrum characteristic of the bR → L transition under conditions used normally to obtain a bR → M difference spectrum (see Figure 1C). Some spectra of other mutants (e.g., D85N) did not exhibit features characteristic of known bR photoproducts and were therefore not useful for making assignments. We have generally limited our comparisons in Figure 1 to those mutants that under standard experimental conditions exhibited predominantly normal difference spectra (including the region from 800 to 1600 cm^{-1} not shown in the figure).

bR → K Difference Spectra. The 1740- and 1733-cm^{-1} peaks (Figure 1A) were initially assigned to the C=O stretch of a glutamic acid residue undergoing a change in environment during the bR → K reaction (Engelhard et al., 1985). However, more recent studies utilizing both [^{13}C]Asp and [^{13}C]Glu demonstrated that these peaks are due to Asp (Eisenstein et al., 1987). Both peaks are eliminated in the D115E and D115N mutants, while they are present in all of the others. We therefore attribute these peaks to a structural alteration of a single residue, Asp-115, during the bR → K photoreaction.

bR → L Difference Spectra. In the wild-type bR → L difference spectrum, there are positive features at 1748 and 1728 cm^{-1} and a larger negative peak at 1741 cm^{-1} with a shoulder at ~1735 cm^{-1}. All of these peaks have previously been assigned to Asp COOH groups, on the basis of shifts observed when bR is suspended in ^2H_2O and when it is labeled with [^{13}C]Asp (Engelhard et al., 1985; Eisenstein et al., 1987). In the D96E and D96N mutants, the negative peak at 1741 cm^{-1} and the positive lobe at 1748 cm^{-1} are gone, while the

[2] Designations for bR mutants make use of the standard one-letter abbreviations for amino acids. Thus "D212E" signifies the mutant in which the aspartic acid at residue 212 has been replaced by glutamic acid, while "D212N" signifies replacement of the same residue by asparagine.

Table I: Preliminary Assignments of Asp COOH Vibrations to Specific bR Residues[a]

	bR	K	L	M
Asp-85	COO^-	COO^-	COO^-	COOH (1761)
Asp-96	COOH (1742)	COOH (1742[b])	$COO^- \rightleftharpoons$ COOH (1748)	$COO^- \rightleftharpoons$ COOH (1748)
Asp-115	COOH (1740[c]/1735[d])	COOH (1733)	COOH (1729)	COOH (1729)
Asp-212	COO^-	COO^-	COO^-	COOH (1738)

[a] The observed COOH vibrations in FTIR difference spectra can be explained by assigning the protonation state of four Asp groups at each photointermediate step as indicated. The COOH frequencies listed under the column labeled bR appear as negative peaks in difference spectra of the wild type (see Figure 1), while those listed under the columns labeled K, L, and M appear as positive peaks. No assignments of COO^- vibrations have been attempted as these peaks are weaker and appear in crowded spectral regions where they are obscured by overlapping bands. [b] No FTIR difference peak observed because this COOH group is unchanged during bR → K photoreaction. [c] At 77 K. [d] Above 170 K.

positive lobe at 1728 cm^{-1} is unaffected and there is a residual negative peak near 1735 cm^{-1}. In the D115N mutant, complementary effects are observed: the positive lobe at 1728 cm^{-1} and the negative component at 1737 cm^{-1} are gone, while the negative peak at 1741 cm^{-1} and the positive lobe at 1748 cm^{-1} are largely unaffected.

On the basis of these results, we assign the positive 1728-cm^{-1} band and a roughly equal-sized negative 1735-cm^{-1} band to Asp-115. Likewise, we attribute a negative band at 1742 cm^{-1} (which contributes most of the intensity to the wild-type peak labeled at 1741 cm^{-1}) and the small positive lobe near 1748 cm^{-1} to Asp-96.

bR → M Difference Spectra. The wild-type bR → M difference spectrum (Figure 1C) exhibits several small positive (1748 cm^{-1} sh, 1728 cm^{-1}) and negative (1743, 1732 cm^{-1}) features at frequencies similar to those observed in the bR → L difference spectrum, along with two distinct new positive peaks at 1761 and 1738 cm^{-1}. It is likely that the first group of smaller features reflects changes in the two COOH groups already observed at the L state, while the two new positive peaks reflect the protonation of two additional Asp groups. In support of this, the former features show sensitivity to the same mutations as observed for the bR → L transition. In particular, the pair of peaks at 1732 and 1728 cm^{-1} is eliminated by substitution of either Asn or Glu at Asp-115, while the pair of peaks at 1748 and 1743 cm^{-1} is eliminated by the substitutions at Asp-96. In the case of the D96E mutant, it appears that the latter pair may be shifted down in frequency. (Only the negative component at 1721 cm^{-1} is labeled in the D96E spectrum in Figure 1C.) This downshift resulting from the additional methylene group in the Glu-96 side chain could reflect either a change in environment or a change in coupling of the C=O stretch with other vibrations of the same residue.

