Contribution of Proton Release to the B2 Photocurrent of Bacteriorhodopsin

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ABSTRACT The contribution of proton release from the so-called proton release group to the microsecond B2 photocurrent from bacteriorhodopsin (bR) oriented in polyacrylamide gels was determined. The fraction of the B2 current due to proton release was resolved by titration of the proton release group in M. At pH values below the pK_a of the proton release group in M, the proton release group cannot release its proton during the first half of the bacteriorhodopsin photocycle. At these pH values, the B2 photocurrent is due primarily to translocation of the Schiff base proton to Asp^{85}. The B2 photocurrent was measured in wild-type bR gels at pH 4.5–7.5, in 100 mM KCl/50 mM phosphate. The B2 photocurrent area (proportional to the amount of charge moved) exhibits a pH dependence with a pK_a of 6.1. This is suggested to be the pK_a of the proton release group in M; the value obtained is in good agreement with previous results obtained by examining photocycle kinetics and pH-sensitive dye signals. In the mutant $Glu^{204}Gln$, the B2 photocurrent of the mutant membranes was pH independent between pH 4 and 7. Because the proton release group is incapacitated, and early proton release is eliminated in the $Glu^{204}Gln$ mutant, this supports the idea that the pH dependence of the B2 photocurrent in the wild type reflects the titration of the proton release group. In wild-type bacteriorhodopsin, proton release contributes approximately half of the B2 area at pH 7.5. The B2 area in the $Glu^{204}Gln$ mutant is similar to that in the wild type at pH 4.5; in both cases, the B2 current is likely due only to movement of the Schiff base proton to Asp^{85}.

INTRODUCTION

Bacteriorhodopsin (bR) is a seven-helical transmembrane protein found in the purple membrane of the archaebacterium *Halobacterium salinarum*. bR contains a retinal chromophore moiety, attached to the apoprotein via a protonated Schiff base linkage with Lys^{216}. Upon photoexcitation of the retinal chromophore, bR undergoes a photocycle characterized by a series of distinct photointermediates, termed K, L, M, N, and O, before returning to the unphotolyzed state. During the photocycle, a single net proton is translocated from the cytoplasmic to the extracellular side of the membrane (reviewed in Ebrey, 1993; Khorana, 1993; Lanyi and Váró, 1995). The resulting transmembrane proton gradient is used by the bacterium for ATP synthesis.

Proton movement during the bacteriorhodopsin photocycle has been investigated using both photocurrent and photovoltage measurements, on bR oriented within lipid bilayers, upon a substrate, or within gels (reviewed in Trissl, 1990; Läuger, 1991; Moltke et al., 1995). Individual charge movements coincide with transitions between the photointermediates of the photocycle. For example, a fast photocurrent or photovoltage signal, with polarity opposite the direction of proton pumping, is coincident with the early transitions of the photocycle (formation of the K intermediate). It has been suggested that this current (sometimes termed the B1 photocurrent; Hong and Montal, 1979) is due to a movement of the protonated Schiff base, or to a change in the charge distribution on the retinal, upon retinal photoisomerization (Trissl and Gärtner, 1987; Groma et al., 1988). The (optical) transitions between the L and M, and the M and N intermediates, are due to proton transfers within the protein and between the protein and the membrane surface (see reviews mentioned above). As such, the photoelectric signals coincident with these spectroscopic transitions (the B2 and B3 currents; Hong and Montal, 1979; Liu, 1990) are likely to be directly linked to these proton movements.

Liu (1990) specifically showed a strong correlation between a photocurrent with an apparent lifetime of $\sim$100 μs (the B2 photocurrent) and the optical transition between the L and M intermediates. It was suggested that at least part of the B2 current is generated by the transfer of the Schiff base proton to the Asp^{85} residue. It is the deprotonation of the Schiff base that gives rise to the characteristic blue-shifted absorption spectrum of the M intermediate (λ_{max} = 410 nm). However, the kinetics and shape of the B2 current in solutions of low ($<$10 mM) ionic strength (Liu et al., 1990) or in the presence of low concentrations of buffers (Liu et al., 1991) are altered in such a manner as to suggest the presence of an additional contribution to the B2 photocurrent. This contribution was suggested to come from proton release from a then unidentified residue (the "proton release group") to the extracellular solution, also coinciding with part of the formation of the M intermediate. Additional evidence for this assertion comes from the behavior of the B2 photocurrent at high pH. Upon deprotonation in the ground state of a group with pK_a 9.5, later identified as the

