Protein Conformational Changes during the Bacteriorhodopsin Photocycle

A FOURIER TRANSFORM INFRARED/RESONANCE RAMAN STUDY OF THE ALKALINE FORM OF THE MUTANT Asp-85 → Asn*

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Bacteriorhodopsin is a light-driven proton pump, which undergoes a photocycle consisting of several distinct intermediates. Previous studies have established that the M → N step of this photocycle involves a major conformational change of membrane embedded α-helices. In order to further investigate this conformational change, we have studied the photocycle of the high pH form of the mutant Asp-85 → Asn (D85Nalk). In contrast to wild type bacteriorhodopsin, D85Nalk has a deprotonated Schiff base and a blue-shifted absorption near 410 nm, yet it still transports protons in the same direction as wild type bacteriorhodopsin (Titror, J., Schweiger, U., Oesterhelt, D. and Bamberg, E. (1994) Biophys. J., 67, 1682–1690). Resonance Raman spectroscopy of D85Nalk and D85Nalk regenrated with retinal labeled at the C-15 position with deuterium reveals the existence of an all-trans configuration of the chromophore. Fourier transform infrared difference spectroscopy shows that the photocycle of this light-activated form involves similar events as the wild type bacteriorhodopsin photocycle including the M → N protein conformational change. These results help to explain the ability of D85Nalk to transport protons and demonstrate that the M → N conformational change can occur even in the photocycle of an unprotonated Schiff base form of bacteriorhodopsin.

EXPERIMENTAL PROCEDURES

Preparation of Protein Samples—Purple membranes from H. salinarium containing either wild type bacteriorhodopsin or the mutant D85N (33) were prepared according to standard methods (34).

Transmission FTIR Difference Spectroscopy—Difference spectra were recorded using methods previously reported (4, 35, 36). Samples of wild type and D85N were prepared in 40 mM Bis-Tris-propane buffer (titrated with 1 M NaOH or 1 M HCl) at pH 6.1 and pH 9.6 (D85Nalk). All chemicals were obtained from Sigma.

ATR-FTIR Difference Spectroscopy—2 μM bR solutions of D85N were
prepared in a buffer containing 1 mM NaP, 1 mM KCl, 0.4 mM MgCl₂, 0.6 mM CaCl₂, and 50 mM NaCl, and 25 μl of each of these samples was uniformly dried using a slow stream of argon onto the surface of a germanium crystal internal reflection element (50 x 20 x 2 mm) (37). The sample films of D85N were equilibrated with a pH 9.6 buffer (40 mM Bis-Tris-propane titrated with 1 mM NaOH) at 7 °C. Additional details of the ATR-FTIR measurements were reported previously (19, 37).

**RESULTS**

Effects of Blue Light Excitation of D85N<sup>alk</sup>—FTIR difference spectra of dark-adapted D85N<sup>alk</sup> excited by blue light are shown in Fig. 1. These averaged difference spectra were obtained by cycling the sample between 10–15 min periods of dark and continuous blue light illumination (see Fig. 1 caption for details). The ATR and transmission FTIR difference spectra are very similar with predominantly positive bands appearing in regions assigned to the C–C stretch modes (1500–1600 cm<sup>-1</sup>) and C–C stretch modes (1150–1250 cm<sup>-1</sup>) of the retinylidine chromophore (42–44). The absence of corresponding negative bands is attributed to the selective photoreaction of the M-like species present in dark-adapted D85N<sup>alk</sup> (28). This M-like species has a 13-cis-retinylidine chromophore with a deprotonated Schiff base in contrast to the more red-shifted photoproducts, which have protonated Schiff bases. Since the intensity of the infrared vibrations of deprotonated retinal Schiff bases are relatively weak compared to the corresponding protonated species (45), only positive chromophore bands appear in the difference spectrum. For example, a similar effect is found in the M → N difference spectrum of wild type bR (Fig. 1) and the mutant T46D (12), where the Schiff base of the M chromophore is deprotonated while that of the N chromophore is protonated. The negative band located near 1516 cm<sup>-1</sup> in the M → N difference spectrum arises from changes in a tyrosine residue as recently established using SDIL combined with resonance Raman spectroscopy—Resonance Raman spectra were measured using a previously described apparatus (38, 39). The sample (250 μl, 40 mM Bis-Tris-propane buffer at pH 9.6) was placed in a quartz spinning cell of 19 mm internal diameter and spun with a time period of 22 ms. Under these conditions the sample was exposed periodically to the probe laser beam for a duration of 1.8 μs every 22 ms, thus preventing contributions to the spectrum from intermediates with lifetimes shorter than 22 ms. Samples were excited by a cylindrically focused 413.1-nm 7-mW CW laser beam from a Krypton ion laser (model 1200-K2, Coherent Inc., Palo Alto, CA). Raleigh scattered light from the sample was blocked by using a 420-nm long pass filter (Corion Corp., Holiston, MA) placed before the entrance to the spectrometer. Spectra were calibrated using a toluene standard. The N-like photoproduct of D85N<sup>alk</sup> was recorded using a dual beam pump-probe method. A 40-mW 413.1-nm laser excitation (pump beam) was used to initiate the photocycle in the sample. This excitation beam was focused at a point upstream from the probe beam such that once the sample was excited, it took approximately 5 ms to sweep past the probe beam (1 mW, 514.5 nm). Probe-only spectra were subtracted from the pump + probe spectra, which resulted in the spectrum of the D85N<sup>alk</sup> photoproducts. KNO₃ (0.5 mM) was added to the sample for spectrometer calibration purposes. The spectrum of the N-intermediate of wild type bR was obtained from a pH 9 purple membrane sample in 3 mM KCl with 2-mW 520.8-nm laser excitation. Details of this are reported elsewhere (40).

