Retinal counterion switch in the photoactivation of the G protein-coupled receptor rhodopsin

Elsa C. Y. Yan*†, Manija A. Kazmi†, Ziad Ganim*, Jian-Min Hou†‡, Douhai Pan*, Belinda S. W. Chang†§, Thomas P. Sakmar†, and Richard A. Mathies*¶

*Department of Chemistry, University of California, Berkeley, CA 94720; and †Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10021

Edited by Jeremy Nathans, Johns Hopkins University School of Medicine, Baltimore, MD, and approved May 29, 2003 (received for review April 9, 2003)

The biological function of Glu-181 in the photoactivation process of rhodopsin is explored through spectroscopic studies of site-specific mutants. Preresonance Raman vibrational spectra of the unphotolyzed E181Q mutant are nearly identical to spectra of the native pigment, supporting the view that Glu-181 is uncharged (protonated) in the dark state. The pH dependence of the absorption of the metarhodopsin I (Meta I)-like photoproduct of E181Q is investigated, revealing a dramatic shift of its Schiff base pK_a compared with the native pigment. This result is most consistent with the assignment of Glu-181 as the primary counterion of the retinylidene protonated Schiff base in the Meta I state, implying that there is a counterion switch from Glu-113 in the dark state to Glu-181 in Meta I. We propose a model where the counterion switch occurs by transferring a proton from Glu-181 to Glu-113 through an H-bond network formed primarily with residues on extracellular loop II (EII). The resulting reorganization of EII is then coupled to movements of helix III through a conserved disulfide bond (Cys110–Cys187); this process may be a general element of G protein-coupled receptor activation.

Rhodopsin, the vertebrate dim-light photoreceptor, is a prototypical member of the G protein-coupled receptor (GPCR) family. The 11-*cis*-retinal chromophore in rhodopsin is linked to the protein through a protonated Schiff base (PSB) whose positive charge is neutralized by the Glu-113 counterion (1–3). After absorption of a photon, isomerization of 11-*cis*-retinal initiates the photoactivation process (Fig. 1) resulting in the formation of metarhodopsin II (Meta II), which binds and activates the G protein, transducin (4–6). Rhodopsin is the only GPCR whose crystal structure has been reported at high resolution (7–9). The rhodopsin structure provides a blueprint to explore and understand the mechanism of rhodopsin activation, which should also provide insights into the general mechanism of GPCR activation (10, 11).

To investigate the photoactivation mechanism of rhodopsin, we have focused on the role of Glu-181. Glu-181 is potentially capable of exerting significant electrostatic interactions on the chromophore. However, until recently, Glu-181 has not been studied in detail because it is situated in extracellular loop II (EII), which was conventionally believed to be on the extracellular membrane surface. The crystal structure of bovine rhodopsin (see Fig. 2) surprisingly revealed that EII folds deep into the transmembrane region and forms an important part of the retinal-binding pocket (7, 9, 12). Glu-181 is only 4.7 Å from C_{12} of 11-*cis* retinal (7), and is highly conserved in vertebrate opsins, blue cone, and UV cone pigments (13). All visual pigments appear to contain either a glutamic acid or an aspartic acid at the position corresponding to 181 in bovine rhodopsin. The exceptions are the green and red cone pigments, which contain His-197 at the corresponding position, which interestingly is part of a chloride ion-binding site (14).

Two experimental mutagenesis studies on the role of Glu-181 in rhodopsin have been performed (13, 15). Terakita *et al.* (13) showed that the E181Q mutation causes an ≈10-nm red shift in the absorption maximum of bovine rhodopsin in the absence of Cl ions and that the residue in squid retinochrome corresponding to Glu-181 in bovine rhodopsin serves as a counterion for the retinal PSB in the dark state. We reported a detailed biochemical study on a full set of Glu-181 mutants (15), revealing three major mutant phenotypes: (*i*) generally increased reactivity toward hydroxylamine, (*ii*) alterations in the decay rates of the Meta II-like photointermediates, and (*iii*) decreased transducin activation rates. E181Q was also found to be the only Glu-181 mutant giving a significant spectral shift in the dark (≈10 nm in the absence of Cl ion and ≈5 nm in the presence of Cl ions). The small magnitude of these shifts together with the insensitivity of

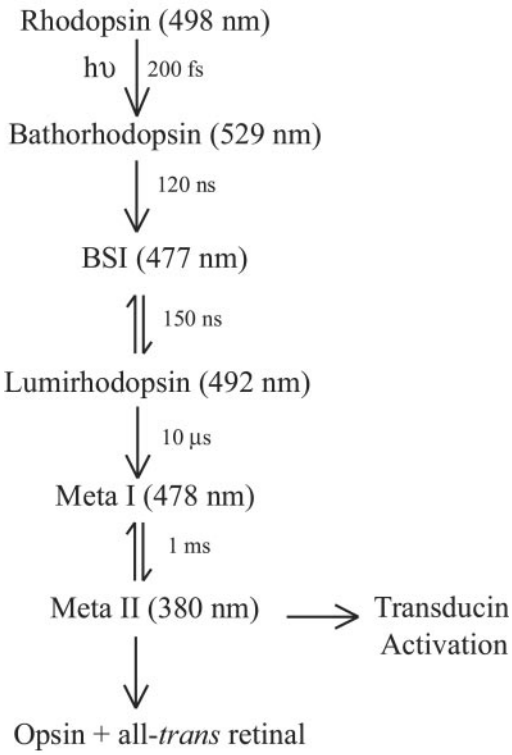

Fig. 1. The photobleaching sequence of rhodopsin.