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proton release group, the apparent M formation kinetics accelerate more than 10-fold. The B2 photocurrent kinetics mirror this acceleration; however, the B2 current area, proportional to the total amount of charge translocated (Trissl, 1990), decreases as this group is titrated (Kono et al., 1993). The proton release group has subsequently been suggested to contain the Glu^{204} residue as a key element (Brown et al., 1995; Richter et al., 1996). It is quite possible that Glu^{204} is part of a complex of residues that together constitute the proton release group; infrared difference bands associated with the deprotonation of Glu^{204} are broad and poorly defined (Brown et al., 1995), and mutation of at least one other residue, Glu^{194}, has effects similar to those of the mutation of Glu^{204} (Balashov et al., 1997). Glu^{194} has recently been suggested to serve as a temporary "way station" for the proton originally released from Glu^{204}; that is, the proton is transferred from Glu^{204} to Glu^{194} during M formation, and released from Glu^{194} to the extracellular solution on a millisecond time scale (Dioumaev et al., 1998). However, experimental evidence has shown that removal of Glu^{204} is sufficient to eliminate proton release during M (Richter et al., 1996; Govindjee et al., 1996), suggesting that Glu^{204} is at least a major component of the proton release group. The Glu^{204}Gln mutant thus serves as a useful system on which to test the contribution of proton release to photocurrents that coincide with M formation.

Lanyi and co-workers have shown that M formation does not occur as a single transition. An in-depth examination of the M formation kinetics suggests that what appears as a single intermediate actually consists of a series of substates with similar, if not identical, spectra. A proposed photocycle scheme containing these multiple M states is shown in Fig. 1 (Zimányi et al., 1992). The L ⇔ M1 transition is identified with the transfer of the Schiff base proton to Asp^{85}. The M2 ⇔ M2′ transition represents the deprotonation of the proton release group. Finally, the M1 ⇔ M2 transition has been identified with the "protonation switch," a conformational or structural change that may transduce the transfer of the Schiff base proton into a drop in the pK_a of the proton release group (Cao et al., 1995; Richter et al., 1996). Note that the proton release group cannot deprotonate in M2 if the pH is lower than its pK_a in M2. In this case, an alternative pathway is followed to complete the photocycle; proton uptake and the reprotonation of the Schiff base occur as usual (during the decay of M), but proton release occurs late in the photocycle (Zimányi et al., 1992), perhaps directly from Asp^{85} (Gerwert et al., 1990). This pathway is also followed if Glu^{204} is mutated to a neutral residue (Richter et al., 1996; Govindjee et al., 1996). The photocycle schemes depicted in Fig. 1 were determined from the pH dependence of the wild-type photocycle kinetics and the extent of accumulation of the different photocycle intermediates (Zimányi et al., 1992; Lanyi and Váro, 1995). The titration of the proton release group in M2 (and the accompanying shift of proton release from the M2 ⇔ M2′ to the O ⇔ bR transition) was verified by monitoring proton release and uptake with pH indicator dyes (Zimányi et al., 1992). The reversal of the dye signal (from proton release preceding proton uptake to proton release after uptake) occurs with a pK_a of ~6 in 150 mM salt, suggesting that the pK_a of the proton release group in M2 is ~6, a substantial decrease from its ground state value of 9.5 (Balashov et al., 1996).

I have used the decrease in proton release from the proton release group during M ("early proton release") as the pH is lowered below its pK_a in M2 to determine the contribution, if any, of proton release to the B2 photocurrent. B2 photocurrents in the wild type (WT) and in the mutant Glu^{204}Gln were investigated. The results show that "early" proton release contributes about half of the B2 signal when the proton release group is able to deprotonate completely. As expected, the B2 photocurrent in the Glu^{204}Gln mutant exhibits no dependence on pH in the appropriate range and is ostensibly due solely to the translocation of the Schiff base to Asp^{85}.