The 15D-all-trans-retinal was obtained as described in Ref. 41 by LiAlD₄ (Aldrich), reduction of all-trans-retinol acid to give 15D-all-trans-retinol. The latter was then oxidized with activated manganese dioxide to give 15D-all-trans-retinal. Crude product was purified on a silica gel column using step gradient of ethyl acetate in hexane and finally recrystallized from petroleum ether. The sample obtained was characterized by ¹H NMR, by analytical high performance liquid chromatography (>96% trans) and by the CI-MS spectrum (M<sup>+</sup> = 285.03; isotopic purity >97%). D85N containing the 15D-all-trans-retinylidine chromophore (15D-D85N) was produced by photobleaching D85N in the presence of 1 mM hydroxylamine, washed two times with bovine serum albumin solution to remove the retinal oxime, and then regenerated with the 15D-all-trans-retinal.

![Fig. 1. FTIR difference spectra of dark-adapted D85N<sup>alk</sup>.](https://example.com/fig1.png)

The blue light excitation (λ<sub>max</sub> < 440 nm) FTIR difference spectra of dark-adapted D85N<sup>alk</sup> were recorded using ATR (a) and transmission (b) methods. Both D85N<sup>alk</sup> spectra were recorded at 2 cm<sup>-1</sup> resolution and represent the average of at least 50 pairs of dark-light cycles (5 min for a and 15 min for b). The ATR spectrum was measured at 7 °C and the transmission spectrum at 22 °C. Further details are given under "Experimental Procedures." Increasing the dark portion of the cycle did not alter the individual difference spectrum significantly, indicating that all the photoproducts decayed within the dark period. Absorbance scale shown (0.00025 absorbance units) is for trace b, the wild type M → N spectrum was recorded under conditions previously reported (12).

FTIR difference spectroscopy.²

Fig. 1 also shows that steady-state illumination of dark-adapted D85N<sup>alk</sup> with blue light produces a mixture of photoproducts that are very similar to the N and O intermediates of the light-adapted bR photocycle. For example, the positive 1510 cm<sup>-1</sup> band and the pair of positive bands at 1200 and 1168 cm<sup>-1</sup> correspond closely to the frequencies of major chromophore bands found in the O intermediate (15, 46). Furthermore, the positive bands at 1530/1554 cm<sup>-1</sup> along with the strong peaks at 1186 cm<sup>-1</sup> are similar to bands assigned to the N intermediate (14, 47). This can be seen by comparing the D85N<sup>alk</sup> difference spectra with the M → N difference spectrum of wild type bR (Fig. 1), where positive bands also appear at 1554, 1530, and 1186 cm<sup>-1</sup>. As discussed later, we believe these photoproducts occur because blue light first light-adapts D85N<sup>alk</sup>, thereby producing an M-like form with an all-trans chromophore (M<sup>all-trans</sup><sup>alk</sup>), which then undergoes a blue light-driven photocycle that includes N- and O-like intermediates.

