Electrostatic Coupling between Retinal Isomerization and the Ionization State of Glu-204: A General Mechanism for Proton Release in Bacteriorhodopsin

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ABSTRACT The pK_a values of ionizable groups that lie between the active site region of bacteriorhodopsin (bR) and the extracellular surface of the protein are reported. Glu-204 is found to have an elevated pK_a in the resting state of bR, suggesting that it corresponds to the proton-releasing group in bR. Its elevated pK_a is predicted to be due in part to strong repulsive interactions with Glu-9. Following *trans-cis* isomerization of the retinal chromophore and transfer of a proton to Asp-85, polar groups on the protein are able to interact more strongly with the ionized state of Glu-204, leading to a substantial reduction of its pK_a. This suggests a general mechanism for proton release in which isomerization and subsequent charge separation initially produce a new electrostatic balance in the active site of bR. Here it is proposed that those events in turn drives a conformational change in the protein in which the ionized state of Glu-204 can be stabilized through interactions with groups that were previously inaccessible. Whether these groups should be identified with polar moieties in the protein, bound waters, or Arg-82 is an important mechanistic question whose elucidation will require further study.

INTRODUCTION

The proton-pumping mechanism of bacteriorhodopsin (bR) involves a series of distinct steps characterized by protein conformational changes and directed proton transfer between ionizable residues. Although spectroscopic and genetic studies have identified many of the groups actively involved in proton pumping, significant uncertainties still remain. Proton pumping is initiated by the absorption of a photon by the retinal chromphore that is bound to Lys-216 in the protein via a protonated Schiff base linkage. Following light absorption, the Schiff base proton is transferred to Asp-85, a nearby group in the active site that is ionized in the ground state of bR but that, after accepting a proton from the Schiff base, remains neutral until late in the photocycle. Therefore, transfer of a proton to Asp-85 must induce some other group to release its proton to the extracellular medium (Siebert et al., 1982; Gerwert et al., 1990; Braiman et al., 1991; Souvignier and Gerwert, 1992). This group has been designated XH (Zimányi et al., 1992; Ebrey, 1993), and although its identity is uncertain, a number of suggestions for its identity have appeared in the literature. The pK_a of XH must be high enough in bR to exist in the neutral form but must be lowered significantly on protonation of Asp-85 to serve as a releasing group. Here we report calculations of the pK_a values of ionizable groups along the likely release pathway of the proton and, on this basis, identify a candidate for XH. Our results also suggest general mechanisms that can be exploited by membrane proteins to direct proton transfer along specific pathways.

Fig. 1 contains a diagram of the structure of bR (Henderson et al., 1990) that highlights ionizable residues that are known to participate in the pumping mechanism or that are close enough to the retinal to be possible candidates for XH. The cytoplasmic region above the Schiff base is narrow, closely packed, and, with the exception of Asp-96, relatively hydrophobic. In contrast, cavities large enough to accommodate a number of water molecules have been identified in the region below the Schiff base (Sampogna and Honig, 1994), and the channel leading to the extracellular surface consists of a network of polar and charged groups. Whereas Arg-82, Asp-85, Asp-212, and the Schiff base have been studied in detail, few experimental data are available on the possible roles of Glu-9, Glu-74, Lys-129, Glu-194, and Glu-204. Inasmuch as these clearly lie between the Schiff base, Asp-85, and the extracellular medium, any of them might play a role in proton pumping. Our results clearly implicate Glu-204 as XH because it is the only acidic residue that is predicted to be protonated in the resting state of bR, and, moreover, our calculations suggest that Glu-204 undergoes a large decrease in pK_a following protonation of Asp-85.

Other suggestions for the identity of XH have appeared in the literature. These include Arg-82 (Mathies et al., 1991), a tyrosine, presumably Tyr-57 or Tyr-185 (Hanamoto et al., 1984; Balashov et al., 1991), and a water molecule interacting with Arg-82 (Braiman et al., 1988) or in a hydrogen-bonded complex associated with other residues (Rothschild et al., 1992; Zimányi et al., 1992; Lanyi, 1994). It has been clearly established that Arg-82, if it is not XH itself, controls the pK_a of XH and that there is a strong coupling between the ionization states of Asp-85 and XH (Balashov et al., 1993). Recent experimental studies based on site-