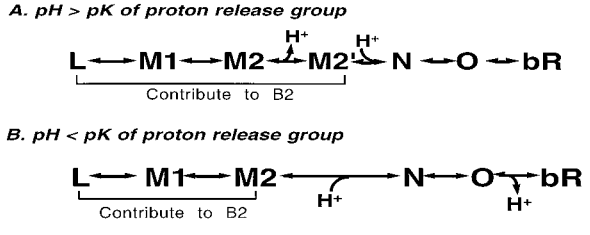

FIGURE 1 Photocycle schemes proposed by Lanyi and co-workers to account for the pH-dependent kinetics of M formation. (*A*) The photocycle at pH values above the pK_a of the proton release group, Glu^{204}. Substates of M in which the proton release group is deprotonated are designated by a′. M1 and M2 are defined as the pre- and postprotonation switch conformations of the protein, respectively. (*B*) Photocycle at pH values below the pK_a of the proton release group. Proton release occurs after proton uptake, ostensibly directly from D85. After Zimányi et al. (1992).

MATERIALS AND METHODS

The mutant Glu^{204}Gln membranes were a kind gift of Rosalie Crouch. WT membranes were purified by the method of Becher and Cassim (1975) with slight modifications. Membranes were oriented in acrylamide gels using the method of Dér et al. (1985). Briefly, membranes were mixed with acrylamide solution in a cast and exposed to an electric field of 10 V/cm for 30 s. *N,N,N′,N′*-tetramethylethylenediamine was added to polymerize the acrylamide, and the electric field was maintained for another 20 s. The gels thus produced (optical density 0.3–0.6) were cut into 10 mm × 5 mm × 5 mm pieces and rinsed with dH_2O. Gel pieces were soaked in 5–10 ml of 100 mM KCl/50 mM phosphate buffer solution at various pH values for 12–24 h before each experiment. Sample pH and resistance were remeasured just before each experiment. Photocurrents were obtained using homemade instrumentation described previously (Liu and Ebrey, 1988). Absorption changes at 410 nm were measured on the same samples, using a homemade kinetic spectrophotometer (Govindjee et al., 1980). A Quanta-Ray DCR-11 Nd:YAG laser (λ = 532 nm, 7 ns pulse width; Spectra-Physics, Mountain View, CA) was used as the source of the actinic flashes. Kinetic absorption traces and photocurrent traces were obtained by averaging signals from 32–128 flashes. All measurements were performed at 20°C. The data were analyzed (using nonlinear least-squares curve fitting and numerical integration) with the Kaleidagraph software package (Synergy Software, Reading, PA).

RESULTS AND DISCUSSION

Considering the relative complexity of the M formation kinetics, is appears that the past conception of the B2 photocurrent in terms of a single component is insufficient. The kinetics of the B2 photocurrent should follow the formation of the M intermediate, which, after all, involves the translocation of the Schiff base proton to Asp^{85}, a photocurrent-generating charge movement. Hence, each observable component of M formation should be associated with a component of the B2 photocurrent. If no additional charge movements are involved, the areas of the different photocurrent components should be proportional to the amplitudes of the corresponding M formation components.

The above interpretation can be expanded by experimental results that link proton release to the B2 photocurrent (Liu, 1990; Liu et al., 1991). The scheme shown in Fig. 1 *A* describes the photocycle at neutral pH (Zimányi et al., 1992); proton release is coincident with an observable component of M formation (because the M2 $\Leftrightarrow$ M2′ transition causes a shift in the L $\Leftrightarrow$ M1 equilibrium). If the rate-limiting step for proton release is the deprotonation step, rather than diffusion of the proton to the extracellular surface of the membrane, this deprotonation will give rise to a photocurrent that is different from those currents due strictly to the movement of the Schiff base proton to Asp^{85}. The photocurrent component with the same kinetics as the M2 $\Leftrightarrow$ M2′ transition will, in this case, contain a contribution from the movement of the Schiff base proton, as well as a contribution from movement of the proton released from the proton release group. Because the M2 $\Leftrightarrow$ M2′ transition is identified with the latter proton movement and limits the rate of the former, the two contributions will have the same apparent time constant and appear as a single photocurrent component. The area of this component will appear disproportionately large relative to the M formation component with the same time constant, and can be identified and characterized on such a basis (Misra et al., 1997).