The 1761-cm^{-1} band in the wild-type spectrum can be assigned to Asp-85, since it shifts only in the D85E mutant. This band appears to downshift to 1725 cm^{-1}, in analogy to the aforementioned shift observed with the D96E mutant. Likewise, the 1738-cm^{-1} positive band is eliminated only in the D212E mutant and is thus tentatively assigned to Asp-212. However, no downshifted Glu-212 peak could be identified in this spectrum.

Discussion

Peak Assignments

All of the carboxylic acid bands observed in FTIR difference spectra of bR with its K, L, and M photoproducts were assigned previously to aspartic acid on the basis of selective isotope labeling (Eisenstein et al., 1987). Preliminary assignments of these bands to specific residues are summarized in Table I. Experiments with other aspartic acid mutants and with isotope labels will be required to confirm these assignments. Additional information can be obtained by analysis of COO^- peaks (Engelhard et al., 1985; Eisenstein et al., 1987), which should also be affected by Asp mutations if these groups cycle through different protonation states as indicated in Table I.

Asp-115. This residue gives rise to the negative/positive lobes observed at 1740/1733 cm^{-1} in the bR → K difference spectrum (Figure 1A) and at ~1732/1728 cm^{-1} in bR → L and bR → M spectra (Figure 1B,C). Asp-115 is expected to have only a single normal mode in this frequency region. The appearance of positive and negative lobes of nearly equal size indicates that this group undergoes a change in environment during the bR → K photoreaction. The downshift in frequency of both the negative and positive components between the bR → K spectrum (obtained at 77 K) and the others (bR → L, 170 K; bR → M, 250 K) indicates a temperature dependence of the C=O vibrational frequency.

Asp-96. As discussed above, a negative/positive pair of peaks at ~1742/1748 cm^{-1} in the bR → L and bR → M difference spectra can be assigned to Asp-96 COOH vibrations. In the bR → L spectrum, the positive component is somewhat smaller than the negative; thus it is unlikely that this pair represents a simple perturbation of a COOH group as in the case of Asp-115. Previous workers concluded that the negative 1742-cm^{-1} peak is due to the deprotonation of an Asp residue (Siebert et al., 1982; Engelhard et al., 1985; Eisenstein et al., 1987; Roepe et al., 1987). Our results are consistent with such a deprotonation of Asp-96 upon L formation. The smaller positive peak at 1748 cm^{-1} could then be explained by the concomitant formation of a small amount of protonated Asp-96 in a different environment than in bR.

Formation of M appears to lead to more complete reprotonation of Asp-96. In the bR → M difference spectrum (Figure 1C), the positive 1748-cm^{-1} and negative 1742-cm^{-1} components are similar in size and therefore cancel each other's intensity. A further reduction in the intensity of the 1742-cm^{-1} negative band probably occurs as a result of overlap with the new positive band at 1738 cm^{-1}.

Asp-85. Our assignment of the large 1761-cm^{-1} peak to Asp-85 is based on its apparent shift to 1725 cm^{-1} in the D85E mutant (Figure 1C). The known correlation of the carboxylic acid C=O stretch frequency with acidity (Bellamy, 1968) indicates that Asp-85 has an unusually low pK_a of ~2.5, as discussed previously (Rothschild et al., 1981). This unusual acidity could be explained partly by the proximity of Asp-85 to a positive charge. In particular, Arg-82 is in a good position to form a salt bridge with Asp-85 since these residues are on the same side of the putative C-helix (Engelman et al., 1985; Mogi et al., 1988).

