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Cooperative phenomena in the photocycle of D96N mutant bacteriorhodopsin

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Abstract The M intermediate decay in the photocycle of D96N mutant bacteriorhodopsin does not depend on the light intensity of the exciting flash. Cooperative phenomena in the photocycle are revealed after addition of azide causing acceleration of the M decay and making it kinetically well separated from the N decay. Increase in the light intensity induces slight deceleration of the M decay and significant acceleration of the N decay. The data obtained directly confirm our recent model [Komrakov and Kaulen (1995) Biophys. Chem. 56, 113–119], according to which appearance of the M_{slow} intermediate in the photocycle of the wild type bR at high light intensity is due to destabilization of the N intermediate leading to the acceleration of the N→M and N→bR reactions.

Key words: Bacteriorhodopsin; Photocycle; Proton transport; Cooperativity; Purple membrane; D96N mutant; *Halobacterium halobium*

1. Introduction

Some experimental works on cooperative phenomena in the bacteriorhodopsin (bR) photocycle were recently published [1–7]. It is generally accepted that the initial bR state restoration from the M intermediate (the so-called M_{fast}) takes place through the N intermediate transient formation at low flash light intensities. However, there is much uncertainty concerning the bR photocycle at high light intensity. Increase in the flash intensity induces slowing-down of the M decay as well as acceleration of regeneration of the bR ground state. Interaction of the excited neighboring molecules is assumed to result in the appearance of the second (parallel) photocycle with the M_{slow} formation instead of the M_{fast}. In this photocycle the M_{slow} converts into the bR initial state without any formation of the N intermediate, and the M_{slow}→bR transition occurs slower than M_{fast}→N but faster than N→bR one [1,3–6]. On the other hand, according to our model [7], the bR protein–protein interaction does not change sequence of the photocycle steps but leads to destabilization of the N intermediate which in turn increases the rates of N→bR as well as reverse N→M transitions. In order to choose between these two models, we have studied cooperative phenomena in the mutant D96N bR photocycle. The data obtained on the influence of exciting light intensity on the N intermediate are compatible with our model.

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Abbreviations: bR, bacteriorhodopsin.

2. Materials and methods

All experiments were carried out using freshly prepared purple membrane from the halobacterial mutant strain D96N. The latter was kindly donated by Prof. D. Oesterhelt (Max-Planck Institute fur Biochimie, Germany).

All measurements were performed on a light-adapted purple membrane suspension at 20°C. The bR photocycle was monitored with a single beam spectrofometer [2,8]. The traces obtained were treated by means of the computer program DISCRETE developed by Provencher [9]. Time-resolved difference spectra were obtained by computer processing of 36 curves of absorption change signals measured within the 350–700 nm interval with a step of 10 nm.

Photoexcitation of bR was carried out with a YG-481 Quantel neodymium laser (λ = 532 nm; pulse half-width, 15 ns; energy, 20 mJ).

3. Results and discussion

It is impossible to accelerate the M decay in the wild-type bR photocycle due to the fixed ratio between an intraprotein donor (D96) and an acceptor (the Schiff base). The mutant D96N gives this opportunity owing to an ability of a number of weak acids (azide, cyanate, nitrite, etc.) to serve as proton donors for the Shiff base [10–15]. The proportional dependence of the rate of the M decay on the concentration of the protonated form of azide indicates that it is a second-order reaction. Thus it is possible to significantly accelerate the M decay and make it much faster than the N decay [13,15] using high concentrations of azide. Moreover, under these conditions M↔N equilibrium should be shifted to the N intermediate and thus simplifies the kinetics analysis. Fig. 1A demonstrates that the M decay in the photocycle of the D96N mutant incubated in the absence of azide does not depend on the light intensity. These data are in accordance with our assumption that the N intermediate is the main target of cooperative action. The N decay is much faster than the previous M decay stage in the absence of azide. Therefore, one can suggest that any slight changes observed in kinetic parameters of the forward reaction to bR or back reaction to M would not affect the M decay. This result is consistent with the absence of pronounced cooperativity in the photocycle of the wild type bR at low pH [16]. Under these conditions, the rate of protonation of the N intermediate becomes faster than the M decay, the latter being independent of external pH due to the fact that it represents an intraprotein proton transfer reaction.

