ELSEVIER

Biochimica et Biophysica Acta 1187 (1994) 191-197

BBA Biochimica et Biophysica Acta

Specific acceleration of de- and reprotonation steps by azide in mutated bacteriorhodopsins

Jörg Tittor, Martin Wahl, Ulrike Schweiger, Dieter Oesterhelt

Max-Planck-Institut für Biochemie, D-82512 Martinsried, Germany

Received 7 March 1994

Abstract

Replacement of Asp-96 by Asn leads to a slowed M-decay (reprotonation of the Schiff base) during the photocycle of bacteriorhodopsin. Addition of azide accelerates the M-decay to the value of the wild-type protein and restores proton pump activity. Under this condition the recovery of the initial state is no longer correlated with the decay of M. Spectroscopic analysis of this situation reveals that the intermediate following M, the N-state, accumulates and its decay gets the rate-limiting step in the photocycle. This is proved by photostationary measurements where a pure N-BR spectrum is measurable. Additional mutation of Leu-93 to Ala leads to decreased decay rates of intermediates N or O, depending on the temperature. Addition of azide has therefore allowed the characterization of the intermediates following M. In mutants lacking Asp-85, a deprotonated Schiff base (410 nm) is the starting point of a photoreaction initiated by blue light leading to a protonated form absorbing maximally at 610 nm. Azide also accelerates the deprotonation of this product. In all cases, the protonated form of azide is the catalytically active species. This suggests that the protonated azide is part of a hydrogen-bond system required for proton movements within the two half-channels of BR.

Key words: Bacteriorhodopsin; Retinal; Photocycle; Schiff base

1. Introduction

Retinal-protein ion pumps translocate protons and halide ions across the cell membrane of archeal halobacteria using light energy (for review see Ref. [1]). Photon absorption causes an isomerization of the retinal moiety bound covalently to the protein part of the pumps, and following this photochemical reaction the proteins can catalyze thermal reisomerization. The energy stored in the chromoproteins is used in part for driving a reaction cycle ending at the initial state and in part for the electrochemical potential of the translocated ion. Retinal is located in about the middle of these membrane-spanning proteins, which form a ring of seven transmembrane helices. The center of the ring is a channel for the diffusion of ions that is divided into half by the retinal's Schiff base, which is protonated in the initial state. After photoisomerization, initial relaxation leads to state L in bacteriorhodopsin (see Scheme 1), the proton pump BR. Scheme 1 shows the widely accepted scheme of the photocycle of BR based on a suggestion from J. Lanyi and co-workers [2].

A proton is transferred from the Schiff base to aspartic acid 85 in the extracellular half-channel (EC) and state MI is formed. While aspartic acid 85 remains protonated, another still unknown group releases a proton to the extracellular surface. The following step in the reaction cycle is critical with respect to vectorial transport: MI is converted in a virtually irreversible reaction into MII. Both of these states contain a 13-*cis* moiety, but in MII the Schiff base can accept a proton

$$\mathrm{BR} \xrightarrow{h\nu} \mathrm{K} \longrightarrow \mathrm{L} \xrightarrow[\text{H to Asp-85}]{} \mathrm{MI} \longrightarrow$$

$$\mathrm{MII} \xleftrightarrow[\text{H from Asp-96}]{} \mathrm{N} \longleftrightarrow \mathrm{O} \longrightarrow \mathrm{BR}$$

Scheme 1

Abbreviations: BR, bacteriorhodopsin; HR, halorhodopsin; EC, extracellular channel; CP, cytoplasmic channel; amino acids are denoted by the standard three-letter code.

Corresponding author. Fax: +49 89 85783557.

SSDI 0005-2728(94)00070-L

exclusively through the cytoplasmic half-channel (CP) while accessibility in MI is solely through the EC. The transition in M changing proton accessibilities is assumed to be linked to protein conformational changes which prepare the Schiff base for protonation by aspartic acid 96 to form state N. Normally the cycle proceeds, after reprotonation of the Schiff base through the CP, by transfer of a proton to aspartic acid 96 from the cytoplasmic side and thermal isomerization to the all-*trans* state O. In the last step of the reaction cycle, under standard conditions, aspartic acid 85 is deprotonated through the EC and by this the initial state is regenerated.

Specific mutagenesis of BR has revealed proteins in which individual photocycle steps were drastically altered. For instance, removal of the carboxylic group at position 96 led to a decrease of the reprotonation rate by a factor of more than 50 compared to wild-type under identical conditions, whereas removal of the carboxylic group at position 85 led to a truncated photocycle after excitation with yellow light, characterized by the lack of the M intermediate. Mutation of Leu-93 to Thr or Ala leads to a decreased rate of N and/or O decay steps, which reflect the reisomerisation of the *13-cis*- to all-*trans*-retinal and restoration of the initial state [3].