A preliminary account of this work was first presented at the 10th International Conference on Retinal Proteins, August, 20–24, 2002, University of Washington, Seattle, WA.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: SB, Schiff base; GPCR, G protein-coupled receptor; PSB, protonated SB; EII, extracellular loop II; DM, *n*-β-dodecyl maltoside.

See commentary on page 9105.

‡Present address: Department of Chemistry, Gonzaga University, 502 East Boone Avenue, Spokane, WA 99258.

§Present address: Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, ON, Canada M5S 3G5.

¶To whom correspondence should be addressed. E-mail: rich@zinc.cchem.berkeley.edu.

www.pnas.org/cgi/doi/10.1073/pnas.1531970100

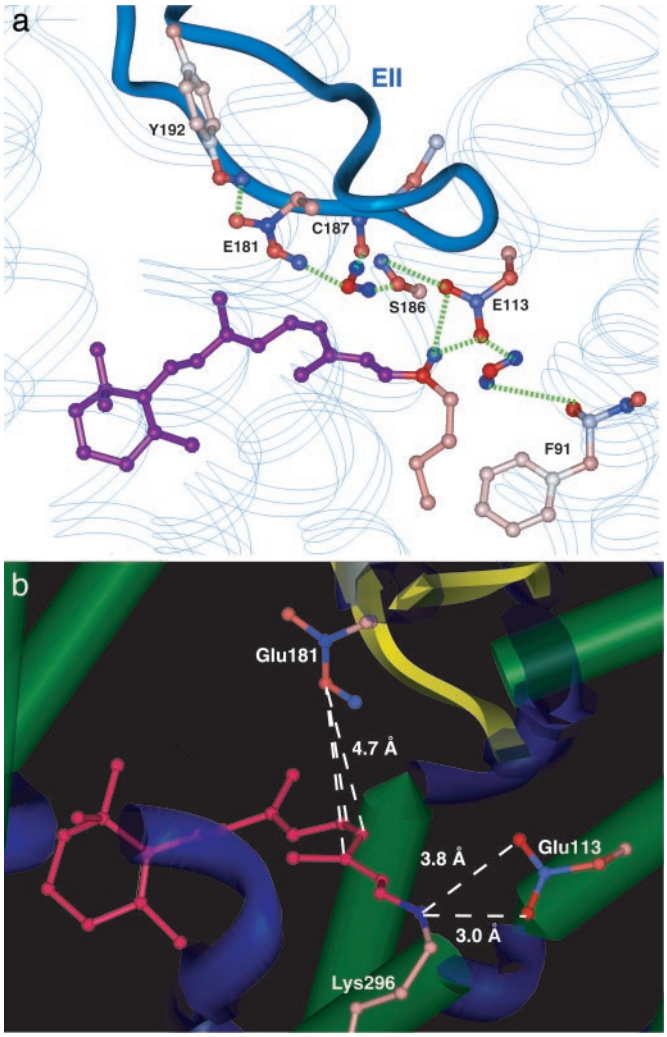

Fig. 2. (*a*) Molecular graphics model of rhodopsin illustrating the H-bond network extending across the retinal chromophore-binding pocket. Extracellular loop II (EII) is shown as the blue ribbon. The side chains of Ser-186 and Tyr-192 together with the backbone of Phe-91 and Cys-187 participating in the H-bond network are shown. (*b*) Glu-181 points toward the center of the polyene chain of retinal and the closest oxygen of Glu-181 is 4.7 Å away from C_{12}.

the λ_{max} of the E181Q rhodopsin pigment to pH led to the suggestion that Glu-181 is neutral in the dark state. This conclusion is consistent with two-photon studies of rhodopsin indicating a neutral chromophore binding site (16).

In the present study, we have explored the functional role of Glu-181 in the photoactivation process. First, preresonance Raman vibrational spectra of Glu-181 mutants were obtained to provide vibrational structural data on the interaction between Glu-181 and the chromophore in the dark state. Second, we studied the pK_a of the retinylidene PSB group for Glu-181 mutants in the Meta I-like and Meta II-like photoproducts to investigate the electrostatic interaction between Glu-181 and the chromophore in the bleaching pathway. We found that E181Q mutation dramatically alters the pH dependence of the Schiff base (SB) group protonation state in the Meta I-like photoproduct. On the basis of these results, we conclude that Glu-181 is the primary counterion of the retinal PSB in the Meta I state. This conclusion requires the existence of a PSB counterion switch during photoactivation of rhodopsin. The primary PSB counterion switches from Glu-113 in the dark state to Glu-181 in the Meta I state. Our observations also provide a mechanistic understanding of Raman and Fourier transform infrared (FTIR) studies revealing an abrupt change of the H-bonding environment of the SB group in the lumirhodopsin (Lumi) state (17–20). A mechanism for counterion switching is proposed and the implications of this counterion switch for the role of the EII loop in GPCR activation are discussed.