Cooperative phenomena in the D96N photocycle become visible only when azide is added. These phenomena are revealed much more obvious at high concentrations of azide (Fig. 1B). Increase in the exciting light intensity induces slight deceleration of the M decay and distinct acceleration of the N decay measured as slow relaxation components in the bR absorption

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maximum region. The N decay analysis as the sum of two exponential components give the following results. The N decay consists of the main component with t = 80–110 ms at low light intensity (five different preparations of mutant bR were tested at 20°C). The contribution of the second fast component with t = 8–12 ms does not exceed 15%. The contribution of the fast component increases to 40–45% at high light intensities. The kinetics of both components do not depend significantly on the exciting light intensity. Note that the ratio between the maximal amounts of the N and M intermediates formed appears to be independent from intensity of the exciting flash. Both these components of the N decay are characterized by the differential spectra similar to the differential spectrum of the N intermediate [13,15,17–19], although two components differ slightly only in the long wavelength region. The latter suggests that there is, probably, O-type intermediate equilibrated with the fast relaxing N component.

According to the model developed by others [1,3–6], appearance of the direct $M_{slow} \rightarrow bR$ transition, and consequently the decrease in amount of the N intermediate without changing of the N decay rate takes place when the light intensity increases. In contrast, according to our model [7], an increase in the light intensity induces the appearance of the fast relaxing N intermediate fraction. Moreover, if the $M \leftrightarrow N$ equilibrium in the photocycle of interacting bR molecules shifts to the N-intermediate (in the presence of the high concentration of azide), then the ratio between the maximal amounts of the N and M intermediate should not change. The data obtained on D96N mutant are strongly compatible with our model.

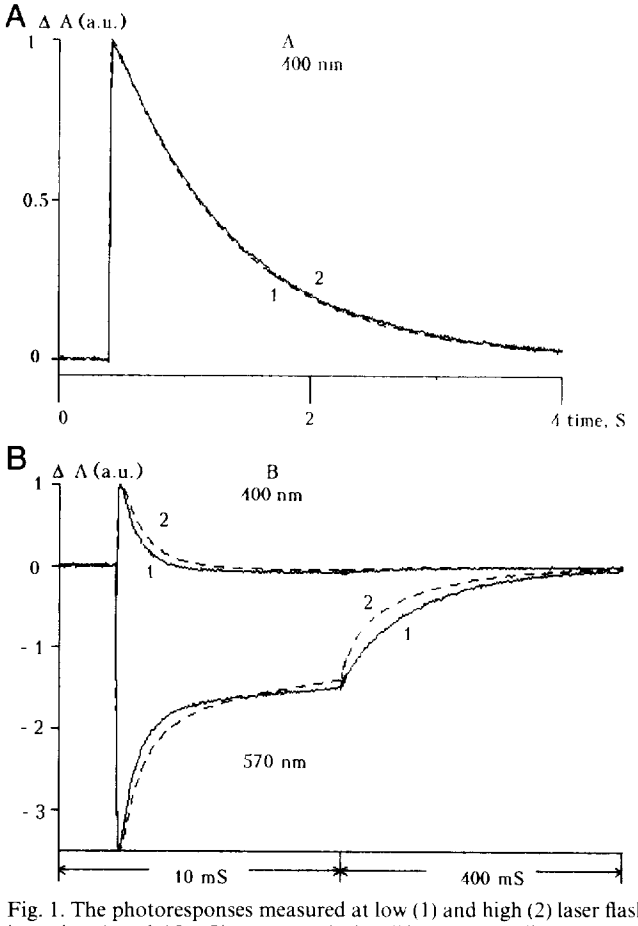

Fig. 1. The photoresponses measured at low (1) and high (2) laser flash intensity (1 and 15 mJ/cm, respectively). The assay medium: 10 mM D96N bR (purple membrane); (A) 1 M NaCl, pH 5; (B) 100 mM sodium azide, pH 5. a.u. = arbitraty units.