A remarkable feature of the D85N<sup>alk</sup> difference spectra is the appearance of a band near 1669/1661 cm<sup>-1</sup> in the amide I region, which have been previously associated with the major conformational change during the M → N transition of bR (12–17) (see Fig. 1). The appearance of these bands is a strong indication that a similar conformational change occurs during blue light excitation of D85N<sup>alk</sup>.³ There is also a negative band at 1742 cm<sup>-1</sup> in the D85N<sup>alk</sup> difference spectra (Fig. 1). In the case of wild type bR, this is assigned to the carboxyl stretch of Asp-96 (13–15) and is associated with the deprotonation of this residue and the reprotonation of the Schiff base during the M → N transition (48). Thus, we conclude that changes in both the conformation and protonation of the protein, similar to those which occur during the M → N step of the wild type bR photocycle, also occur in the blue light-

² C. F. C. Ludlam, S. Sonar, G. J. Ludlam, and K. J. Rothschild, unpublished results.

³ An earlier FTIR study on blue light excitation of D85N<sup>alk</sup> did not detect these protein bands (20). However, these measurements were made at 170 K, where this conformational change is most likely blocked.
The photocycle of Light-adapted D85N<sub>alk</sub> at Low Temperature—In analogy with wild type bR, we found that it is possible to trap the photoproducts of light-adapted D85N<sub>alk</sub> by illuminating the sample at temperatures below 0°C with blue light. Under these conditions, the initial blue light illumination of dark-adapted D85N<sub>alk</sub> produces a difference spectrum (Fig. 2, trace a) similar to that shown in Fig. 1 (trace b). However, unlike measurements made above 0°C, almost no decay of the blue light photoproducts was observed over a 1-h period at −3 °C, as indicated by the almost featureless FTIR difference spectrum (Fig. 2, trace c).

The photochemistry of the N- and O-like blue light photoproducts of D85N<sub>alk</sub> trapped at low temperature were studied by recording the FTIR difference spectra produced by using different wavelengths of illumination. Illumination with green light (500 nm) followed by photoreversal with red light (650 nm) produces a difference spectrum (Fig. 2, trace c) that compares well with the O → N difference spectrum measured for wild type bR (17) and the mutant Y185F (15, 49) (Fig. 2, trace d). For example, the difference bands in the 1150–1250 cm<sup>−1</sup> fingerprint region are highly characteristic of an all-trans to 13-cis isomerization of the chromophore (44, 50). This effect can be explained by the selective photoreaction of the low temperature trapped O-like photoproduct with red light to form an N-like species. A similar effect has been previously observed for the O intermediate formed by the bR mutant Y185F (39). At room temperature, the O photocyte appears to involve a K-like intermediate and a long-lived N intermediate (49, 51). The N species formed in D85N can also be photoreversed back to O using green light. Notice that photoreversal between O and N produces a characteristic switch in the protein conformation as indicated by the bands at 1669 and 1649 cm<sup>−1</sup>. This is again similar to the photocycle of wild type bR, where this conformational change occurs between M → N and is reversed during the N → O step (15).

Interestingly, we also observed a difference spectrum similar to the O → N difference spectrum (green → red) shown in Fig. 2 when blue light was used for excitation. A possible explanation for this effect is that the O-like species, which contains an all-trans protonated chromophore, exists in equilibrium with an M-like species, which contains an all-trans chromophore and deprotonated Schiff base (M<sup>−</sup>all-trans). Photocconversion by blue light of M<sup>−</sup>all-trans to N would then be expected to cause a depletion of the O-like species and the appearance of the negative O-like chromophore bands.

Resonance Raman Spectroscopy of D85N<sub>alk</sub>—The resonance Raman spectra of D85N<sub>alk</sub> containing a normal retinylidene chromophore (D85N<sub>alk</sub>) and a retinylidene chromophore labeled with deuterium at the C-15 position (15D-D85N<sub>alk</sub>) are shown in Fig. 3 (panel A) along with the spectrum of the M intermediate of wild type bR. The main ethylenic modes (C<sub>9</sub>≡C-C=O) of both D85N<sub>alk</sub> and 15D-D85N<sub>alk</sub> appear at 1563 cm<sup>−1</sup>, close to that of the M intermediate (1567 cm<sup>−1</sup>) (50, 52). The D85N<sub>alk</sub> spectrum also exhibits a band at 1621 cm<sup>−1</sup>, typical of the C=N stretch mode of an unprotonated Schiff base chromophore such as found in the M intermediate (50, 52). As expected, this band downshifts in 15D-D85N<sub>alk</sub> due to the expected 15D isotope effect on the C=N stretch mode. In the M spectrum of 15D substituted wild type bR, the C=N mode appears as a weakly scattering band centered at 1604 cm<sup>−1</sup> (50). In our 15D-D85N<sub>alk</sub> spectrum, we do not see a distinct band at 1604 cm<sup>−1</sup>, probably due to its weak intensity buried under the profile of the ethylenic band.