Experimental Procedures

Preparation of Pigment Samples. Site-directed mutants were prepared by using the QuikChange method (Stratagene) as described earlier and verified by automated sequencing (21). The mutant pigments were transiently expressed in COS-1 cells, harvested and regenerated with 11-*cis*-retinal. The regenerated pigments were purified by an immunoaffinity adsorption procedure (22, 23). Pigments were prepared in 100 mM NaCl and 0.1% (wt/vol) *n*-β-dodecyl maltoside (DM) in 50 mM Tris·HCl buffer at pH 6.8, and concentrated to OD ≈1.5 for Raman measurements.

The pigments used for the Meta I measurements were prepared in digitonin solution. The exchange of detergent from DM to digitonin was accomplished through the washes during the immunoaffinity adsorption procedure before elution with 0.5 mM Tris·HCl buffer and 100 mM NaCl. HCl or NaOH was then added to the pigment solution to achieve the desired pH. The pH was predetermined by trials on the 0.5 mM Tris buffer in the absence of pigment. UV-visible spectroscopy was performed on a Perkin–Elmer λ-800 spectrophotometer where temperature was regulated to 4°C. Pigments were photobleached by illumination with light of $\lambda > 495$ nm. The final pH was measured after the UV-visible measurements.

The pigments used for the Meta II titration were prepared in 100 mM NaCl, 0.1% DM in 0.5 mM Tris-HCl buffer at pH 6.8. Adjustment of the pH was achieved by additions of phosphate buffer at various pH values to a final concentration of 50 mM. The pigment was photobleached with light of $\lambda > 495$ nm at room temperature. The pH was measured before and after the experiments.

Raman Spectroscopy. Spectra of rhodopsin were obtained by using 765 nm illumination from a Ti:Sapphire laser (Lexel 479) pumped by the all-lines output of an Ar ion laser (Spectra-Physics 2025) (24). The excitation beam (30 mW) was focused with a 500-mm focal length lens onto ≈6 μl of pigment solution contained in a 400-μm inside diameter circular capillary. Nitrogen gas running through a dry ice/propanol bath was used to cool the sample to ≈0°C to prevent thermal degradation. No significant sample bleaching due to thermal or photochemical processes was observed during the experiment. The scattered photons were dispersed by a double-spectrograph (Spex 1400) and detected by a charge-coupled device (CCD) (LN/CCD-1100/PB, Princeton Instruments). The spectrum was calibrated by recording spectra of cyclohexane and cyclohexanone. The reported frequencies are accurate to within 2 cm^{-1}, and the spectral resolution is 2 cm^{-1}.

Results

Fig. 3 presents preresonance Raman vibrational spectra of the E181D and E181Q mutants together with the WT rhodopsin spectrum. The spectrum of E181D is the same as that of WT rhodopsin within experimental error. This finding shows that the E181D mutation does not alter the vibrational structure of the chromophore in the binding pocket. The spectrum of E181Q reveals an ≈4-cm^{-1} upshift of the C=N stretching frequency indicating an indirect effect on the SB dipolar environment. E181Q also exhibits an ≈4-cm^{-1} downshift for the C=C stretching frequency corresponding to a lower C=C bond order and a more delocalized electronic structure. This observation implies

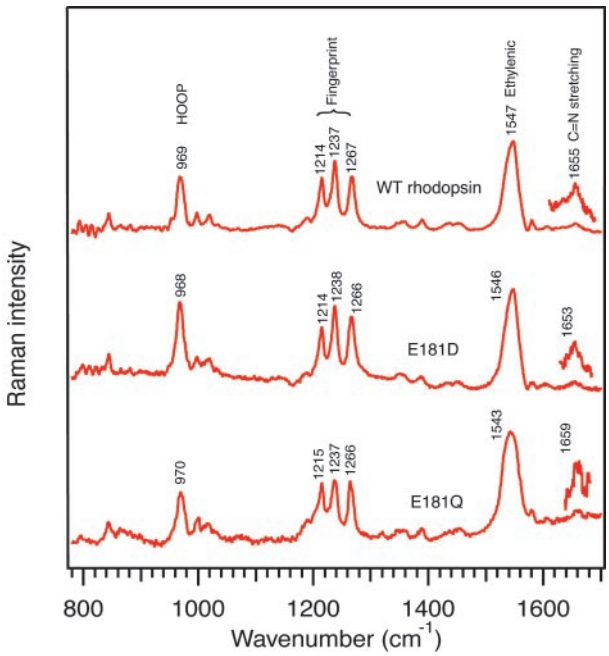

Fig. 3. Preresonance Raman spectra of WT rhodopsin and its E181D and E181Q mutants. The spectra were taken by using preresonance excitation (30 mW, 765 nm) at a temperature of ≈0°C.

a red shift in the absorption spectrum of E181Q, which is consistent with the previously reported results (13, 15). The fingerprint (C–C stretching) and hydrogen out-of-plane modes of E181Q are the same as those of WT rhodopsin. The modest magnitudes of the spectral shifts in E181Q are most consistent with a neutral (protonated) Glu-181 residue in the WT rhodopsin.

We next examined the pH dependence of the SB in E181Q, E113Q, and WT rhodopsin in digitonin solution at 4°C under conditions that stabilize native Meta I (λ_{max} = 480 nm) (25–28). The UV-visible spectra of the dark and bleached pigments at various pH values are presented in Fig. 4. The dark spectrum (blue) of WT rhodopsin at pH 6.8 exhibits the expected band at 500 nm in Fig. 4*b*. Illumination with light of $\lambda > 495$ nm removes the 500-nm absorbance and produces the characteristic 480-nm band of Meta I (red trace). The photobleached form of WT rhodopsin absorbs at 480 nm regardless of pH, showing that the SB stays protonated under all of the experimental conditions (Fig. 4 *a–c*). The apparent pK_a of the SB for native rhodopsin and for Meta I is thus >9.