Azide was described to catalyse the light-induced de- and reprotonation step in halorhodopsin, a retinal protein closely related to BR. This reaction normally does not occur during the photocycle of this protein, but is a side reaction leading to a decrease of halorhodopsin's activity as a chloride pump [4]. Because the amplitudes of the formation of the deprotonated form are not changed, whereas the rates of de- as well as reprotonation are increased drastically, azide was considered as a catalyst for both reactions in this protein [5]. Furthermore, azide is a substrate of one anion-binding site in HR [6].

In BR, azide was first described to reactivate proton pump activity of BR Asp-96 → Asn due to its ability to accelerate the reprotonation of the Schiff base, which is the rate-limiting step in the photochemical cycle of this mutant [20]. This finding was confirmed by several other groups [7–9] and azide is now commonly used in investigation of mutated BRs if de- and reprotonation steps are affected by a mutation.

Blue light-driven stationary proton currents are also strongly enhanced by addition of azide in mutants lacking Asp-85 [10]. The corresponding photochemical cycle of proton translocation (see Scheme 2) starts with a deprotonated form (M*trans*), undergoes isomerization upon capture of a photon, and thermally converts to a protonated form absorbing maximally around 600 nm (BR6XX) by passing an intermediate absorbing maximally at 450 nm. (The exact absorbance maxima are determined by the respective substitution of Asp-85.) The repopulation of the initial deprotonated form is now strongly affected by the presence of azide.

$$\mathrm{M}\mathit{trans} \xrightarrow{h\nu} \mathrm{M}\mathit{cis} \longrightarrow 450 \longrightarrow \mathrm{BR6XX} \xrightarrow[\text{azide}]{} \mathrm{M}\mathit{trans}$$

Scheme 2

In this contribution we describe the effects of azide in more detail in mutated bacteriorhodopsins Asp-96 → Asn, Leu-93 → Ala/Asp-96 → Asn and Asp-85 → Asn or Thr. Two different types of action of azide are presented:

(i) in reprotonation steps of the photochemical cycle, i.e., in mutants where carboxylic group Asp-96 is absent and

(ii) in deprotonation steps, i.e., in mutants where carboxylic group Asp-85 is absent.

2. Material and methods

Due to limited space we refer to our previous published work and references cited therein, from which all necessary information can be obtained [10–12].

3. Results and discussion

3.1. Azide's action in the reprotonation reaction

A. Bacteriorhodopsin Asp-96 → Asn

Mutant bacteriorhodopsin Asp-96 → Asn has the same absorption maximum and the same light-dark adaptation time as wild-type protein [11]. The primary photochemical reaction and following events in the mutant protein are very similar to that in wild-type protein up until the formation of intermediate M [7,12]. However, the decay of M is drastically slowed, and concomittant to this pH-dependent process, the initial state is reformed. Addition of azide not only accelerates the decay of M but also decouples repopulation of the initial state from M decay. This fact facilitates the spectroscopic analysis of late intermediates. Fig. 1 shows absorption changes of the mutant protein at selected wavelengths. 10 ms after flash excitation the M intermediate has quantitatively decayed, yet only some of the absorption changes at 570 nm are reversed (Fig. 1, time window A). A global fit analysis revealed that three time constants are necessary and sufficient to describe all absorbance changes at different wavelengths simultaneously at temperatures between 30°C and 12°C. At 19°C, the absorbance change at 410 nm is reverted monophasically with a time constant $\tau_2 = 1.8$ ms and synchronously an absorption increase at 570 nm occurs with an amplitude of 49% of the initial flash-induced decrease. The residual 51% of the ab-

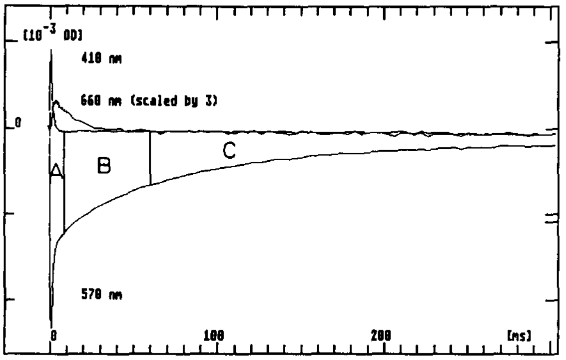

Fig. 1. Time-dependent absorbance changes at 410, 570 and 600 nm of mutant BR Asp-96 → Asn in the presence of 100 mM azide. Purple membranes (10 μM) were suspended in 10 mM sodium phosphate pH 5.6 at 20°C. The photocycle was initiated by a single dye laser flash at 590 nm (3 mJ, 18 ns). Three different time-domains, A–C, are indicated which correspond to three rate constants, used for global fit analysis of the kinetics.

sorption change recovers with two time constants, $\tau_3 = 7.8$ ms (26%) and $\tau_4 = 29$ ms (Fig. 1, time window B and C). Absorption changes at 660 nm are small compared to those at 410 and 560 nm, and are characterized by τ_2 for the rise and τ_3 for the decay. (Fig. 1, time window B). The difference spectra in Fig. 2 characterize the transitions which take place in the time ranges A to C in Fig. 1. Fig. 2A shows difference spectra with a time interval of 110 μs, beginning 600 μs and ending 3 ms after the laser flash. A positive absorption feature is seen with a difference maximum at 410 nm decreasing concomittantly with an increase of absorption around 570 nm. The isosbestic point of this transition is at 460 nm and therefore almost the same as for the M to BR transition. This indicates that M decays to a species absorbing very similarly to the initial state but having a lower extinction coefficient between 500 and 600 nm. This is typical for the N-intermediate of the photocycle [13].