If proton migration into the extracellular solution (or to Glu^{194}) is slower than the deprotonation process itself, the observed photocurrents will be qualitatively different. Once again, a photocurrent component corresponding to every M formation component should be observed. In this case, the strict proportionality between the areas of the photocurrent components and the amplitudes of the corresponding M formation components will be maintained. However, an additional photocurrent component, reflecting the directed movement of a proton from the neighborhood of the proton release group into the solution, will be present. This component would not coincide with any component of M formation, but would instead be slower than all of the observed components of M formation.

Titration of B2 at acid pH reveals a proton release component

The above analysis is predicated on whether the deprotonation of Glu^{204} is manifested as a photocurrent at all. If the dielectric constant in the Glu^{204} environment is large, little or no photocurrent will be generated by the deprotonation of this residue, because the photocurrent is proportional to the dielectric distance traveled by the charge (Trissl, 1990). Thus determining the contribution of proton release to the B2 photocurrent is important, especially because prior evidence connecting the B2 current to proton release has been indirect (Liu et al., 1991; Kono et al., 1993). Liu (1990) observed a small pH dependence of the B2 photocurrent area between pH 5 and pH 7, amounting to ~20% of the maximum, but did not discuss the origins of this dependence. Because the pK_a of Asp^{85} at the salt concentration used is only ~2.5, this pH dependence could not have been due to titration of the pigment into its nonpumping blue form (protonation of Asp^{85}). M formation was, at the time, not yet characterized as consisting of multiple components (the kinetics of M being such that, at pH 7, it can be adequately fit as a single ~85-μs exponential component). Thus the possible origins of a pH dependence in the range between 5 and 7 would have been obscure at the time.

As shown by Zimányi et al. (1992), the observed M amplitude increases as the pH is raised "through" the pK_a of the proton release group in M2. One would expect the associated B2 photocurrent to increase in this pH range, if only because more total M accumulates. However, any contribution of proton release to the B2 photocurrent should also increase as the pH is increased. Thus, as the proton release group is titrated, the B2 photocurrent area should increase disproportionately relative to the absorbance change at 410 nm due to formation of M.

M formation and B2 photocurrents measured in the WT, between pH 4.5 and 7.5, are shown in Fig. 2. The L $\Leftrightarrow$ M1 transition appears as a fast ($\tau \approx 1$ μs, essentially unresolved on the time scale shown) optical component with relatively low amplitude (<10% of the total signal, similar to results reported previously; Cao et al., 1995). The time constants of two additional optical components have been reported to be 40 μs and 140 μs (Cao et al., 1995) or 65 μs and 250 μs (Drachev et al., 1992; Komrakov and Kaulen, 1994) at pH 7; there is some uncertainty about the exact values, especially because both groups reported performing their experiments at 20°C. An additional complication is that the observed time constant of the third component (M2 $\Leftrightarrow$ M2′ equilibration in the scheme of Fig. 1) should vary with pH (because of the dependence of the rate constant of Glu^{204} protonation, but not the rate of deprotonation, on the proton concentration).

Fig. 2 shows that both the total amount of M and the photocurrents increase in amplitude between pH 5 and pH 7. Unfortunately, the signal-to-noise ratio in my measurements is not sufficient to resolve three components of M formation and of the photocurrents; curve fits of the data are overdetermined, and the second and third components cannot be fit in a consistent, model-independent fashion. It is still possible, however, to examine whether proton release contributes to the observed photocurrents, by looking at the dependence of the area of the photocurrent (proportional to the amount