Asp-212. The positive 1738-cm^{-1} band in the wild-type bR → M difference spectrum (Figure 1C) is assigned to Asp-212 because only mutations at this residue specifically eliminate this peak. In the D212N mutant, no M is formed under the conditions normally used to observe this intermediate (see Figure 1C); rather, only L appears to be present. The D212E mutation also causes the bR → M spectrum to appear somewhat abnormal; however, the presence of characteristic

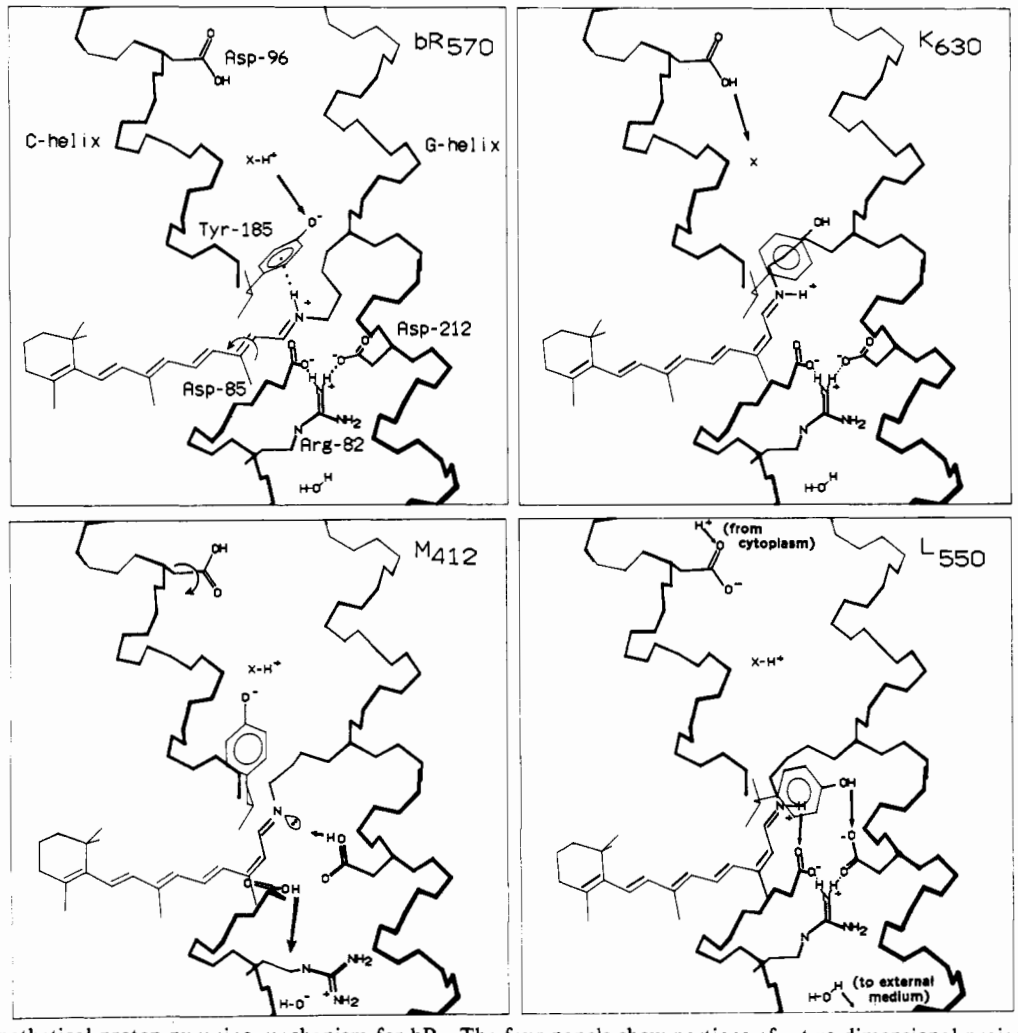

FIGURE 2: A hypothetical proton-pumping mechanism for bR. The four panels show portions of a two-dimensional projection of proposed structures for the bR_{570}, K_{630}, L_{550}, and M_{412} intermediates. In each panel, the protonation states of Asp-85, Asp-96, and Asp-212 are as indicated in Table I. Arrows indicate changes that occur to form the subsequent intermediate. $\mathbf{bR}_{570}$: The positions of the helix backbones and side chains are from a proposed three-dimensional model of the retinylidene binding site (K. J. Rothschild and M. S. Braiman, unpublished), which is based on a previous transmembrane folding model (Huang et al., 1982). Additional groups that are involved in the retinal binding site but are not shown here include Trp-182 and Trp-189. Ionization of Tyr-185 occurs at netural pH, due to its interaction with the protonated Schiff base and with a second group ($X-H^+$), which could be (for example) a protein side chain or a hydrated metal ion. The two nearby positive charges act to lower the effective pK_a of the nearby tyrosine. Positively charged groups (Arg-82 and the chromophore Schiff base with Lys-216) also help to maintain Asp-85 and Asp-212 in an ionized form. Altogether, three groups—Tyr-185, Asp-85, and Asp-212—serve as counterions for the chromophore-protonated Schiff base. $\mathbf{K}_{630}$: Light-induced isomerization about the $C_{13}=C_{14}$ bond disrupts the interaction between Tyr-185 and the Schiff base. As a result, Tyr-185 undergoes a protonation from the $X-H^+$ group. $\mathbf{L}_{550}$: During the K → L transition, Asp-96 becomes ionized; its proton will ultimately be transferred to Asp-212. Rotation of Tyr-185 about its $C_\alpha-C_\beta$ bond allows the chromophore to relax back to a planar conformation and brings the Schiff base group closer to Asp-85. $\mathbf{M}_{412}$: The rate-limiting step for M formation is a conformational relaxation allowing Asp-85 to accept a proton from the Schiff base. Deprotonation of the Schiff base destabilizes the Asp-212 anion and thereby induces it to accept a proton (indirectly) from Asp-96. There are probably multiple pathways available for this transfer, although only one is indicated (via Tyr-185 and the $X-H^+$ group). As a consequence of the charge neutralization of Asp-85 and Asp-212, the positively charged Arg-82 moves away from these residues and toward a water molecule which responds by losing a proton to form a hydroxide ion. This proton is subsequently transported through a hydrogen-bonded chain leading to the extracellular medium. During the lifetime of M_{412}, Asp-96 also takes up a proton from the cytoplasmic medium. The M_{412} structure returns back to bR through the N and O intermediates, as described in the text.