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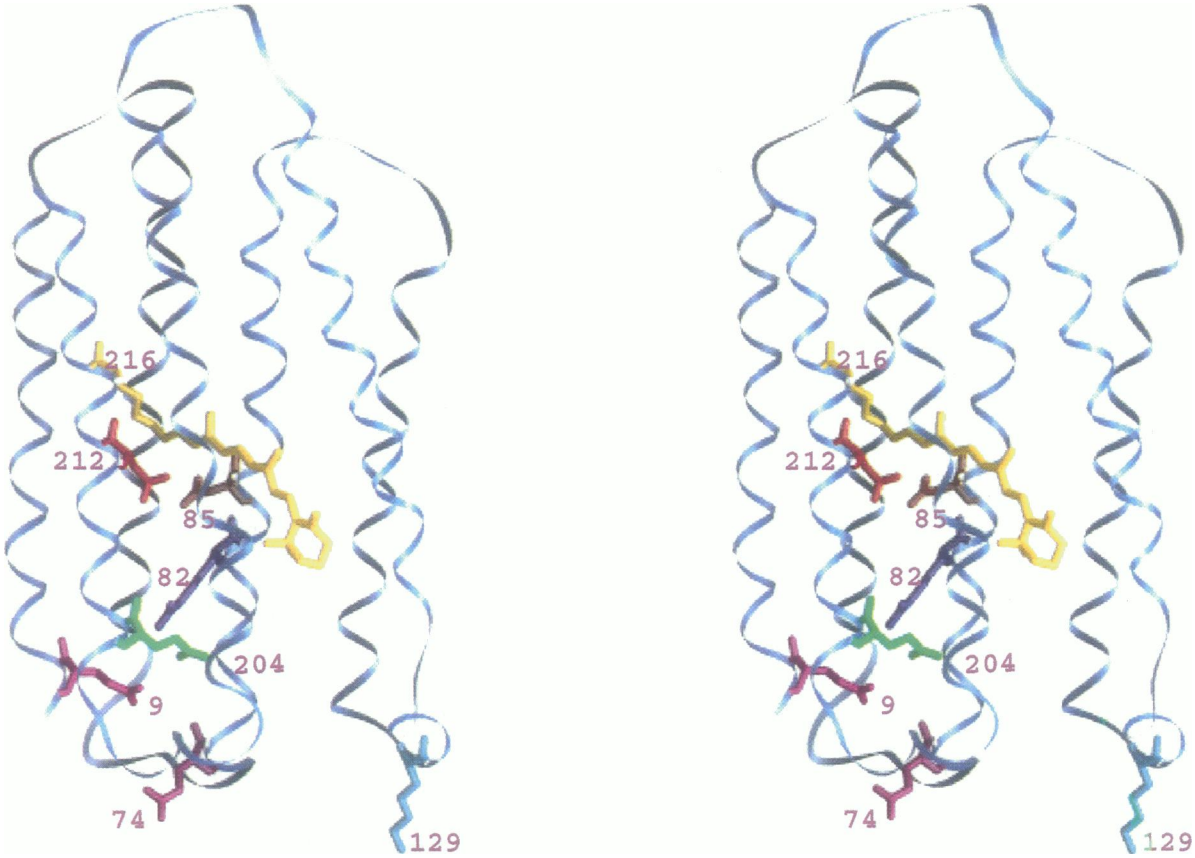

FIGURE 1 Stereoview of ionizable residues in the electron diffraction structure determined by Henderson et al. (1990). Figure generated with GRASP (Nicholls et al., 1991).

directed mutagenesis strongly implicate Glu-204 as XH (Brown et al., 1995).

Independent theoretical studies have also implicated Glu-204 as the protein-releasing group. Glu-204 was shown to undergo strong electrostatic interactions with Arg-82 (Sampogna and Honig, 1994), and it was found that Glu-204 undergoes a large decrease in pK_a following protonation of Asp-85 (Sampogna, 1995). Recent theoretical calculations of pK_a values based on the DelPhi program used in this study have also identified Glu-204 as the proton-releasing group (Scharnagl et al., 1995). A detailed model for proton release was described in which Arg-82 functions as a switch that leads to the deprotonation of Glu-204. The possible role of other amino acids near Glu-204, such as Glu-9, Glu-74, Lys-129, and Glu-194, was not considered. Here we show that Glu-204 is the only residue along the proton transfer pathway that exhibits the behavior of a releasing group and that proton release from Glu-204 does not require that Arg-82 function as a switch. Rather, the mechanism proposed by Scharnagl et al. (1995) appears to be one of a number of plausible possibilities. More generally, we report on the nature of the interactions that produce the unique behavior of Glu-204.