The spectra shown in Fig. 4 *d–f* demonstrate the titration of the SB in the E113Q mutant pigment in the dark state (blue) and after illumination (red). Previous studies have established that the E113Q mutation removes the native counterion, thereby lowering the pK_a of the rhodopsin PSB as a result of the altered electrostatic environment of SB (1–3). As expected, the dark rhodopsin absorption shifts from a single 500-nm band at pH 4.3 to a pair of bands at 380 and 500 nm at pH 6.8, and finally to a fully titrated form at pH 9.0. On photobleaching E113Q with light, the acid form (λ_{max} = 500 nm) is activated and photoconverted to the 480-nm absorbing species, whereas the basic form (λ_{max} = 380 nm) is not effectively bleached.

The absorption spectrum of E181Q in the dark state (blue) is not affected by pH (Fig. 4 *g–i*). This observation is consistent with the previous conclusion that Glu-181 does not directly and significantly influence the PSB through electrostatic interaction in the dark state as well as the idea that Glu-181 is protonated in the dark state (15). The apparent pK_a of the SB in the dark form of E181Q is thus >9. At low pH, photobleaching of E181Q produces exclusively a 480-nm form (Fig. 4*g*), characteristic of native Meta I. At neutral pH, the 500-nm dark spectrum is transformed into two absorption bands at 480 and 380 nm corresponding to protonated and deprotonated SB products, respectively (Fig. 4*h*). At high pH, the spectrum of photobleached E181Q exhibits an increased 380-nm band and a decreased 480-nm band (Fig. 4*i*). Under conditions that produce native Meta I, we observe that the SB for E181Q is titrable with a $pK_a \approx 6$. This is very similar to the behavior observed for E113Q in the dark state that was used to assign the Glu-113 counterion of rhodopsin (1–3).

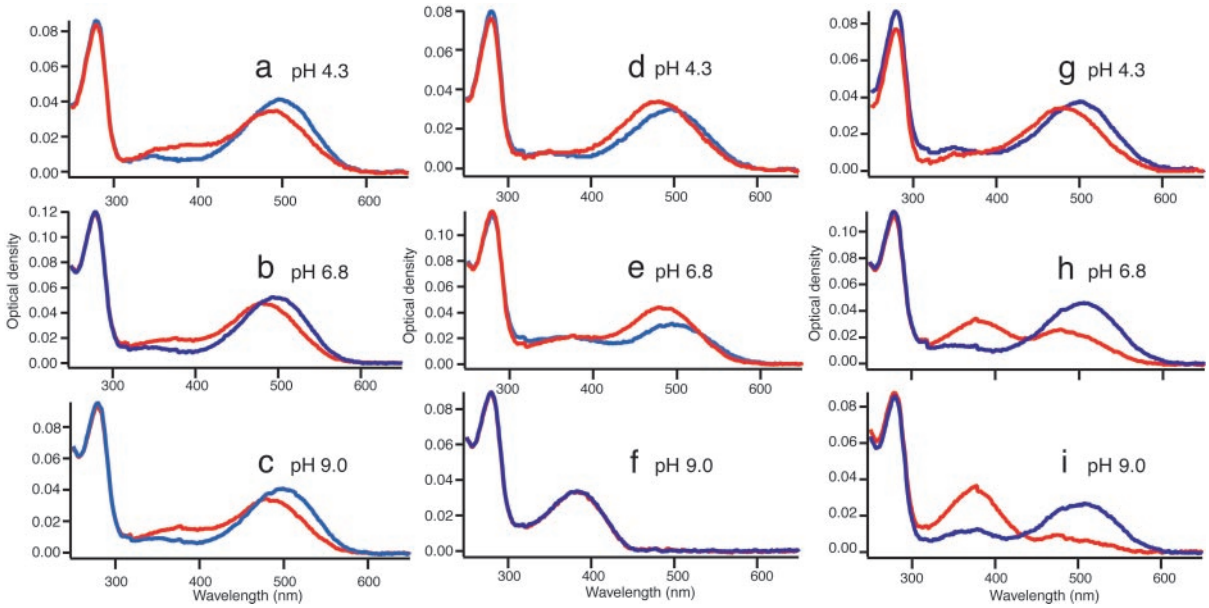

Fig. 4. The pH dependence of the UV-visible absorption spectra of the WT rhodopsin (*a–c*), the E113Q mutant (*d–f*), and the E181Q mutant (*g–i*) before (blue) and after (red) photobleaching with light $\lambda > 495$ nm. The pigments were in 100 mM NaCl, 0.1% digitonin, and 0.5 Tris buffer at 4°C, a condition that traps native Meta I (λ_{max} = 480 nm). Two fully independent experiments produced identical spectral results.