bands due to the M chromophore (e.g., positive band at 1567 cm^{-1}) indicates that an M-like species is formed. Furthermore, this spectrum exhibits the bands in the 1720–1760-cm^{-1} region that were assigned above to Asp-85, -96, and -115, although their positions and intensities are somewhat altered. While the disappearance of the positive 1738-cm^{-1} peak in the D212E spectrum might arise from secondary effects on another Asp residue, a simpler explanation is provided by our assignment of this band to an Asp-212 group undergoing a protonation reaction during the bR → M transition.

A Model for the bR Proton-Pumping Mechanism

In Figure 2, we present a model for proton transport that is consistent with our FTIR spectra of bR mutants and with other experimental results. A more complete justification of the details of this model will be published elsewhere. Our model features a proton relay, with most of the tightly coupled steps involving proton transfers between groups located in an "active site" near the chromophore Schiff base. In this model the *connectivity* of the proton path is cytoplasm ⇒ Asp-96 ⇒ Asp-212 ⇒ retinal–lysine Schiff base ⇒ Asp-85 ⇒ external medium. However, as indicated in the figure, the proton transfers must occur in a different *temporal* sequence in order to account for the protonation states of Asp-85, -96, and -212 presented in Table I. The model accounts for almost all of the COOH carbonyl stretch features observed in FTIR difference spectra of bR photointermediates. The remainder are assigned to Asp-115, which does not appear to change its

protonation state during the photocycle. The Asp-115 perturbation signals could arise from changes in an interaction with chromophore during the photocycle.[3]

The proton pathway given in the preceding paragraph is abbreviated; more transfers than indicated must occur to span the thickness of the lipid bilayer and to account for the sequence of observed protonation changes. Proton transport over portions of these gaps could be accounted for by an icelike conduction mechanism through a small number of water molecules and/or threonine residues in short hydrogen-bonded chains. Additionally, we have hypothesized Tyr-185 and an unidentified cation ($X-H^+$) as carriers of a proton between Asp-96 and Asp-212, in order to account for Tyr-185 protonation changes deduced from FTIR and UV/vis difference spectroscopy (Rothschild et al., 1986; Roepe et al., 1987; Dollinger et al., 1986; Braiman et al., 1987, 1988; Ahl et al., 1988). However, the indicated role for Tyr-185 is clearly not obligatory, since the Y185F mutant is capable of carrying out proton pumping at 40% of the efficiency of the wild type (Mogi et al., 1987).

Figure 2 shows structures for intermediates only up to M. We have no FTIR data on protonation states of residues at the N and O intermediates. However, resonance Raman spectroscopy has shown that the chromophore is reprotonated during the M → N transition and is reisomerized during the N → O step (Fodor et al., 1988). We propose that Asp-212 is responsible for reprotonating the Schiff base during the M → N transition. This is plausible on structural grounds since Asp-212 is one turn of an α-helix away from Lys-216. The ~5-ms time constant for this reprotonation might reflect a conformational rearrangement of the protein, i.e., a "reprotonation switch" (Fodor et al., 1988), required to bring the two groups in closer proximity. Once the Schiff base is protonated at N, the transition to O requires a rapid (≤5-ms) thermal reisomerization around the chromophore's $C_{13}=C_{14}$ bond. A slight conformational rearrangement of the N structure could position one or more nearby anionic side chains (Asp-212, Asp-85, and Tyr-185) close to C_{13}. This would lower the energy barrier to rotation about the adjacent double bond, as indicated by recent calculations and experiments on retinylidene model compounds (Seltzer, 1987).