METHODS

Details of the finite-difference Poisson–Boltzmann method used in this study to calculate values of pK_a are described elsewhere (Bashford and Karplus, 1990; Yang et al., 1993). We used the DelPhi program to obtain electrostatic free energies (Nicholls and Honig, 1991) and then employed the same formalism used in previous studies of bR (Bashford and Gerwert, 1992; Sampogna and Honig, 1994). Atomic charges and radii were taken from the DISCOVER force field (Hagler et al., 1973), except for the retinal chromophore, for which charges calculated by Tavan et al. (1985) were used. The protein was defined by its solvent accessible surface and assigned a dielectric constant of 4. In addition to regions outside this surface, all interior cavities and clefts were assigned a dielectric constant of 80 unless they were filled with explicit water molecules. An extensive discussion of the effects of treating explicit water molecules in protein cavities as low dielectric regions and the exclusion of a lipid bilayer can be found in our previous paper (Sampogna and Honig, 1994).

This study utilizes three bR models derived from the structure determined by Henderson and co-workers (Henderson et al., 1990). These are referred to as $bR_{in}(\epsilon)$, $bR_{out}(wat)$, and $bR_{in/cis}(\epsilon)$. Their generation was described fully in a previous paper (Sampogna and Honig, 1994). The Arg-82 sidechain in the experimentally determined structure model faces the extracellular surface of the protein (Fig. 1). This position has been modified in $bR_{in}(\epsilon)$ so that the Arg-82 sidechain is associated with the active site and forms a salt bridge with Asp-85 (Fig. 2). In $bR_{out}(wat)$, internal cavities near the Schiff base are filled with five water molecules, whereas heavy atom positions are identical to those determined by Henderson et al. (1990). In the electrostatic calculations, these waters are treated explicitly as part of the protein and are thus associated with a low dielectric region.

One structure, $bR_{in/cis}(\epsilon)$, contains a modified chromophore in which the retinal has been rotated about the 13,14 double bond into the 13-*cis* conformation. $bR_{in/cis}(\epsilon)$ is derived from $bR_{in}(\epsilon)$ and contains the Arg-82 sidechain in close association with Asp-85 and Asp-212 in the active site. This structure is intended to represent the state of the protein following