In the time range from 3 ms to 16 ms (B in Fig. 1), a single transition of positive absorption between 630 and 700 nm ($\lambda_{\max} = 660$ nm) to a species absorbing between 500 and 630 nm ($\lambda_{\min} = 570$ nm) is observed (Fig. 2B). The isosbestic point of this transition is located at 621 nm. The difference absorption maximum at 660 nm is commonly assigned to the O intermediate [14,15], but alternatives exist. A red-shifted intermediate K610 of the *13-cis* photocycle [16] seems to be excluded because light-adaptation in the mutant protein Asp-96 → Asn leads, as in the wild-type protein, to more than 95% all-*trans* retinal. A second alternative to the O-intermediate causing absorption changes around 660 nm would be the L-intermediate. This possibility will be considered in more detail below. After the reversal of absorption changes around 660 nm, the final transition to the initial state becomes observable (Fig. 2C). Absorption with a minimum at 570 nm in the difference spectra increases to its initial value with the time constant τ_4. This spectroscopic feature was attributed to an intermediate between M and BR and was called R360 [17] or P [18]. Detailed measurements especially at high pH have revealed that P and R are identical to N [13]. The slow decay of N to BR in the mutant protein causes a stationary accumu-

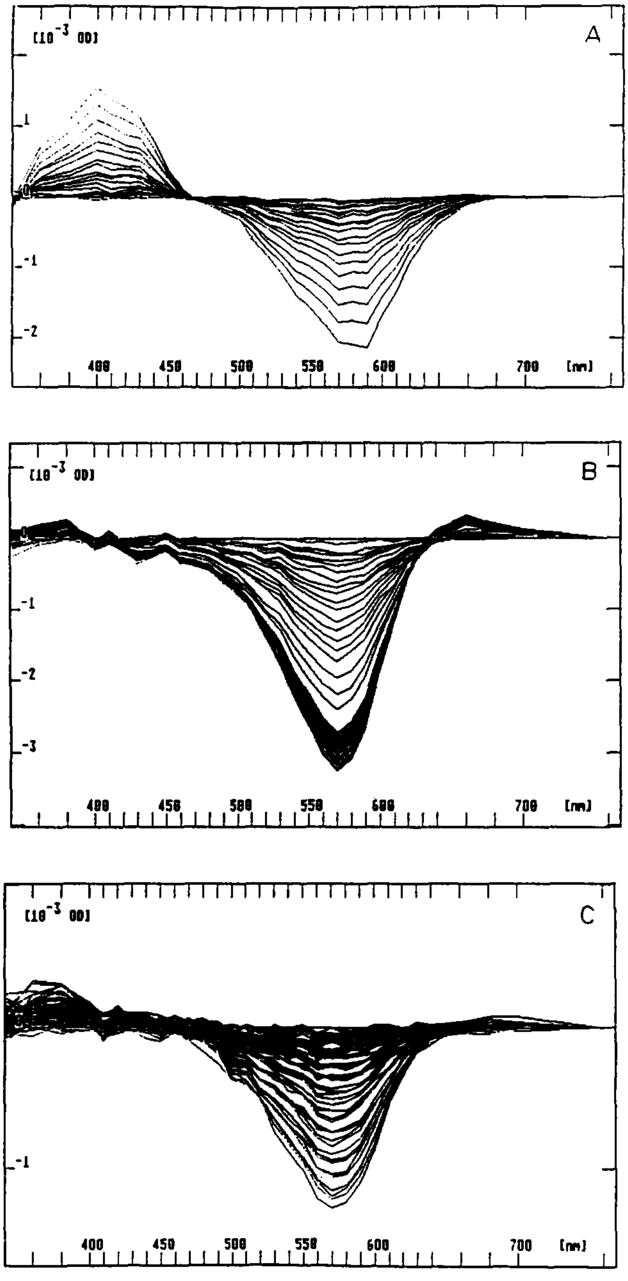

Fig. 2. Time-resolved difference spectra in the three different time-domains A–C indicated in Fig. 1. The spectrum at the begin of each time domain is taken as the reference to illustrate the transitions occuring in each time domain exclusively. Experimental conditions are the same as described in Fig. 1. (A) Difference spectra were recorded every 110 μs between 110 μs and 3 ms after the laser flash. (B) Difference spectra every 110 μs between 3 ms and 15 ms after the flash. (C) Difference spectra every ms between 15 ms and 30 ms after the flash.