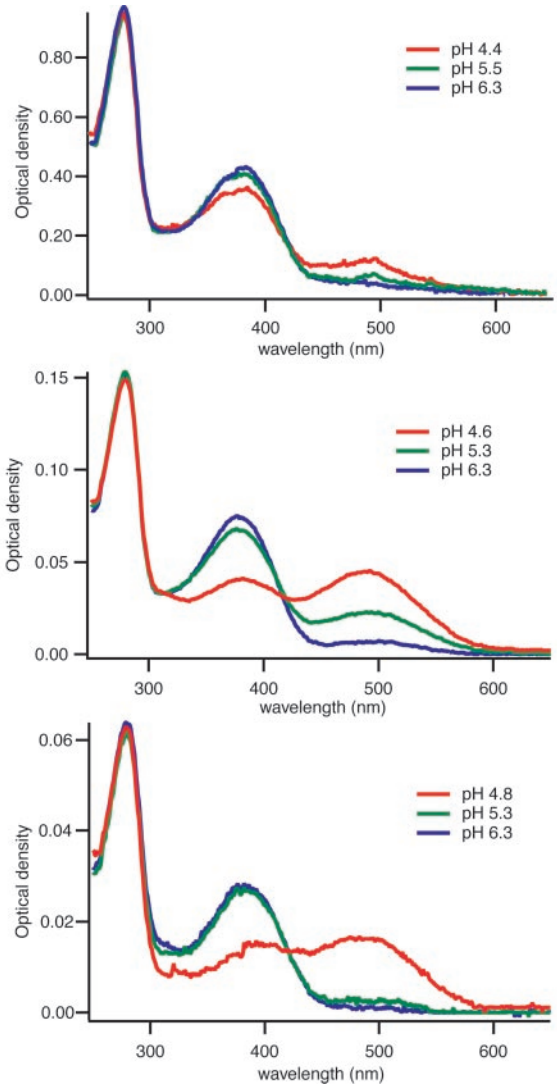

Fig. 5. The pH dependence of UV-Vis absorption spectra of photobleached WT rhodopsin (*Top*), E113Q (*Middle*), and E181Q (*Bottom*) pigments. The pigments were studied in 100 mM NaCl, 0.1% DM at room temperature, a condition that stabilizes Meta II (λ_{max} = 380 nm) for the native pigment at neutral pH.

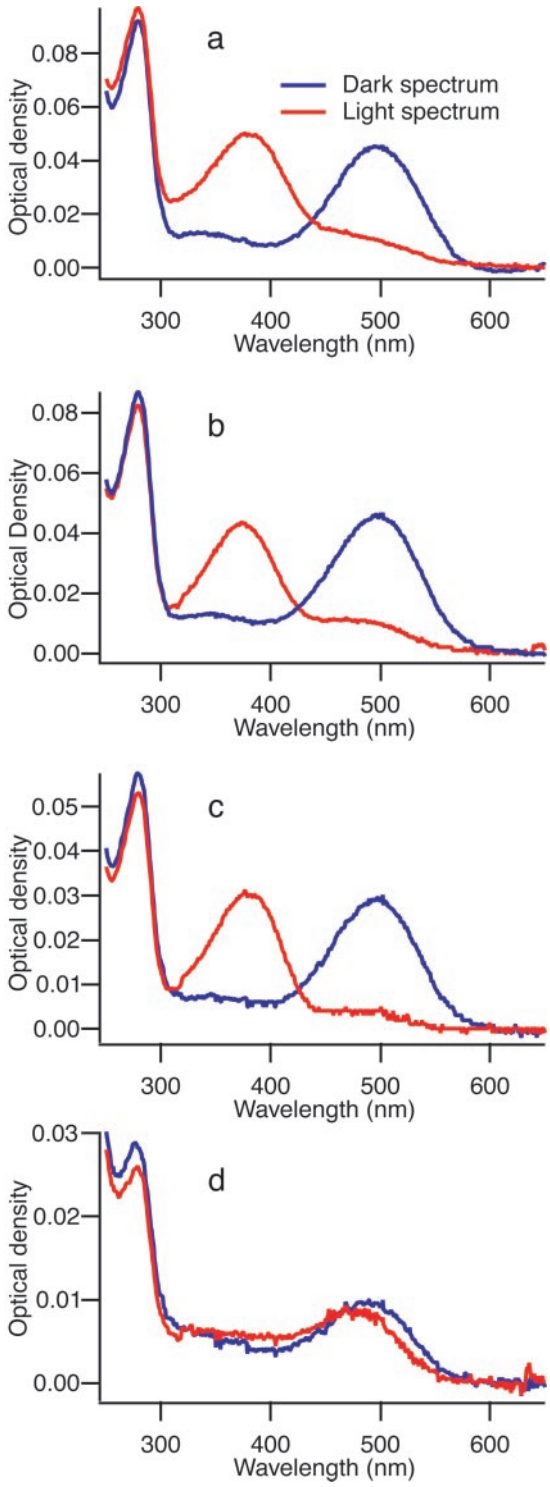

Fig. 6. UV-Vis spectra of the WT rhodopsin (*a*), and its S186A (*b*), S186I (*c*), and S186D (*d*) mutants purified in 0.1% DM in the dark (blue) and after illumination with light $\lambda > 495$ nm (red) at room temperature.