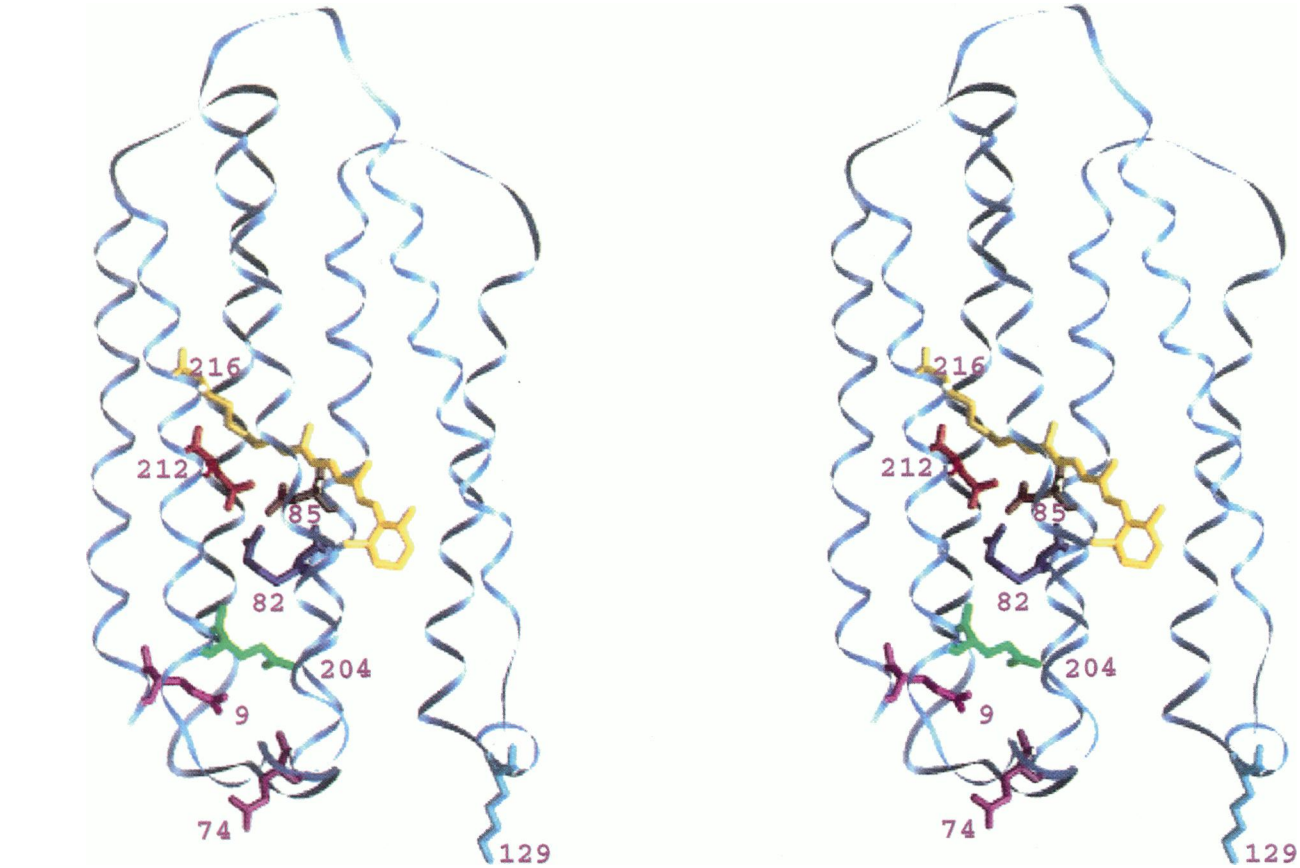

FIGURE 2 Stereoview of ionizable residues in the $bR_{in}(\epsilon)$ structure. Figure generated with GRASP (Nicholls et al., 1991).

light absorption, although no attempt is made to identify it with specific states of the photocycle. Energy minimization and molecular dynamics calculations were carried out on the *cis* species to relax sterically strained regions in the protein resulting from isomerization and to maximize hydrogen bonding and charge–charge interactions (Sampogna and Honig, 1994). Helices F and G were found to move in response to the retinal isomerization, shifting slightly toward the extracellular side.

pK_a shifts arise from differential electrostatic interactions between the free ionizable residue located in solution and the same group when it is located in the protein environment. The factors that contribute to the shift can be broken down into three separate free-energy contributions (Bashford and Karplus, 1990; Yang et al., 1993). The first, known as the reaction field free energy, $\Delta\Delta G_i^{rf}$, results primarily from the loss of stabilizing interactions with the solvent on transfer of a titratable group, i, from solvent into the protein environment. The resulting "desolvation penalty" can be compensated for by interactions with dipolar groups in the protein, particularly those that form hydrogen bonds with the titratable group, yielding a term referred to as $\Delta\Delta G_i^{dip}$. Finally, pH-dependent interactions with other titratable residues in the protein (charge–charge interactions) also affect the pK_a of group i, with the corresponding free-energy contribution, $\Delta\Delta G_i^{ttr}$.

The pKa of a group, i, is given by the expression

$$pK_{ai} = pK_i^{\circ} - \gamma(i)[\Delta\Delta G_i^{rf} + \Delta\Delta G_i^{dip}]/2.3kT + \Delta pK_i^{ttr} = pK_i^{int} + \Delta pK_i^{ttr}, \quad (1)$$

where pK_i°, the reference pK_a, is the pK_a of the isolated group in water and $\gamma(i)$ is -1 or 1 for an acidic or a basic group, respectively. The intrinsic pK_a of a group in a protein, pK_i^{int}, is defined as the pK_a of residue i when all other groups are neutral. Because ΔpK_i^{ttr} is pH dependent, the average charge of each group at a specific pH is determined from a statistical mechanical average of the possible protonation states of the entire protein (Bashford and Karplus, 1990; Yang et al., 1993).

RESULTS

Table 1 lists calculated electrostatic interactions and pK_a values for residues located in the extracellular region of the structures studied in this research (Figs. 1 and 2). These include Glu-9, Glu-74, Arg-82, Lys-129, Glu-194, and Glu-204. Whereas the removal of a charged group from solvent (as given by $\Delta\Delta G^{rf}$) tends to shift pK_a values to those that favor the neutral form, compensating interactions with local dipolar groups (given by $\Delta\Delta G^{dip}$) can stabilize the charged form of a buried residue. Thus, the first column in Table 1 is always positive and the second column is generally negative. Depending on the relative values of the two terms, pK_i^{int} will be shifted toward either the ionized or the neutral form of a particular residue. In addition, favorable charge–charge interactions will stabilize the ionized form of a titratable group, whereas interactions between like charges will have the opposite effect. In general, the strength of electrostatic interactions that occur between buried protein residues is large compared with such interactions mediated by solvent. This is largely responsible for the coupling observed between strongly interacting ionizable residues