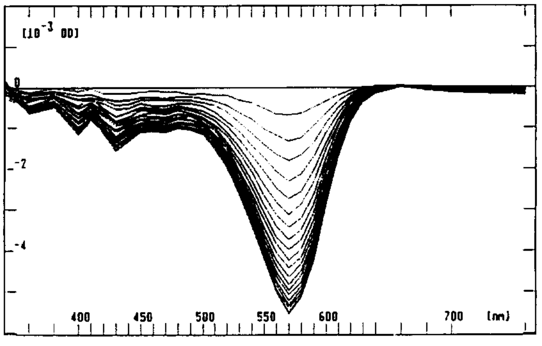

Fig. 3. Difference spectra of absorbance changes induced by the measuring light in BR mutant Asp-96 → Asn and taken every 10 ms. Experimental conditions were as described in legend of Fig. 1.

lation of N under the conditions of intense measuring light. Fig. 3 shows the difference spectra during accumulation of N in the photostationary state. Within the limit of accuracy, the difference spectra in Figs. 2C and 3 are similar in the visible range, but differences are seen below 500 nm where no positive absorption is seen in the BR-N difference spectrum taken under stationary conditions. Clearly, the absorption band of the N intermediate is entirely covered by the absorption band of BR. The fact that the difference maximum is at 570 nm indicates that the absorption maximum of N is slightly blue-shifted compared to the maximum of BR at 568 nm.

Additional electrical component in presence of azide.

Time-resolved photocurrents of oriented bacteriorhodopsin and the mutant Asp-96 → Asn immobilized in polyacrylamid gels revealed a largest time constant of 30 ms in the presence of azide, compared to 300 ms in the absence of azide. Furthermore, a second time constant of 1.5 ms is only observable in the presence of azide. As a control, the experiment was also performed with wild-type bacteriorhodopsin and no difference in presence or absence of azide was found. By comparison with the spectroscopic experiments shown above (Figs. 1 and 2) it is obvious that the 30 ms time-constant reflects the decay of the N intermediate and its electrogenicity shows that the N decay is correlated with the release of the azide anion from the protein through CP. The electrical component with 300 ms (without azide) and 1.5 ms (in azide) are correlated to the M decay in the mutant and reflect proton transfer to the Schiff base.

pH dependence of the azide effect.

A second characteristic of mutant BR Asp-96 → Asn is the strong pH dependence of M decay. The corresponding time constant varies from 16 ms up to 10 s in the pH range of 2 to 10 [19–21] and is also strongly affected by the ionic strength of the bulk phase [21]. The pH dependence of the repopulation kinetics of the initial state at 570 nm and the M-decay at 410 nm reveal a pH dependence of both rates at 10 and 100 mM azide (Fig. 4A, B). The reaction rate describing M-decay displays a stronger pH-dependence than that for N-BR transition, and both become equal at pH 8.2 for 10 mM and at pH 9.2 for 100 mM azide (dashed lines in Fig. 4). This obviously reflects the fact that azide needs to be protonated to act as a catalyst and this effect vanishes as pH increases (pK_a of 4.9). Moreover, the ratio of M intermediate and initial state in the photostationary state should decrease as the azide concentration increases because the rate-limiting

Table 1
Amplitudes and time constants of electric currents:
10 mM sodium phosphate (pH 5.1) and, if added, 10 mM NaN_3. Purple membranes of BR Asp-96 → Asn were oriented and immobilized with an optical absorbance of 0.5 at 570 nm

BR without azide:	60 nA, 350 μs	6 nA, 10 ms	
BR with azide:	60 nA, 300 μs	12 nA, 11 ms	
Mutant without azide:	40 nA, 300 μs		0.1 nA, 300 ms
Mutant with azide:	60 nA, 300 μs	12 nA, 1.5 ms	0.3 nA, 30 ms

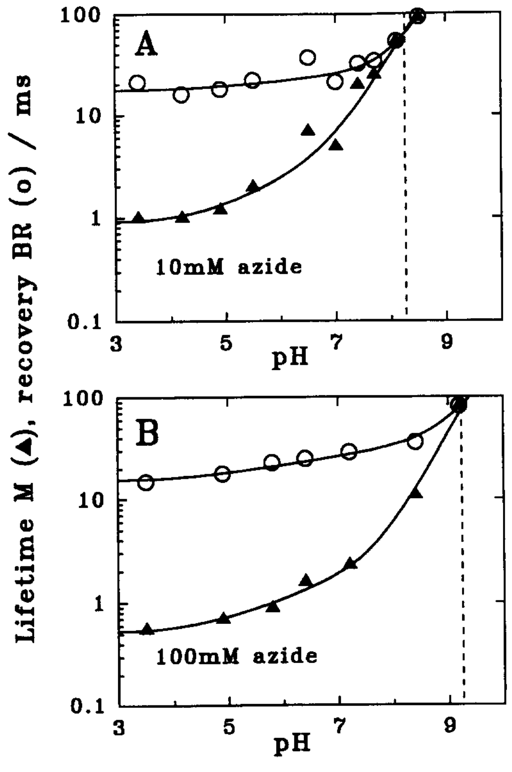

Fig. 4. pH dependence of M decay (triangles) and BR recovery (circles) at 10 mM azide (A) and 100 mM azide (B). Purple membranes of mutant BR Asp-96 → Asn were suspended in a 1:1 mixture of 10 mM sodium phosphate and Tris buffer. pH was adjusted by adding 0.1 M HCl or NaOH. Temperature was kept constant at 19°C. Actinic light was from a dye laser at 585 nm (3 mJ, 18 ns). The dashed vertical line indicate at which the two rates merge and therefore the azide effect vanishes.