Under conditions that stabilize native Meta II, the pH dependence of the absorption spectra of the E181Q and E113Q mutants was also investigated along with a WT rhodopsin control (Fig. 5). At pH 6.3, WT rhodopsin converts completely to deprotonated Meta II with a characteristic 380-nm absorption. However, at pH 4.4 the 500/380 ratio is ≈1/3, suggesting that the pK_a of the Meta II intermediate of WT rhodopsin under the experimental condition is slightly below 4.4. Fig. 5 *b* and *c* shows that at pH 4.6 and 4.8, the acidic and basic forms of the Meta II-like photoproducts of E113Q and E181Q contribute almost equally to the absorption spectra. The apparent pK_a values of the photobleached forms of E181Q and E113Q are thus ≈5. This mutation-induced pK_a shift implies that both Glu-181 and Glu-113 play roles in determining the electrostatic environment and hence the pK_a of the SB in Meta II. These interactions could be an important factor in maintaining an unprotonated SB in native Meta II.

Fig. 6 presents the dark and light spectra of the S186A, S186I, and S186D mutants as well as the WT rhodopsin control. The spectra were taken at pH 6.8 in the presence of 0.1% DM and 100 mM NaCl at room temperature, conditions that should stabilize Meta II. The S186A and S186I mutant pigments have an absorption maximum at 500 nm in the dark. After being bleached with light >495 nm, the S186A and S186I mutant pigments exhibit an absorption band at 380 nm corresponding to the Meta II-like photoproduct. These observations are identical to those for WT rhodopsin. On the other hand, Fig. 4*d* shows that

the dark spectrum of the S186D mutant peaks at ≈495 nm, ≈5 nm blue shifted from WT rhodopsin. On illumination, S186D fails to form a normal Meta II state. Instead, it produces a very broad band having $\lambda_{max} \approx 480$ nm, exhibiting a characteristic absorption of Meta I_{480} with a protonated SB. It is evident that the addition of a carboxylic acid residue situated between Glu-113 and Glu-181 (Fig. 2) has altered the normal transition from rhodopsin to Meta II.

Discussion

The absorption and Raman spectra presented here permit an assignment of the protonation state of rhodopsin's Glu-181 residue in the dark state. Compared with WT rhodopsin, there are only modest shifts in the SB and ethylenic vibrational modes for the E181Q mutant. The fingerprint region and hydrogen out-of-plane modes are also unperturbed by E181Q mutation. If Glu-181 were charged in the WT pigment, E181Q mutation would introduce a neutral residue in its place and be expected to produce a more significant perturbation. Because of the proximity of Glu-181 to retinal (see Fig. 2) the mild perturbations to the vibrational structure caused by E181Q mutation are consistent only with a neutral (protonated) Glu-181. This conclusion is in agreement with previous biochemical studies (15).

The UV-visible spectra of photobleached E181Q are strongly pH dependent under conditions that trap the native Meta I intermediate. This observation is strikingly similar to the observed pH dependence of the E113Q mutant in the dark state that was used to assign the Glu-113 counterion (1–3). The E181Q mutation evidently removes the native counterion in the Meta I state, and hence changes the pK_a value of the SB into the range of physiological pH. This result is most consistent with the conclusion that Glu-181 is the primary counterion of the PSB in the Meta I state.

The UV-visible spectra of the Meta II-like photoproducts of E113Q and E181Q demonstrate that both mutations raise the pK_a from ≤4.4 in WT rhodopsin to ≈5. This observation reveals that both Glu-181 and Glu-113 interact with the SB in the Meta II state. In addition to the known anion effects (29), these interactions could be a determining factor controlling the deprotonation of the SB in native Meta II. This suggests that, as indicated by the structure, Glu-181 and Glu-113 are not interacting independently with the chromophore in the later intermediates such that the possibility of a complex counterion as observed in bacteriorhodopsin must be considered (30, 31).

The identification of Glu-181 as the primary counterion of the PSB in the Meta I state requires the existence of a counterion switch from Glu-113 in the dark state to Glu-181 in the Meta I state on photoactivation. How might this switch occur? According to the recent refined crystal structure (9), there is an H-bond network extending across the binding pocket, which involves Glu-113 and Glu-181 (Fig. 2*a*). We propose that the switch is accomplished by transferring a proton from Glu-181 to Glu-113 through a structurally evolving H-bond network as depicted in Fig. 7. Fig. 7*a* shows the binding pocket of the chromophore from the crystal structure of rhodopsin in the dark state (9). Two water molecules and Ser-186 form an H-bond chain between Glu-113 and Glu-181. Glu-113, being closest to the PSB, is the primary counterion neutralizing the positive charge in rhodopsin. After photolysis, the chromophore isomerizes to form bathorhodopsin (Batho), which has a characteristic twisted ethylenic chain. In the blue-shifted intermediate (BSI) state, the torsion along the ethylenic chain in Batho has partially relaxed (17), and this interaction presumably changes the conformation of the H-bond chain to prepare for proton transfer. In the Lumi state, the PSB group has shifted away from Glu-113 and the identity of a formal counterion has been lost because the negative charge becomes delocalized along the H-bond chain. The idea that Lumi is the transition state in the counterion switching process is supported