In conclusion, our model incorporates as a central mechanism proton transfers between protein side chains—a concept elaborated by a number of researchers [see, for example, Nagle and Morowitz (1978)]. We have obtained experimental results that allow us to specify which side chains are involved and which transfers occur at each step in the photocycle. The model also provides detailed explanations of how the primary photoisomerization is coupled to proton movement and why Asp-85, -96, and -212 are required for proton-pumping activity. It should be possible to test this model and to fill in additional details with further spectroscopic studies on bR mutants.

[3] We cannot exclude the possibility that, along with a change in its environment, Asp-115 undergoes both deprotonation and reprotonation during one or more steps in the photocycle.

References

Ahl, P. L., Stern, L. J., Düring, D., Mogi, T., Khorana, H. G., & Rothschild, K. J. (1988) *J. Biol. Chem. 263*, 13594–13601.

Bagley, K., Dollinger, G., Eisenstein, L., Singh, A. K., & Zimanyi, L. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4972–4976.

Bellamy, L. J. (1968) *The Infrared Spectra of Complex Molecules. Vol. 2. Advances in Infrared Group Frequencies*, 304 pp, Chapman and Hall, London.

Braiman, M. S., & Rothschild, K. J. (1988) *Annu. Rev. Biophys. Biophys. Chem. 17*, 541–570.

Braiman, M. S., Ahl, P. L., & Rothschild, K. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5221–5225.

Braiman, M. S., Mogi, T., Stern, L. J., Hackett, N. R., Chao, B. H., Khorana, H. G., & Rothschild, K. J. (1988) *Proteins: Struct., Funct., Genet. 3*, 219–229.

Dollinger, G., Eisenstein, L., Lin, S.-L., Nakanishi, K., & Termini, J. (1986) *Biochemistry 25*, 6524–6533.

Eisenstein, L., Lin, S.-L., Dollinger, G., Odashima, K., Termini, J., Konno, K., Ding, W.-D., & Nakanishi, K. (1987) *J. Am. Chem. Soc. 109*, 6860–6862.

Engelhard, M., Gerwert, K., Hess, B., Kreutz, W., & Siebert, F. (1985) *Biochemistry 24*, 400–407.

Engelman, D. M., Henderson, R., McLachlan, A. D., & Wallace, B. A. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2023–2027.

Fodor, S. P. A., Ames, J. B., Gebhard, R., van den Berg, E. M. M., Stoeckenius, W., Lugtenburg, J., & Mathies, R. A. (1988) *Biochemistry 27*, 7097–7101.

Huang, K.-S., Radhakrishnan, R., Bayley, H., & Khorana, H. G. (1982) *J. Biol. Chem. 257*, 13616–13623.

Mogi, T., Stern, L. J., Hackett, N. R., & Khorana, H. G. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5595–5599.

Mogi, T., Stern, L. J., Marti, T., Chao, B. H., & Khorana, H. G. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 4148–4152.

Nagle, J. F., & Morowitz, H. J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 298–302.

Roepe, P., Ahl, P. L., Das Gupta, S. K., Herzfeld, J., & Rothschild, K. J. (1987) *Biochemistry 26*, 6696–6707.

Rothschild, K. J., & Marrero, H. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4045–4049.

Rothschild, K. J., Zagaeski, M., & Cantore, W. A. (1981) *Biochem. Biophys. Res. Commun. 103*, 483–489.

Rothschild, K. J., Roepe, P., Lugtenburg, J., & Pardoen, J. A. (1984) *Biochemistry 23*, 6103–6109.

Rothschild, K. J., Roepe, P., Ahl, P. L., Earnest, T. N., Bogomolni, R. A., Das Gupta, S. K., Mulliken, C. M., & Herzfeld, J. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 347–351.

Seltzer, S. (1987) *J. Am. Chem. Soc. 109*, 1627–1631.

Siebert, F., & Mäntele, W. (1983) *Eur. J. Biochem. 130*, 565–573.

Siebert, F., Mäntele, W., & Kreutz, W. (1982) *FEBS Lett. 141*, 82–87.

Smith, S. O., Lugtenburg, J., & Mathies, R. A. (1985) *J. Membr. Biol. 85*, 95–109.

Stoeckenius, W., & Bogomolni, R. A. (1982) *Annu. Rev. Biochem. 52*, 587–616.