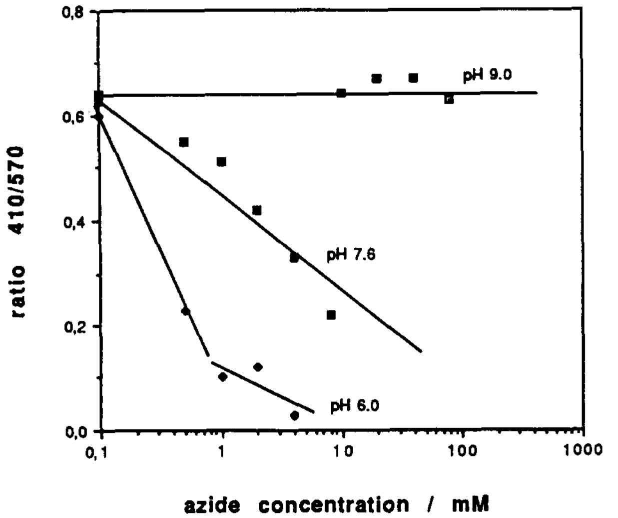

Fig. 5. Ratio of 410 and 570 nm absorbance changes in the photostationary state at different azide concentrations and pH values of 9.0, 7.6 (100 mM Tris-HCl) and 6.0 (100 mM Mes). Lines are drawn to guide the eye.

step shifts from M-decay to N-decay. In Fig. 5 the ratio of the absorbances at 410 to 570 nm versus increasing azide concentration is shown at pH values of 6.0, 7.6 and 9.0. Due to the shift of the rate-limiting step from M-decay to N-decay, this ratio can decrease from 0.64 (if only M is present) to values smaller than 0.1 (at pH 6.0). Again, due to the p*K* of azide of 4.9, addition of that substance is more effective at pH 6.0 than at higher pH values, whereas no effect of azide (up to 100 mM) can be demonstrated at pH 9.0.

B. Bacteriorhodopsin Leu-93 → Ala, Asp-96 → Asn

(a) Photostationary behavior.

Replacement of Leu-93 by Ala or Thr in BR was reported to slow down the N/O* decay. Resonance Raman experiments revealed the existence of a long-lived *13-cis*-retinal in this mutant [3]. We take this as an arguement that this O could be in 13-*cis* and therefore mark it as O* to distuinguish between the wild-type O which was shown to have an all-*trans*-retinal [22]. It is evident that this assumption has to be clarified by further experimentation. Therefore, a double mutation, Leu-93 → Ala in addition to replacement of Asp-96 by Asn, was carried out and the resulting protein investigated for its spectroscopic properties. The absorption maximum of this mutant protein was blue-shifted to 542 nm compared to 568 nm in wild-type and the Asp-96 → Asn mutant. The content of retinal isomers in this mutant was 80% all-*trans* and 20% *13-cis* after 12 h at 4°C in the dark. After 10 min illumination with yellow light ($\lambda > 511$ nm, 110 mW/cm^2) the isomeric ratio was determined as 90% all-*trans* and 10% *13-cis*.

Illumination of this protein with white light results in a difference spectrum between the inital state (difference absorbance minimum at 540 nm) and the M intermediate (absorbance maximum at 410 nm) with a minor contribution from the O* intermediate (absorbance at 610 nm). This is shown in Fig. 6A. The difference spectra are shown at time intervals of 10 ms (zero time at the baseline) indicating at the largest amplitude the establishment of the photostationary state. Addition of 10 mM azide to this sample alters the photostationary state mixture significantly. The difference spectrum now shows a minimum at 540 nm and a maximum at 600 nm with an isosbestic point at 565 nm (Fig. 6B). This is attributed to a pure BR-O* difference spectrum. The same difference spectrum is also observed at 40°C. Apparently no N/BR difference spectrum can be observed under continuous illumination with white light. A possible explanation is a photochemical conversion of N leading to accumulation of O*.

(b) Kinetic behavior.