In order to determine the isomeric composition of D85N<sub>alk</sub>, we examined the 15D induced changes in the structurally sensitive fingerprint region (1100–1400 cm<sup>−1</sup>). In the case of model compounds containing an all-trans unprotonated retinal Schiff base, a band appears at 1178 cm<sup>−1</sup> and downshifts to 1158 cm<sup>−1</sup> for the 15D isotope substitution (50). However, no such isotope shift is observed for the 13-cis unprotonated Schiff...
base. In addition, 15D substitution causes a characteristic 1225 cm\(^{-1}\) band in the spectrum of 13-cis unprotonated Schiff base to become more intense. In contrast, only a small band that is isotope-insensitive is observed at this frequency for all-trans unprotonated Schiff base compounds.

In the case of 15D-D85N\(^{\text{alk}}\), we observe isotope effects that are typical of the all-trans unprotonated retinal Schiff base but which are not observed for 13-cis unprotonated retinal Schiff bases. First, a decrease in the intensity of the 1175 cm\(^{-1}\) band occurs along with the appearance of a distinct band at 1157 cm\(^{-1}\) characteristic of the all-trans configuration. This effect is clearly observable in the scaled subtracted spectrum of 15H- and 15D-D85N\(^{\text{alk}}\) (see inset to Fig. 3, panel A). Second, unlike the M-intermediate, no isotope effects are seen at 1225 cm\(^{-1}\) in 15D-D85N\(^{\text{alk}}\) compared to the 15D analog (50). These results strongly indicate that the D85N\(^{\text{alk}}\) chromophore exists in a predominantly all-trans configuration with an unprotonated Schiff base. However, since we do not observe a complete disappearance of the 1175 cm\(^{-1}\) band in 15D-D85N\(^{\text{alk}}\) and a drop also occurs in the band near 1200 cm\(^{-1}\) as seen in 13-cis model compounds (50), a species with a 13-cis chromophore configuration may also be present (28, 30). This may be due to the presence of a fraction of D85N\(^{\text{alk}}\), which is not light-adapted by the 413.1-nm exciting light.

The N Photoproduction of D85N\(^{\text{alk}}\) at Room Temperature—The room temperature photochemistry of D85N\(^{\text{alk}}\) was also investigated by resonance Raman spectroscopy. Using a 413.1-nm pump and a 514.5-nm probe beam, we obtained a spectrum of D85N\(^{\text{alk}}\) that has all the characteristic bands of the N intermediate of wild type bR (Fig. 3, panel B). Especially notable are the two double ethylenic bands at 1530 and 1549 cm\(^{-1}\) (47). The bands at 1187, 1198, 1328, 1439, and 1644 cm\(^{-1}\) are also highly characteristic of the N intermediate. Thus, in agreement with the low temperature FTIR studies, the D85N\(^{\text{alk}}\) photo-cycle includes an N-like photointermediate even at room temperature.

DISCUSSION

FTIR difference spectroscopy indicates that a major conformational change involving membrane buried \(\alpha\)-helical structure occurs during the M \(\rightarrow\) N transition and is reversed during the N \(\rightarrow\) O transition of bacteriorhodopsin (12–17). These events are correlated with the transfer of a proton from Asp-96 to the Schiff base (M \(\rightarrow\) N) and the reprotonation of Asp-96 along with the isomerization of the chromophore from a 13-cis to an all-trans configuration (N \(\rightarrow\) O) (15, 17, 46, 48, 53). Structural changes in the C-D loop near Asp-96 are also detected by site-directed spin labeling, which are correlated with the decay of the M intermediate (54).