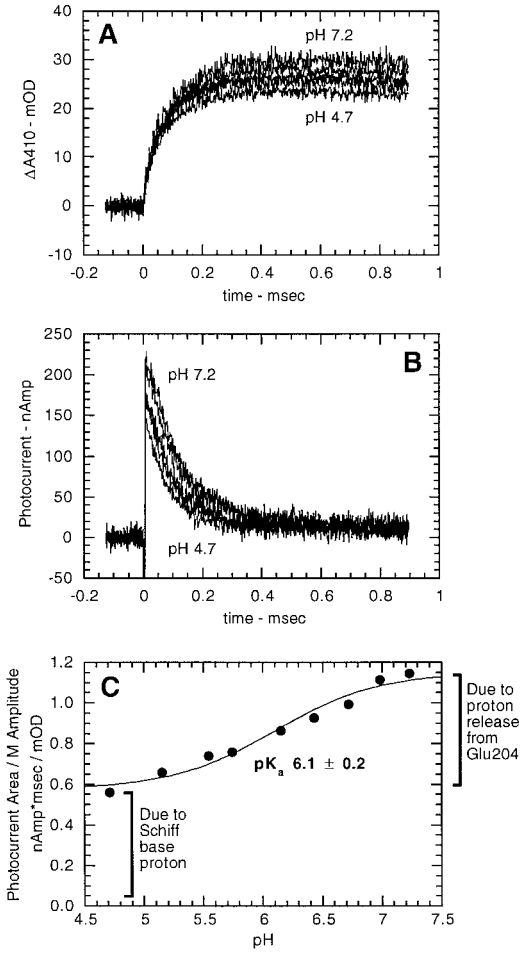

FIGURE 2 Titration of the proton release group in M. (*A*) Representative traces of M formation, at pH 4.7, 5.5, 6.2, 6.7, and 7.2, showing an increase in amplitude with increasing pH. (*B*) Representative submillisecond (B2) photocurrents at the same pH values. The photocurrent area increases disproportionately relative to the amount of increase in total M, suggesting that an additional charge is translocated at pH 7 which is not present at pH 4.5. This is more clearly seen in *C*, which shows the variation of the ratio of photocurrent area to the amount of M. About twice as much effective charge is translocated at neutral pH as at pH 4.5. The additional charge is contributed by deprotonation of the proton release group during the M2 ⇔ M2′ transition, i.e., early proton release.

of charge translocated) on pH. The area of the microsecond photocurrents, calculated by numerical integration (independently of the time constants of the M1 ⇔ M2 and M2 ⇔ M2′ transitions), was divided by the M amplitude. The result, shown in Fig. 2 *C*, implies that about twice as much charge is translocated at pH 7.5 than at pH 4.5 relative to the amount of M formed. The pK_a for the increase in charge translocation is 6.1 ± 0.2, similar to the pK_a for the increase in total M amplitude in this pH region (data not shown; see also Misra et al., 1997). The observed pK_a, presumably the pK_a of the proton release group in M2, is in good agreement with the result obtained by Lanyi and co-workers in their analysis of M formation kinetics and their experiments on the order of proton release and uptake using pH-sensitive dyes (Zimányi et al., 1992).

No photocurrent components were observed that were slower than M formation, but more rapid than the currents associated with N formation (proton translocation from Asp^{96} to the Schiff base and proton uptake from the cytoplasmic side of the membrane). This suggests that proton release from the proton release group is indeed limited by the deprotonation process itself, rather than by diffusion of the proton to the extracellular surface or to Glu^{194} (Dioumaev et al., 1998).

Absence of proton release contribution to B2 photocurrent in $Glu^{204}Gln$

The replacement of Glu^{204} by a neutral residue causes proton release during the M intermediate ("early proton release") to be abolished (Brown et al., 1995; Richter et al., 1996). Proton release instead occurs late in the photocycle, during the O ⇔ bR transition ("late proton release"), and ostensibly occurs directly from Asp^{85} (Govindjee et al., 1996). In addition, one would not expect to observe an increase in the total M amplitude (because of titration of the proton release group in M2) in such a mutant. The absence of this pH dependence in the $Glu^{204}Gln$ mutant has in fact been demonstrated experimentally (Richter et al., 1996; Govindjee et al., 1997). As stated above, the pH dependence of the ratio between the WT B2 photocurrent area and the corresponding M amplitude can be ascribed to the titration of the proton release group in M2. To provide additional evidence for this assertion, the pH dependence of the microsecond photocurrents in the $Glu^{204}Gln$ mutant was examined.

Fig. 3 shows the dependence of the M amplitude and the associated photocurrent on pH. The kinetics are biphasic; the two phases are most readily identified with the L ⇔ M1 and M1 ⇔ M2 transitions, respectively. Interestingly, the amount of the total M signal assumed by L ⇔ M1 (~25%) is larger than in the WT (<10%), suggesting that the L ⇔ M1 equilibrium is shifted more toward M1 in the mutant than in the WT (see Misra et al., 1997). The apparent time constant of the M1 ⇔ M2 transition (slower component of M formation in Fig. 3 *A*) is ~25 μs, and thus the overall kinetics of M formation are about three times as rapid in $Glu^{204}Gln$ as in the WT. The kinetics of the microsecond photocurrents (Fig. 3 *B*) match the kinetics of M formation; in addition, neither the photocurrent amplitude nor kinetics demonstrate a dependence on pH. Hence the ratio between the photocurrent area and M amplitude is independent of pH, as hypothesized (Fig. 3 *C*).