Time-resolved difference spectra after flash excitation of BR Leu-93 → Ala, Asp-96 → Asn in presence of 10 mM azide are shown in Fig. 7. At 22°C (Fig. A) the M intermediate ($\lambda_{\max}$ at 410 nm) decays in 1.2 ms and concomitantly N rises ($\lambda_{\max}$ at 530 nm). The decay of N is concomitant to the recovery of the initial state,

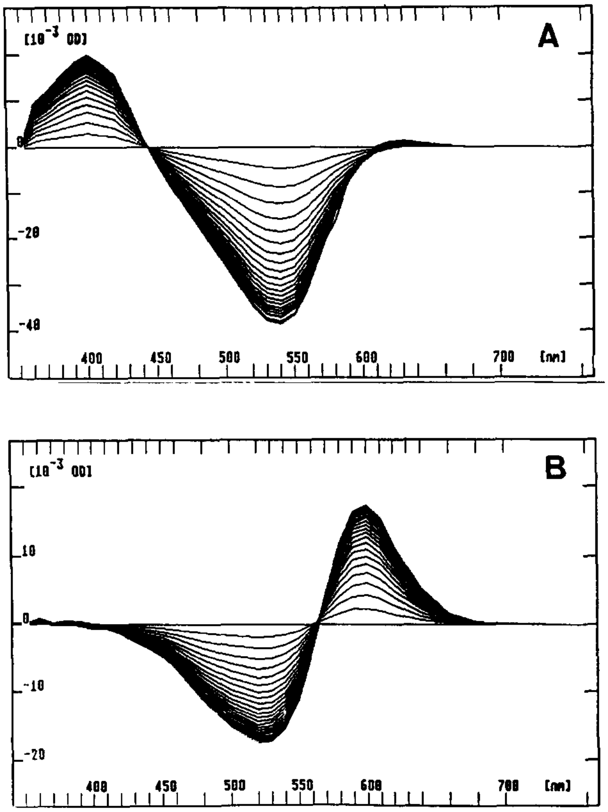

Fig. 6. Photostationary difference spectra of BR Leu-93 → Ala,Asp-96 → Asn upon illumination with white light (11 mW/cm^2) at 22°C. BR (0.8 μM) was suspended in 10 mM Tris (pH 7.0) in the absence (A) and in the presence of 10 mM azide (B).

with a negative difference maximum at 590 nm, not clearly seen in the three-dimensional representation in Fig. 7A. The fast decaying absorbance band around 610 nm (0.8 ms) is most probably due to a photoproduct of the *cis*-cycle and not discussed in this context, as no correlating rate of conversion of any other intermediates was found. Therefore, this fast decaying 610 nm form is considered to result from a photoreaction parallel to that of all-trans retinal. Increasing the temperature to 45°C leads to a shift of the absorbance traces after M decay (0.9 ms). M decays under this condition to O* (difference absorbance maximum at 610 nm) which then decays in 116 ms to the initial state. The negative absorbance of the initial state is at 530 nm. It is concluded that the ratio of rate constants leading to and from N and O* change with temperature, thereby leading to the respective accumulation of N and O*.

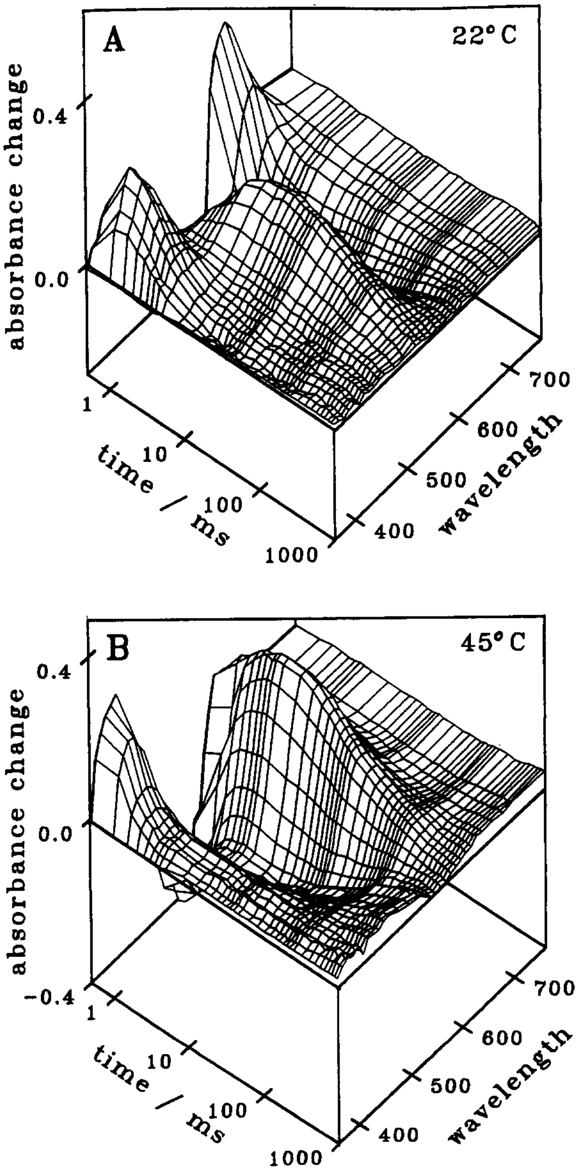

Fig. 7. Three-dimensional representation of flash-induced difference spectra of BR Leu-93 → Ala,Asp-96 → Asn. 0.8 μM BR were suspended in 10 mM sodium phosphate (pH 6.0) at 22°C (A) and at 45°C (B). Laser excitation (single flash) was at 590 nm with 3 mJ. Spectra shown are averages of 10 measurements to improve the signal-to-noise ratio.