TABLE 1 Electrostatic interactions in bacteriorhodopsin

Site	$\Delta\Delta G^{rf}$	$\Delta\Delta G^{dip}$	Charge–Charge Interactions (kcal/mol)						Calc. pKa
			Glu-9	Glu-74	Arg-82	Lys-129	Glu-194	Glu-204	
$bR_{in}(\epsilon)$									
Glu-9	5.0	−6.5	–	0.7	−2.3	0.0	0.1	5.1	2.0
Glu-74	0.5	−0.1	0.7	–	−0.1	−0.1	0.0	0.4	4.6
Arg-82	9.8	−1.4	−2.3	−0.1	–	0.0	−0.1	−2.0	26.8
Lys-129	0.1	−0.1	0.0	−0.1	0.0	–	0.0	0.0	10.8
Glu-194	1.4	−0.9	0.1	0.0	−0.1	0.0	–	0.5	4.8
Glu-204	8.7	−9.2	5.1	0.4	--2.0	0.0	0.5	–	7.8
bR_{out}(wat)									
Glu-9	5.2	−3.2	–	1.1	−5.0	0.0	0.1	5.4	1.2
Glu-74	0.2	−0.2	1.1	–	−0.4	−0.1	0.0	0.7	4.8
Arg-82	8.5	−2.6	−5.0	−0.4	–	0.0	−0.1	−5.6	20.2
Lys-129	0.1	−0.1	0.0	−0.1	0.0	–	0.0	0.0	10.8
Glu-194	0.3	0.2	0.1	0.0	−0.1	0.0	–	0.2	4.6
Glu-204	9.4	−5.8	5.4	0.7	−5.6	0.0	0.2	–	9.0
$bR_{in/cis}(\epsilon)$									
Glu-9	4.3	−5.1	–	0.9	−1.3	0.0	0.1	3.9	5.8
Glu-74	0.5	−0.3	0.9	–	−0.1	−0.1	0.0	0.3	4.2
Arg-82	9.2	1.3	−1.3	−0.1	–	0.0	−0.1	−2.1	28.0
Lys-129	0.1	−0.1	0.0	−0.1	0.0	–	0.0	−0.1	10.8
Glu-194	1.2	0.1	0.1	0.0	−0.1	0.0	–	0.5	5.6
Glu-204	7.6	−14.4	3.9	0.3	−2.1	−0.1	0.5	–	−2.2

$\Delta\Delta G^{rf}$, $\Delta\Delta G^{dip}$, and charge–charge interactions are in kcal/mol.

and the resulting highly anomalous titration behavior (Bashford and Gerwert, 1992; Balashov et al., 1993, 1995; Sampogna and Honig, 1994). Note that the charge–charge interactions shown in Table 1 describe only interactions between residues that are both ionized. These will not contribute at a pH when at least one of the pair is in its neutral form.

The following subsections describe the pK_a values of the residues in the extracellular region for the three states of bR listed above.

$bR_{in}(\epsilon)$

In $bR_{in}(\epsilon)$ large unfavorable reaction field energies are calculated for Glu-9, Arg-82, and Glu-204, indicating that they are buried within the protein. The large desolvation penalty for Arg-82 is compensated for by strong attractive interactions with active site residues Asp-85 and Asp-212 (Sampogna and Honig, 1994). Although the large positive reaction field energies calculated for both Glu-9 and Glu-204 would significantly raise their pK_a values, both desolvation penalties are compensated for by local hydrogen bonding. This effect alone would be sufficient to allow both residues to exist in their ionized forms; i.e., each has an intrinsic pKa below 4. However, the calculations reveal that only Glu-204 has an elevated pK_a. This is due to a strong electrostatic interaction between the two glutamates (5.1 kcal/mole), which are located ~6.4 Å apart. This unfavorable interaction destabilizes the doubly ionized state relative to states in which only one of the two groups is charged. Our calculations indicate that Glu-9 is easier to ionize than Glu-204 because the sum of $\Delta\Delta G^{dip}$ and $\Delta\Delta G^{rf}$ for this residue is more negative than the comparable sum for Glu-204 by 1 kcal/mole. Consequently, the intrinsic pK_a of Glu-204 is ~0.7 unit higher than that of Glu-9 (1 pK_a unit = 1.4 kcal/mole). Thus, the latter residue is easier to ionize.