Notably, both the increase in the M amplitude and the increase in the ratio of the B2 photocurrent area to the M amplitude are abolished in the $Glu^{204}Gln$ mutant. The former occurs in the WT because of shifting of the L ⇔ M1 equilibrium in response to the increasing forward bias of the M2 ⇔ M2′ transition as the pH is increased above the pK_a of the proton release group in M2. The latter is due to an increasing contribution to the B2 photocurrent of proton

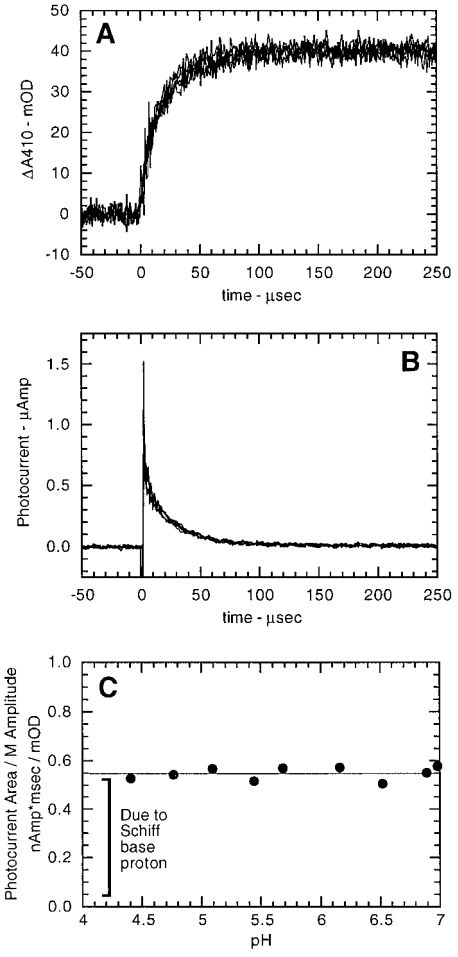

FIGURE 3 M formation and the associated B2 photocurrents in Glu^{204}Gln are independent of pH between 4 and 7. (*A*) Representative traces of M formation, at pH 4.4, 5.1, 5.7, 6.5, and 7.0. (*B*) Representative submillisecond (B2) photocurrents at the same pH values. (*C*) The ratio of the photocurrent area to the M amplitude is independent of pH. Elimination of Glu^{204} removes the M2 ⇔ M2′ transition, and abolishes the increase in the M amplitude and photocurrent area observed in the wild type in this pH range (Fig. 2).

release from the proton release group, in addition to the movement of the Schiff base proton. Both processes will be eliminated in the mutant, if they are indeed caused by the deprotonation of the proton release group. Whereas the invariance of the M amplitude with pH has been verified previously (Govindjee et al., 1997), photocurrents in Glu^{204}Gln have not been examined up to now. The results shown in Fig. 3 support the assertion that part of the increase in photocurrent area in the WT between pH 5 and pH 7 is due to an increasing contribution from proton release.

It is interesting to note that the B2 photocurrent area (normalized to the M amplitude) in the mutant is similar to that in the WT at pH 4.5 (0.5–0.6 nAmp/mOD in our samples). This may suggest that the Schiff base environment in the mutant is not significantly different from that of the WT, and that the difference in photocurrents in the two species is due solely to the absence or incapacity of the proton release group in the mutant.