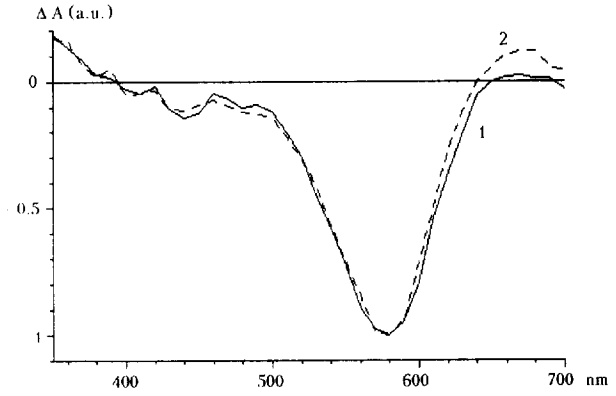

Fig. 2. The difference spectra of the slow (1) and fast (2) components of the N decay. Conditions as in Fig. 1B. 1 = the spectrum was measured in 150 ms after flash. 2 = the spectrum was obtained as the difference between the spectrum measured in 10 ms after flash and the spectrum 1, multiplied by the factor determined from the relative contribution of the fast and slow relaxation components at 570 nm.

It is interesting to note a slight decrease of the M decay rate at the high light intensity (Fig. 1B, Fig. 2). The M decay occurs with a characteristic time of 1–2 ms and has no components comparable with that of the N decay. Thus, this effect is impossible to explain by the same reasons as the slowing down of the M decay in the wild type bR photocycle. Formally this effect indicates to a decrease in the rate constant of the azide interaction with bR. According to our unpublished data (Radionov and Kaulen, in preparation), there is high similarity between the effects of varies agents on the protonation of the Shiff base by D96 and azide. Thus, this effect might indicate that besides an increase in the rate of the back reaction $N \rightarrow M$, the rate of the forward $M \rightarrow N$ slightly decreases as the result of interactions of bR molecules.

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References

[1] Tokaji, Zs. and Dancshazy, Zs. (1991) FEBS Lett. 281, 170–172.
[2] Danshina, S.V., Drachev, L.A., Kaulen, A.D. and Skulachev, V.P. (1992) Photochem. Photobiol. 55, 734–740.
[3] Dancshazy, Zs. and Tokaji, Zs. (1993) Biophys. J. 65, 823–831.
[4] Tokaji, Zs. (1993) Biophys. J. 65, 1130–1134.
[5] Hendler, R.W., Dancshazy, Zs., Bose, S., Shrager, R.I. and Tokaji, Zs. (1994) Biochemistry 33, 4604–4610.
[6] Tokaji, Zs. (1993) FEBS Lett. 357, 156–160.
[7] Komrakov, A.Yu. and Kaulen, A.D. (1995) Biophys. Chem. 56, 113–119.
[8] Drachev, L.A., Kaulen, A.D., Skulachev, V.P. and Zorina, V.V. (1987) FEBS Lett. 226, 139–144.
[9] Provencher, S.W. (1976) Biophys. J. 16, 27–50.
[10] Tittor, J., Soell, C., Oesterhelt, D., Butt, H.-J. and Bamberg, E. (1989) EMBO J. 8, 3477–3482.
[11] Otto, H., Marti, T., Holz, T., Mogi, M., Lindau, M. Khorana, H.G. and Heyn, M.P. (1989) Proc. Natl. Acad. Sci. USA 86, 9228–9332.
[12] Cao, Y., Varo, G., Chang, M., Ni, B., Needleman, R. and Lanyi, J.K. (1991) Bochemistry 30, 10972–10979.

[13] Danshina, S.V., Drachev, L.A., Kaulen, A.D., Khorana, H.G., Marti, T., Mogi, T. and Skulachev, V.P. (1992) Biokhimiya (in Russian) 57, 1574–1585.
[14] Drachev, L.A., Kaulen, A.D. and Komrakov, A.Yu. (1992) FEBS Lett. 313, 248–250.
[15] Tittor, J., Wahl, M., Schweiger, U. and Oesterhelt, D. (1994) Biochim. Biophys. Acta 1187, 191–197.
[16] Danshina, S.V., Drachev, L.A., Kaulen, A.D. and Skulachev, V.P. (1992) Biokhimiya (in Russian) 57, 738–748.
[17] Dancshazy, Zs., Govindjee, R., Ebrey, T.G. (1988) Proc. Natl. Acad. Sci. USA 85, 6358–6361.
[18] Kouyama, T., Nasuda-Kouyama, A., Ikegami, A., Mathew, M.K. and Stoeckenius, W. (1988) Biochemistry 23, 5855–5863.
[19] Zimanyi, L., Cao, Y., Nedleman, R., Ottolenghi, M. and Lanyi, J.K. (1993) Biochemistry 32, 7669–7678.