bR_{out}(wat)

Glu-9 and Glu-204 are found to have smaller reaction field energies in bR_{out}(wat) than in the $bR_{in}(\epsilon)$ model. However, the corresponding values for $\Delta\Delta G^{dip}$ are smaller than for the $bR_{in}(\epsilon)$ model, leading to the prediction that both groups have elevated intrinsic pK_a values. Both ionic species are strongly stabilized by charge–charge interactions with Arg-82, whereas the doubly ionized state is destabilized by large repulsive interactions between the two glutamates. As was the case for the $bR_{in}(\epsilon)$ structure, Glu-9 is predicted to have a low pK_a and the pK_a for Glu-204 is raised to 9.

$bR_{in/cis}(\epsilon)$

trans-cis isomerization of the retinal chromophore has a profound effect on the protein structure and on calculated pK_a values. Although they are ionized in the ground state, both the Schiff base and Asp-85 are neutral in the photoisomerized state (Sampogna and Honig, 1994), which may then be regarded as a model for M. The ease with which proton transfer occurs is due to strong coupling between the two groups that is extremely sensitive to small changes in the environment of each. Because of conformational

changes in the protein resulting from retinal isomerization, the pKa calculated for Glu-204 is significantly lower than the value obtained for the corresponding ground-state model, $bR_{in}(\epsilon)$ (Table 1). By far, the biggest effect results from a large increase in stabilizing hydrogen bonding interactions, which lower its intrinsic pK_a by ~5 units, in good agreement with the measured shift in the pK_a of XH following protonation of Asp-85 (Balashov et al., 1993). Specifically, improved hydrogen-bonding interactions are formed between Glu-204 and polar groups of Gly-197, Ile-198, Ile-203, Thr-205, Phe-208, and especially Gly-192 and Ser-193 (Fig. 3*A* and *B*). In addition, the distance between Glu-9 and Glu-204 is also increased, decreasing their repulsive electrostatic interaction by ~1 kcal/mol. However, this interaction still raises the pK_a value of Glu-9 1.5 units from its reference pK_a (pK°).

DISCUSSION

An ionizable residue located in the protein interior and excluded from water tends to have its pK_a shifted in a direction that favors its neutral form. Because the majority of titratable groups in bR are totally or partially removed from solvent, other compensating interactions must overcome this desolvation effect so as to stabilize the charged form. This stabilization can be provided by polar groups in the protein, which form strong hydrogen bonds with the charged state of the residue, and by pH-dependent electrostatic interactions between titratable groups. In a membrane protein such as bR, such interactions generally occur within its low dielectric interior and are thus magnified in comparison with those that occur through solvent or at the protein surface. As a result, electrostatic potentials become remarkably sensitive to the local environment. For example, previous studies demonstrated that the ionization state of the Schiff base is extremely sensitive to the protonation state of Asp-85. In addition, the large difference in the titration behavior between active site residues Asp-85 and Asp-212 arises solely from differences in their immediate polar environments (Sampogna and Honig, 1994; Sampogna, 1995).

In addition to the possibility of their exhibiting unstable titration behavior, desolvated residues, especially Arg-82, Asp-85, Glu-204, Asp-212, and the Schiff base, can have markedly shifted pK_a values (Sampogna and Honig, 1994). In contrast, groups such as Glu-74, Lys-129, and Glu-194, which are relatively solvent exposed, display small desolvation penalties and negligible pK_a shifts. Furthermore, potentials generated by these groups are propagated through and shielded by solvent, resulting in pairwise interactions that are only of the order of 1 kcal/mol or less (Gilson and Honig, 1988). The electrostatic potential calculated within the interior of bR is characterized by a large contiguous negative region that includes the active site and extends to the extracellular region (Sampogna, 1995). This potential contributes significantly to the elevated pK_a values calculated for Arg-82 and Glu-204 in the ground state.