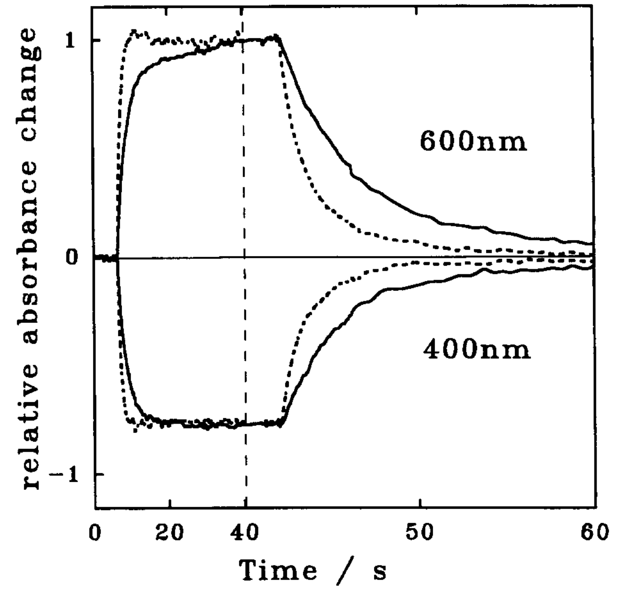

Fig. 8. Photostationary state of yellow and decay of the blue form of BR Asp-85 → Asn after blue-light excitation (laser at 390 nm, 1 mJ, 20 Hz repetition rate) in absence (solid line) and presence of 160 mM azide (dashed line). BR Asp-85 → Asn (6 μM) was suspended in 10 mM Tris/Hepes at pH 9.0 and 100 mM NaCl.

3.2. Azide also affects deprotonation reactions in BR

Mutants of BR lacking the carboxylic group at position 85 show a reduced pK_a of their Schiff base to a value of about 8.5 compared to > 13 in wild-type [23]. The deprotonated form absorbing maximally at 410 nm was shown to generate stationary proton currents upon continuous blue-light illumination [10]. This current has the same sign as wild-type BR, indicating a proton translocation pathway which starts by protonation of the Schiff base and ends with a deprotonation reaction. In the photostationary state, continuous blue-light illumination produces a form which absorbs maximally at 610 nm and decays to the initial form in the time range of seconds. This decay can be accelerated from 4.5 s to 1.4 s at pH 9.0 by addition of azide (Fig. 8). This acceleration demonstrates that azide affects the deprotonation of the Schiff base in BR. Because proton translocation has the same direction as in wild-type and the 610 nm intermediate must be a protonated Schiff base, the transition from 610 to 410 form shown in Fig. 8 must be a deprotonation reaction through the EC which is catalyzed by azide. The pH dependence of this azide-dependent deprotonation is shown in Fig. 9. Again, the effect of azide vanishes at higher pH values,

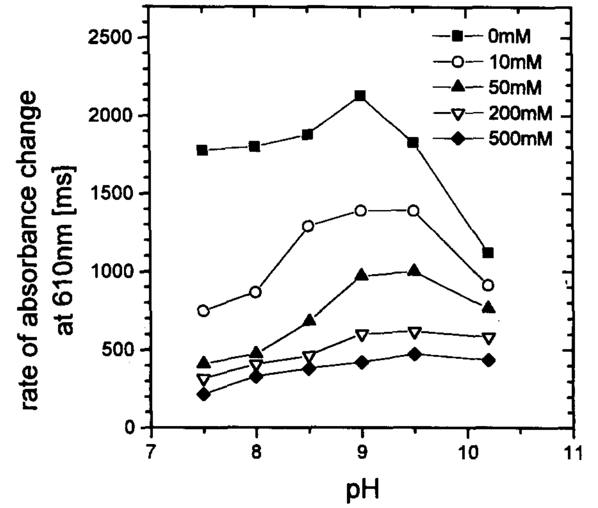

Fig. 9. pH dependence of the azide effect shown in Fig. 8. The numbers in the upper right-hand corner indicate the azide concentration in mM.

indicating that the protonated azide is responsible for the acceleration.

4. Conclusions

Azide was shown to increase the rate of two different types of protonation changes. Mutants which are kinetically defective in the reprotonation step in the photocycle can be restored or even enhanced in their natural rate of reprotonation by addition of azide. In the absence of an additional mutation, the protein then accumulates in the N state. However, if the isomerisation step is affected by a second mutation at position Leu-93, the protein also accumulates in the N state at 22°C but in the O* state if the temperature is increased to 45°C. These findings enable the study the pure intermediates M, N and O* in one and the same protein. Azide also increases the rate of the deprotonation of the Schiff base in a mutant where the natural proton acceptor Asp-85 is lacking. In both cases the pH dependence of the azide effect indicates that protonated azide is responsible for this function. This can be interpreted as azide being part of an intraprotein hydrogen bond system which would be a prerequisite for efficient proton transfer in the two half-channels of BR [24–27]. Earlier measurements have already shown that the entropy of activation for reprotonation is enhanced in mutant Asp-96 → Asn and azide actually restores this entropic defect to a value found in wild-type [20]. This indicates the necessity of an ordered state in the CP channel for efficient proton transfer which is lacking in mutant Asp-96 → Asn and restored in presence of azide. Further experimentation will have to show whether this explanation describes in fact the molecular events which occur during the function of BR as a light-driven proton pump.