Alternative sources for the B2 photocurrent

The above analysis of the B2 photocurrent is to some degree dependent on the presence of two charge movements on the microsecond time scale: movement of the Schiff base proton and movement of the proton from the proton release group. Other charge movements may occur, although they would not substantially change the conclusions from the experimental results reported here. It has been suggested that a movement or "swinging" of the Arg^{82} residue from the neighborhood of Asp^{85} to the neighborhood of Glu^{204} occurs during M formation (Scharnagl et al., 1995), although it is not clear whether this movement should be identified with the M1 ⇔ M2 (protonation switch) transition, or whether it is coincident with or strongly coupled to the deprotonation of the proton release group (the M2 ⇔ M2′ transition). If the former is the case, and if the movement of this charged residue generates a photocurrent, then part of the current that I have ascribed to movement of the Schiff base proton is actually due to movement of Arg^{82}. This contribution to B2, however, would still be pH-independent (like the contribution from the Schiff base proton) and would not alter the conclusions presented above. In the second case, if movement of Arg^{82} is a concerted process that is kinetically inseparable from deprotonation of the proton release group, the pH-dependent contribution to the B2 photocurrent would be due to both charge movements. The two charge movements, however, would exhibit the same pH dependence, and the conclusions presented in the previous sections would not be altered in any way other than to ascribe the pH-dependent portion of B2 to several charge movements rather than solely to the deprotonation of the proton release group. A previous study using methods identical to those presented in this paper found that, in the mutant Arg^{82}Gln, a photocurrent linked to proton release is still present (Govindjee et al., 1996). The area of this component is roughly similar to that associated with faster photocurrents linked to transfer of the Schiff base proton and the protonation switch, suggesting that the absence of Arg^{82} in this mutant does not result in a substantial decrease in any of the components of the B2 photocurrent.

Glu^{194} has recently been identified as a regulator of the proton release group (Balashov et al., 1997), and there is a strong possibility that it may participate in the transfer of the proton from the proton release group to the extracellular medium by acting as a proton acceptor from the proton release group and, subsequently, as a proton donor to the medium (Dioumaev et al., 1998). If this is the case, the above discussions of the proton release current should be understood to mean the transfer of the proton from the proton release group (possibly including Glu^{204}) to Glu^{194}. The proton transfer from Glu^{194} to the extracellular medium is suggested to occur on a millisecond time scale (Dioumaev

et al., 1998), and would not be resolved in the current study. It would coincide with the B3 photocurrent, which has heretofore been suggested to be due primarily to proton uptake to Asp^{96}, but may well contain a component from proton transfer from Glu^{194} into the solution. Titrations of the B3 photocurrent have so far yielded data that are not readily interpretable (unpublished observations). Examination of photocurrents in mutants of Glu^{194} is currently under way. Because of the proximity of Glu^{194} to the extracellular surface of the membrane, proton transfer from this residue to the medium may be shielded by water molecules and ions (that is, the local dielectric constant may be high) and may therefore generate only a small photocurrent signal.

It is interesting that the titration of the proton release group results in a decrease in the B2 photocurrent area by a factor of 2, suggesting that the two proton movements, from the Schiff base to Asp^{85} and from the proton release group to the membrane surface (or Glu^{194}), generate about equal amounts of photocurrent. The distance between the Schiff base and Asp^{85} is ~4 Å; if the proton release group is identified with Glu^{204}, the distance between it and Glu^{194} or the nearby membrane surface is ~6 Å (Kimura et al., 1997; Pebay-Peyroula et al., 1997). This suggests that the dielectric constant in the extracellular half of the membrane, in a several-angstrom-thick layer near the membrane solvent, is only slightly greater than that near the retinal binding pocket. This agrees with findings that the retinal binding pocket, although buried in the interior of the protein, contains several water molecules (Maeda et al., 1997) and an extensive hydrogen bonding network, whereas the part of the molecule closer to the extracellular surface is relatively poorly hydrated (Pebay-Peyroula et al., 1997), leading to relatively similar local environments for the two proton transfers leading to these photocurrents.

CONCLUSIONS

The proton release process is reflected in the B2 photocurrent. It appears as an "extra" contribution to the particular photocurrent component corresponding to that phase of M formation with which the proton release process is coincident, i.e., the $M2 \Leftrightarrow M2'$ transition. The B2 photocurrent component in question contains contributions from M formation (transfer of the Schiff base proton) and from proton release (deprotonation of the proton release group). The other B2 components are presumably caused solely by the translocation of the Schiff base proton to D85. Elimination of Glu^{204} removes the contribution of early proton release to the B2 photocurrent, as shown by the absence of a pH-dependent fraction of the B2 area in the Glu^{204}Gln mutant.

This paper is dedicated to Dr. Matthew Davidson.

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