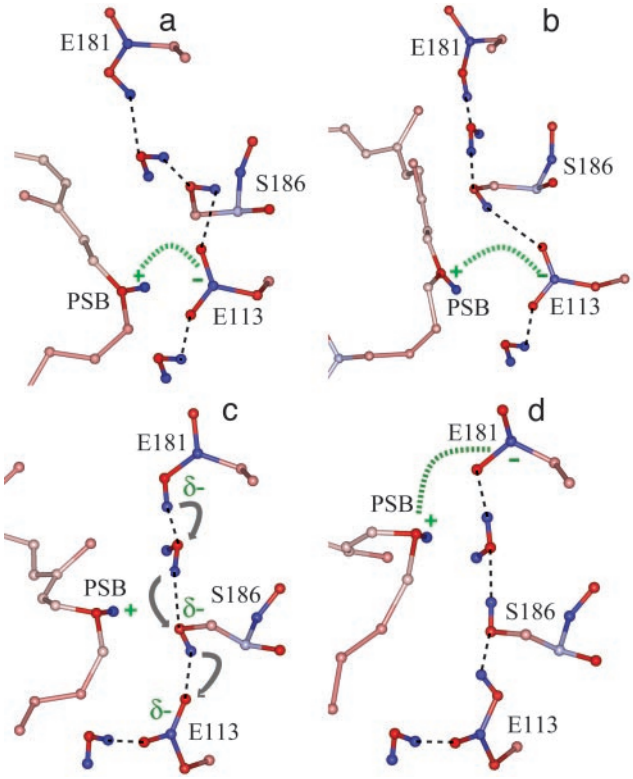

Fig. 7. Schematic of the proposed proton transfer mechanism for switching the PSB counterion in rhodopsin. (*a*) Rhodopsin: two water molecules and Ser-186 form a H-bond chain between Glu-113 and Glu-181. Electrostatic interaction between the PSB and Glu-113 is indicated by the green dashed line (9). (*b*) Blue-shifted intermediate: after photoisomerization, the H-bond chain evolves so that the two water molecules and Ser-186 are lined up to prepare for the proton transfer and the PSB has shifted relative to Glu-113. (*c*) Lumirhodopsin: the PSB shifts further away from Glu-113 toward Glu-181. The gray arrows indicate a possible proton transfer pathway. Lumi is the transition state for the counterion switch as suggested by previous Raman and FTIR studies showing that the SB has moved from a strong H-bond environment to a very weak H-bond environment in the Lumi state (17–20). (*d*) Metarhodopsin I: proton transfer is completed. The PSB group is now close to Glu-181 to establish the electrostatic interaction (green dashed line) with the new counterion. In the Meta intermediates, it is possible that the counterion environment about Glu-181 is complex as has been observed and discussed in the case of bacteriorhodopsin (30, 31).

by our recent Raman studies (17, 18) revealing that the H-bond of the SB dramatically weakens in the BSI-to-Lumi transition before forming a more normal H-bond with some residue in the Lumi-to-Meta I transition. Finally, in the Meta I state, the SB group has moved toward Glu-181. Because the distance from the SB to Glu-181 in rhodopsin is only ≈7 Å compared with ≈3 Å for Glu-113, we believe only a modest conformational change that alters the spatial relationship between EII and helix III (HIII) would be required to allow the formation of the new salt bridge between Glu-181 and the PSB in Meta I.

In our model, Ser-186 serves as a proton carrier between Glu-113 and Glu-181. It seems likely that in the S186D mutant the Asp at position 186 provides a carboxylic group to replace Glu-181 as a proton donor, which introduces a "short circuit" for the proton movement and prevents formation of a normal Meta II intermediate. Preliminary results suggest that the transducin activation of S186D is significantly reduced compared with that of WT rhodopsin.

Our proposed counterion switch model provides insight into the broader biological role of EII in GPCRs. EII, consisting of

the β3 and β4 strands, is particularly interesting because alignments of the amino acid sequence among GPCRs have revealed that β4 is high conserved in vertebrate visual pigments but varies significantly in other classes of receptors (10, 11, 32). This implies that the function of the β4 strand is to provide specific recognition for agonist ligands. EII is also important for the formation of the H-bond network in the binding pocket. Among the six residues participating in the H-bond network in rhodopsin (Phe-91, Glu-113, Glu-181, Ser-186, Cys-187, and Tyr-192) (9), the latter three are located on β4, and Glu-181 is located at the linker between β3 and β4.

Why are the important residues participating in the H-bond network in the binding pocket situated on the loop structure of EII? Our counterion switch mechanism suggests an answer to this question. First, the loop structure provides flexibility to allow the β4 residues to accommodate the newly formed or bound agonist leading to an active conformation that can couple to the corresponding G protein. Second, EII is linked covalently to transmembrane HIII through a highly conserved disulfide bond (Cys-110–Cys-187) (33). Extensive biochemical and mutagenesis studies revealed that this disulfide bond is essential for a fully functional rhodopsin (34–38). Mutant pigments with Cys-110–Cys-185 disulfide crosslinks displayed markedly altered bleaching behaviors (34). The new Glu-181 counterion in Meta I is on the EII loop with Cys-187, whereas Glu-113 is exactly one helical turn away from Cys-110 on HIII. Considering the high conservation of Glu-113, Glu-181 and the disulfide bond in visual pigments, we speculate that the EII and HII movements triggered by the counterion switch and coordinated by the disulfide bound could be a general photoactivation mechanism in visual pigments and presumably in all GPCRs. Further analyses of the structural dynamics of rhodopsin's later intermediates guided by the counterion switch model should lead to a better understanding of the general mechanism of GPCR activation.

We thank James W. Lewis, David S. Kliger, Reiner Vogel, and Friedrich Siebert for valuable discussions. This research was supported in part by National Institutes of Health Grant EY02051 (to R.A.M.). T.P.S. is an investigator of the Howard Hughes Medical Institute and an Ellison Foundation senior scholar.