Although the exact nature of the structural changes occurring upon N formation are not yet known, most available data are consistent with the reorientation of membrane-embedded \(\alpha\)-helices (12). For example, electron diffraction detects structural changes in the br photocycle that involve a tilt of a portion of the F and C helices on the cytoplasmic side of the protein (55). Furthermore, the 1669 cm\(^{-1}\) band observed in the M \(\rightarrow\) N FTIR difference spectrum (Fig. 1) exhibits out-of-plane dichroism which is consistent with the tilt of \(\alpha\)-helices away from the membrane normal.\(^4\) Recent studies based on SDIL have established that a portion of this band contains contributions from the amide carbonyl group of Tyr-185 and may reflect structural rearrangements in the Tyr-185/Pro-186 amide bond (19). Changes in this bond are detected as early as the br \(\rightarrow\) K phototransition (56), which is most likely directly triggered by the all-trans \(\rightarrow\) 13-cis isomerization of the retinylidene chromophore. Thus, one possibility is that the initial chromophore isomerization initiates structural changes in the Tyr-185/Pro-186 region of helix F that results later in the photocycle in a reorientation of part of this as well as other helices in the protein.

In order to explore further the nature of this conformational change (termed here the RT-conformational change)\(^\text{\footnote{In analogy with earlier work (22), we designate the two conformations of the protein as R (resting) and T (triggering). This corresponds to the T (trans) and C (cis) designations, respectively, used by Mathies and co-workers (59).}}\) and its functional significance in the bacteriorhodopsin proton pump, we have focused on the alkaline form of the mutant Asp-85 \(\rightarrow\) Asn in this work. Earlier studies have established that blue light-driven D85N\(^{\text{alk}}\) exhibits proton pumping in the same direction as wild type bR (30, 32), despite the fact that it has a deprotonated Schiff base. It was also predicted in these works that the proton pumping occurs due to the photocycle of an M\(^{\text{all-trans}}\) form of D85N\(^{\text{alk}}\). In analogy with light-adapted bR (bR\(_{\text{467}}\)), it might then be expected that the photocycle of D85N\(^{\text{alk}}\) would include a step similar to the RT-conformational change.

The results of our FTIR and resonance Raman measurements establish the following. (i) Light-adapted D85N\(^{\text{alk}}\) contains a retinylidene chromophore with an all-trans configuration (M\(^{\text{all-trans}}\)). (ii) The photocycle of light-adapted D85N\(^{\text{alk}}\) involves formation of N- and O-like intermediates. (iii) The photocycle of light-adapted D85N\(^{\text{alk}}\) involves an RT-conformational change and proton transfer from Asp-96 to the Schiff base. (iv) The N- and O-like photoproducts of the light-adapted D85N\(^{\text{alk}}\) photocycle can be trapped at low temperatures and exhibit a photoreversible N \(\rightarrow\) O conversion, which also involves reversal of the RT-conformational change. A simplified scheme similar to one proposed earlier (30) which accounts for these findings and the ability of light-adapted D85N\(^{\text{alk}}\) to pump protons in the same direction as wild type bR is shown in Fig. 4. Blue light adaptation of dark-adapted D85N\(^{\text{alk}}\) (species not shown) produces an equilibrium mixture of two light-adapted species containing all-trans retinal chromophores, M\(^{\text{all-trans}}\) and O. Both of these species exist in the R conformation and differ mainly by the Schiff base protonation state. Blue light absorption by the M\(^{\text{all-trans}}\) species triggers a photocycle that consists of a number of early intermediates (not shown) including an M-like intermediate with a deprotonated Schiff base and 13-cis retinal configuration (20, 31) and later, an N-like species containing a 13-cis\(=\text{NH}\) chromophore. Formation of the N-like species involves a net change in protein conformation from the R to T state and the internal movement of a proton from Asp-96 to the Schiff base. The thermal decay of the N-like to O-like species involves the reisomerization of the chromophore, uptake of a proton by Asp-96 from the cytoplasmic medium, and a switch of the protein conformation from T to R. The Schiff base of the O-like species then deprotonates releasing a proton into the external medium, thereby reestablishing its thermal equilibrium with the M\(^{\text{all-trans}}\) form.

An important feature of the proposed D85N\(^{\text{alk}}\) photocycle is its similarity to the br photocycle. In both photocycles, an RT-conformational change is observed, which is accompanied by proton transport from Asp-96 to the Schiff base. In the case of br, this involves deprotonation of the Schiff base and ejection of a proton to the external medium. However, in the case of D85N\(^{\text{alk}}\), proton ejection does not occur at this stage since M\(^{\text{all-trans}}\) already has a deprotonated Schiff base. The late photocycles in both cases involve the reprotonation of Asp-96 via uptake of a proton from the cytoplasmic medium, a reversal in
protein conformation, and reisomerization of the chromophore to an O-like species. Finally, both photocycles involve the decay of the O-like species. However, unlike native bR where light-adapted bR (bR570) has an all-trans chromophore with a protonated Schiff base, the M\textsuperscript{all-trans} form of D85N\textsuperscript{alk} contains a deprotonated Schiff base.