The most important conclusion of this study is that Glu-204 is predicted to have a pK_a above 7 in both the $bR_{in}(\epsilon)$ and the bR_{out}(wat) models for the resting state of bR. These results strongly suggest that Glu-204 should be identified

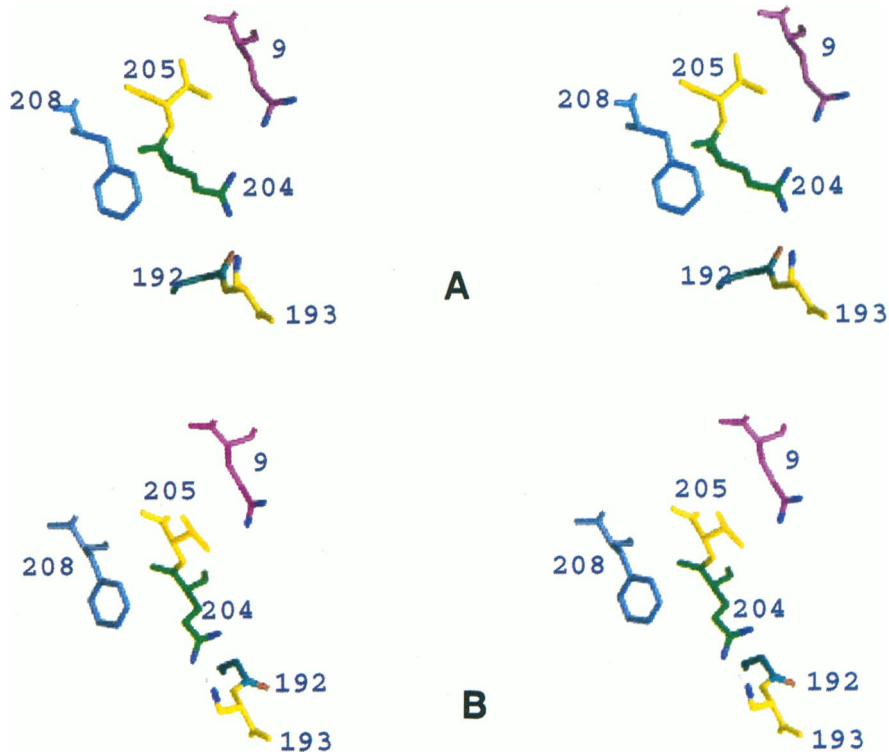

FIGURE 3 (A) Stereoview of polar groups surrounding Glu-204 in the $bR_{in}(\epsilon)$ model. (B) Stereoview of polar groups surrounding Glu-204 in the $bR_{in/cis}(\epsilon)$ model. Polar groups, especially of residues of Gly-192 and Ser-193, are in close contact with Glu-204. In addition, the distance between Glu-9 and Glu204 is ~1.5 Å greater than that in the $bR_{in}(\epsilon)$ model.

with XH, the proton-releasing group to the extracellular medium. In either model, the elevated pK_a of Glu-204 results in large part from its interaction with Glu-9. Thus, our calculations suggest that a mutant bR in which Glu-9 has been replaced with a neutral residue would exhibit substantially modified proton-release kinetics.

Despite the fact that both models yield comparable pK_a values the bR_{out}(wat) model encounters a serious difficulty when it is considered in detail. The coordinates in this model were obtained directly from the experimental structure (Henderson et al., 1990) and were not energy minimized as was done with the $bR_{in}(\epsilon)$ structure. When an energy minimization of bR_{out}(wat) is carried out (results not shown) a strong ion pair is formed between Glu-204 and Arg-82 that has the effect of lowering the pK_a of Glu-204 to below 4. Thus it is unlikely that Glu-204 would be ionized in the bR_{out}(wat) structure.

To determine how *trans-cis* isomerization of the chromphore might affect proton release, we have calculated pK_a values in the $bR_{in/cis}(\epsilon)$ structure. Clearly, the most striking result is that Glu-204 is predicted to have a low pK_a, undergoing a shift of $\sim$10 pK_a units relative to the $bR_{in}(\epsilon)$ structure (Table 1). This shift is due in part to a reduced electrostatic repulsion with Glu-9. However, the main effect appears due to polar main-chain and sidechain groups located in the loop region between helices F and G, which rearrange to stabilize negative charge on Glu-204 (Fig. 3). In the ground state, Glu-204 is strongly destabilized by the loss of reaction field energy and by a repulsive interaction with Glu-9, which is magnified within the protein interior. Upon improved stabilization by polar groups in the isomerized form, the charged form of Glu-204 is favored substantially and its pK_a is lowered significantly.

It has been proposed that Arg-82 may be the proton donor to the extracellular medium (Mathies et al., 1991) or at least that it controls the pKa of some other group that is directly involved in proton release (Balashov et al., 1993). Our calculations argue against the former suggestion. Although Arg-82 is inaccessible to solvent, strongly negative potential in the interior of bR and direct interactions with Asp-212 maintain its charged form at lower pH values. As pH is increased, the titration of other negatively charged groups, Asp-85 and Glu-204, for example, provides further stabilization (Sampogna and Honig, 1994; Sampogna, 1995). Hence, calculated pK_a values for Arg-82 are extremely high in all our models. Based on this finding, we rule out Arg-82 as the proton donor to the extracellular medium and suggest, instead, that Glu-204 is the ultimate proton-releasing group.