Acknowledgements

We gratefully acknowledge the skillfull technical assistance of Christina Haupts and the expertise of Dr. H.-J. Butt in measuring the time-resolved electrical currents. Special thanks are due to Dr. Johannes Rudolph for improving the English style.

References

[1] Lanyi, J.K. (1993) Biochim. Biophys. Acta 1143, 142–161.
[2] Varo, G. and Lanyi, J.K. (1991) Biochemistry 30, 5016–5022.
[3] Subramaniam, S., Greenhalgh, D.A., Rath, P., Rothschild, K.J. and Khorana, H.G. (1991) Proc. Natl. Acad. Sci. USA 88, 6873–6877.
[4] Bamberg, E., Hegemann, P. and Oesterhelt, D. (1984) Biochim. Biophys. Acta 733, 53–60.
[5] Hegemann, P., Oesterhelt, D. and Steiner, M. (1985) EMBO J. 4, 2347–2350.
[6] Lanyi, J.K. (1986) Biochemistry 21, 6706–6711.
[7] Holz, M., Drachev, L.A., Mogi, T., Otto, H., Kaulen, A., Heyn, M.P., Skulachev, V. and Khorana, H.G. (1989) Proc. Natl. Acad. Sci. USA 86, 2167–2171.
[8] Cao, Y, Varo, G., Chang, M., Ni, B., Needleman, R. and Lanyi, J.K. (1991) Biochemistry 30, 10972–10979.
[9] Danshina, S.V., Drachev, L.A., Kaulen, A.D., Khorana, H. G, Marti, T. Mogi, T. and Skulachev, V.P. (1992) Biokhimiya 57, 1574–1585.
[10] Tittor, J., Schweiger, U., Oesterhelt, D. and Bamberg, E. (1994) Biophys. J. (submitted).
[11] Soppa, J., Otomo, J., Straub, J., Meeßen, S. and Oesterhelt, D. (1989) J. Biol. Chem. 224, 13049–13056.
[12] Butt, H.J., Fendler, K., Bamberg, E., Tittor, J. and Oesterhelt, D. (1989) EMBO J. 8, 1657–1663.
[13] Kouyama, T., Nasuda-Kouyama, A., Ikegami, A., Mathew, M.K. and Stoeckenius, W. (1988) Biochemistry 23, 5855–5863.
[14] Dencher, N. and Wilms, M. (1575) Biophys. Struct. Mech. 1, 259–271.
[15] Lozier, R.H., Bogomolni, R.A. and Stoeckenius, W. (1975) Biophys. J. 11, 955–963.
[16] Hofrichter, J., Henry, E. and Lozier, R.H. (1989) Biophys. J. 56, 693–706.
[17] Dancshazy, Z., Govindje, R., Nelson, B. and Ebrey, T.G. (1986) FEBS Lett. 169, 44–48.
[18] Drachev, L.A., Kaulen, A.D., Skulachev, V.P. and Zorina, V.V. (1986) FEBS Lett. 169, 316–320.
[19] Otto, H., Marti, T., Holz, M., Mogi, T., Lindau, M., Khorana, H.G. and Heyn, M.P. (1989) Proc. Natl. Acad. Sci. USA, 86, 9288–9232.
[20] Tittor, J., Soell, C., Oesterhelt, D., Butt, H.J. and Bamberg, E. (1989) EMBO J. 8, 3477–3482.
[21] Miller, A. and Oesterhelt, D. (1990) Biochim. Biophys. Acta 1016, 57–64.
[22] Smith, S.O., Pardoen, J.A., Mulder, P.P.J., Curry, B., Lugtenburg, J. and Mathies R.A. (1983) Biochemistry 18, 6141–6148.
[23] Druckmann, S.M., Ottolenghi, M., Pande, A., Pande, J. and Callender, R.H. (1982) Biochemistry 17, 4953–4959.
[24] Hildebrandt, P. and Stockburger, M. (1984) Biochemistry 19, 5539–5548.
[25] Olejnik, J., Brzezinski, B. and Zundel, G. (1992) J. Mol. Struct. 231, 157–173.
[26] Maeda, A., Sasaki, J., Yamazaki, Y., Needleman, R. and Lanyi, J.K. (1994) Biochemistry 33, in press.
[27] LeCoutre, J. and Gerwert, K. (1994) in Time-resolved Vibrational Spectroscopy (Liu, A., Siebert, F. and Warncke, W., eds.), Springer Proceedings in Physics 74, pp. 256–257.