It can also be seen from Fig. 4 that the major differences between native bR and D85N\textsuperscript{alk} arise as a natural consequence of the Asp\textsuperscript{ALK} substitution, which acts to lower the Schiff base pK\textsubscript{a} from about 13 in wild type bR down to 8.7 in D85N (26-28, 30). This drop in pK\textsubscript{a} causes the all-trans retinal Schiff base to deprotonate and produces the observed equilibrium between the all-trans protonated (O-like) and deprotonated (M\textsuperscript{all-trans}) forms of the chromophore. Note that spectroscopic and stop-flow measurements show the transition between these two forms is not rapid (20, 32). This may be due to the ability of the Asn residue in D85N to form as strong a hydrogen bond as Asp in wild type bR (38).

An additional conclusion from our work is that light-adapted wild type bR and D85N\textsuperscript{alk} both exist in the R conformation despite the fact that the protonation state of the Schiff base is different. In particular, we observe the RT-conformational change in the photocycle of both M\textsuperscript{all-trans} and bR\textsubscript{570}. This also implies that the switch from the R to T conformation is not dependent on the disruption of the Schiff base-counterion interaction.\textsuperscript{6} This contrasts with a recent study of D85N\textsuperscript{alk} based on x-ray scattering measurements of D85N at high pH (\~11) along with the effects of azide on the photocycle of D85N and D85N/D96N (20). This study concluded that D85N\textsuperscript{alk} exists in the "C" conformation in contrast to D85N at lower pH and light-adapted bR, which exists in the "E" conformation.\textsuperscript{7} It was also deduced from these experiments that the switch between the E and C conformations is due mainly to the disruption of ionic interactions of the positively charged Schiff base inside the retinal binding pocket (20, 21). However, recent FTIR-ATR studies show that a global conformational change occurs in the wild type bR (57) above pH 11, which is concomitant with ionization of Asp-96. Thus, the C conformation of D85N measured at pH 11 by x-ray might be more related to the high pH disruption of interactions near Asp-96 rather than at the Schiff base. Recent, pH induced ATR-FTIR difference measurements on D85N support this possibility.\textsuperscript{8}

In conclusion, our results establish that the photocycle of D85N\textsuperscript{alk} includes as a key step a conformational change, which has previously been observed to occur in wild type bR between the M and N intermediates. While the equivalence of this RT-conformational change and the required EC switch for bacteriorhodopsin has not yet been established, such an equivalence would help to account for the observed proton pumping of D85N\textsuperscript{alk} in the same direction as wild type bR (30). Our results also indicate that electrostatic interactions in the Schiff base region are not essential for stabilization of the two major conformations of bR observed by FTIR spectroscopy. In contrast, the configuration of the chromophore appears to play an essential role, since only the isomerization of retinal from an all-trans to 13-cis configuration has been associated with trigger-

\textsuperscript{6} There also exists a number of examples where bR undergoes an RT-conformational change, yet the Schiff base remains protonated and Asp-85 is still available as a counterion. For example, both D212N and Y57D exhibit blocks in their photocycles, where a proton has not been transferred from the Schiff base to Asp-85, yet the RT-conformational change still occurs (60-62).

\textsuperscript{7} The E-C transition corresponds to the required step in all active ion pumps (20, 22-25), namely the closing of an ion binding site(s) (conformation E) and formation of an ion pathway leading from the cytoplasmic side of the protein to the ion binding site(s) (conformation C) (20, 21, 30).

\textsuperscript{8} A. Nilsson, P. Rath, and K. J. Rothschild, unpublished results.
Bacteriorhodopsin Protein Conformational Changes

29751

...ing the RT-conformational change. This can be simply understood if the all-trans-C=C anti and 13-cis-C=C syn forms of retinal act to stabilize the light-adapted conformation of br, whereas the 13-cis-C=C anti conformation causes strong steric interactions in the retinal binding pocket eventually causing the protein to change conformations. Future studies which combine FTR, site-directed mutagenesis, and site-directed isotope labeling (58) are likely to shed further light on the nature of this conformational change and the chromophore-protein interactions that trigger it.

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