1. Nathans, J. (1990) *Biochemistry* **29,** 9746–9752.
2. Sakmar, T. P., Franke, R. R. & Khorana, H. G. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8309–8313.
3. Zhukovsky, E. A. & Oprian, D. D. (1989) *Science* **246,** 928–930.
4. Hofmann, K. P., Jager, S. & Ernst, O. P. (1995) *Israel J. Chem.* **35,** 339–355.
5. Fung, B. K. K. & Stryer, L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2500–2504.
6. Hargrave, P. A., Hamm, H. E. & Hofmann, K. P. (1993) *BioEssays* **15,** 43–50.
7. Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., *et al.* (2000) *Science* **289,** 739–745.
8. Okada, T., Ernst, O. P., Palczewski, K. & Hofmann, K. P. (2001) *Trends Biochem. Sci.* **26,** 318–324.
9. Okada, T., Fujiyoshi, Y., Silow, M., Navarro, J., Landau, E. M. & Shichida, Y. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 5982–5987.
10. Menon, S. T., Han, M. & Sakmar, T. P. (2001) *Physiol. Rev.* **81,** 1659–1688.
11. Ballesteros, J. A., Shi, L. & Javitch, J. A. (2001) *Mol. Pharmacol.* **60,** 1–19.
12. Teller, D. C., Okada, T., Behnke, C. A., Palczewski, K. & Stenkamp, R. E. (2001) *Biochemistry* **40,** 7761–7772.
13. Terakita, A., Yamashita, T. & Shichida, Y. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 14263–14267.
14. Wang, Z., Asenjo, A. B. & Oprian, D. D. (1993) *Biochemistry* **32,** 2125–2130.
15. Yan, E. C. Y., Kazmi, M. A., De, S., Chang, B. S. W., Seibert, C., Marin, E. P., Mathies, R. A. & Sakmar, T. P. (2002) *Biochemistry* **41,** 3620–3627.
16. Birge, R. R., Murray, L. P., Pierce, B. M., Akita, H., Balogh-Nair, V., Findsen, L. A. & Nakanishi, K. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4117–4121.
17. Pan, D., Ganim, Z., Kim, J. E., Verhoeven, M. A., Lugtenburg, J. & Mathies, R. A. (2002) *J. Am. Chem. Soc.* **124,** 4857–4864.
18. Pan, D. & Mathies, R. A. (2001) *Biochemistry* **40,** 7929–7936.
19. Siebert, F. (1995) *Israel J. Chem.* **35,** 309–323.
20. Ganter, U. M., Gartner, W. & Siebert, F. (1988) *Biochemistry* **27,** 7480–7488.
21. Marin, E. P., Krishna, A. G., Archambault, V., Simuni, E., Fu, W.-Y. & Sakmar, T. P. (2001) *J. Biol. Chem.* **276,** 23873–23880.
22. Min, K. C., Zvyaga, T. A., Cypess, A. M. & Sakmar, T. P. (1993) *J. Biol. Chem.* **268,** 9400–9404.
23. Han, M., Lin, S. W., Minkova, M., Smith, S. O. & Sakmar, T. P. (1996) *J. Biol. Chem.* **271,** 32337–32342.
24. Lin, S. W., Sakmar, T. P., Franke, R. R., Khorana, H. G. & Mathies, R. A. (1992) *Biochemistry* **31,** 5105–5111.
25. Resek, J. F., Farahbakhsh, Z. T., Hubbell, W. L. & Khorana, H. G. (1993) *Biochemistry* **32,** 12025–12032.
26. Hubbard, R. & Kropf, A. (1958) *Proc. Natl. Acad. Sci. USA* **44,** 130–139.
27. Shichi, H., Kawamura, S., Muellenberg, G. G. & Yoshizawa, T. (1977) *Biochemistry* **16,** 5376–5381.
28. König, B., Welte, W. & Hofmann, K. P. (1989) *FEBS Lett.* **257,** 163–166.
29. Vogel, R., Fan, G. B., Siebert, F. & Sheves, M. (2001) *Biochemistry* **40,** 13342–13352.
30. Kusnetzow, A., Singh, D. L., Martin, C. H., Barani, I. J. & Birge, R. R. (1999) *Biophys. J.* **76,** 2370–2389.
31. Stuart, J. A., Vought, B. W., Zhang, C. F. & Birge, R. R. (1995) *Biospectroscopy* **1,** 9–28.
32. Sakmar, T. P. (2002) *Curr. Opin. Cell Biol.* **14,** 189–195.
33. Karnik, S. S. & Khorana, H. G. (1990) *J. Biol. Chem.* **265,** 17520–17524.
34. Hwa, J., Reeves, P. J., Klein-Seetharaman, J., Davidson, F. & Khorana, H. G. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 1932–1935.
35. Hwa, J., Klein-Seetharaman, J. & Khorana, H. G. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 4872–4876.
36. Davidson, F. F., Loewen, P. C. & Khorana, H. G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4029–4033.
37. Karnik, S. S., Sakmar, T. P., Chen, H.-B. & Khorana, H. G. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8459–8463.
38. Ridge, K. D., Lu, Z., Liu, X. & Khorana, H. G. (1995) *Biochemistry* **34,** 3261–3267.