A recent study proposes that, to initiate deprotonation of Glu-204, the Arg-82 sidechain must break its interaction with Asp-85 and reorient itself to pair with Glu-204 before the M state (Scharnagl et al., 1995). Our studies, however, do not support this model for proton release. We find that, on transfer of a proton from the Schiff base to Asp-85 in our $bR_{in}(\epsilon)$ model, the sidechain of Arg-82 moves closer to Asp-212 to interact with this newly unpaired buried charge. Thus, we are not able to identify a suitable electrostatic driving force that would cause Arg-82 to leave the active site following isomerization of the chromphore.

It is important to emphasize that the structural models presented here are not intended to provide a detailed model for the proton-release mechanism of bR. We have not, for example, attempted to generate new structures based on molecular dynamics calculations similar to those reported by Humphrey et al. (1994) and Scharnagl et al. (1995) but rather have chosen to introduce minimal perturbations into the experimentally derived structure. That Glu-204 is predicted to have an elevated pK_a both in our $bR_{in}(\epsilon)$ model and in the model of Scharnagl et al. (1995) suggests that there may be crucial interactions involved that are independent of the specific model that is adopted. The results reported here suggest that the electrostatic repulsion between Glu-9 and the ionized form of Glu-204 may provide a common feature in both models. A recent study by Govindjee et al. (1996) demonstrates that Arg-82 raises the pK_a of Glu-204 in the photolyzed state by $\sim$1.7 units. This corresponds to a direct electrostatic interaction between the two residues of ~ -2.4 kcal/mol, in good agreement with the value of -2.1 kcal/mol reported for the $bR_{in/cis}\epsilon$ structure in Table 1. This result suggests that, although Arg-82 accelerates the rate of proton release from Glu-204, its role is not essential, in agreement with the results of this study. On the other hand, the interaction between Glu-204 and Arg-82 may be complex, as indicated by the fact that the pK_a of Glu-204 is lowered in the unphotolyzed R82Q pigment.

Given the uncertainties about the detailed mechanism, it is of interest to consider whether there are general features in the proton-release mechanism that are also model independent. In this regard, our calculations suggest a model wherein the electrostatic balance among the four active site residues in the ground state of bR is disrupted by isomerization of the chromphore and subsequent transfer of the Schiff base proton to Asp-85. Calculations (unpublished) suggest that both retinal isomerization and proton transfer to Asp-85 are required so that the relaxation processes leading to the stabilization of the ionized form of Glu-204 can occur. These processes cause the structure to relax because the detailed balance of electrostatic forces in the active site has now been significantly modified. In the $bR_{in/cis}(\epsilon)$ model presented here, a consequence of this relaxation is that, because of the displacement of the G and F helices, polar groups in the vicinity of Glu-204 are now better able to stabilize the ionized form of this residue. One might imagine a closely related model in which bound water molecules in the active site become free to reorient following chromophore isomerization. Indeed, the model of Scharnagl et al. in which Arg-82 swings out of the active site to stabilize the ionized form of Glu-204 can also be grouped in this category. The general features of this model are consistent with the isomerization–charge-separation model (Honig et al., 1979) wherein isomerization of the chromophore and concomitant charge separation lead to modified pK_a values and the resulting displacement of protons.

The new feature proposed here is that the charge-separation process can lead to conformation changes that may play a significant functional role.

In summary, the results of this and our previous study (Sampogna and Honig, 1994) suggest that the essential feature of the proton-release mechanism of bR is due to the strong coupling between the protonation states of ionizable groups. First, the Schiff base and Asp-85 are strongly coupled, and a strong mutual Coulombic interaction is required for both groups to be ionized. Their separation as a result of chromphore isomerization then leads to the stabilization of the doubly neutral state in which a proton has been transferred to Asp-85. The continuation of the release mechanism involves conformational changes in the protein in response to the new ionization state, which yields new interactions that stabilize the ionized form of Glu-204. The specific location of Glu-204 within the bR structure thus allows direct electrostatic and physical coupling both to active site groups and to the extracellular medium, so that this residue provides a crucial link between the protein interior and the aqueous phase.

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