Volume 281, number 1,2, 170–172 FEBS 09583 April 1991
© 1991 Federation of European Biochemical Societies 00145793/91/$3.50
ADONIS 001457939100293s

Light-induced, long-lived perturbation of the photocycle of bacteriorhodopsin

Zsolt Tokaji and Zsolt Dancsházy

Institute of Biophysics, Biology Research Centre, Szeged, PO Box 521, H-6701 Hungary

Received 28 January 1991

The relative weight of the slowly decaying M intermediate of the photocycle of bacteriorhodopsin increases upon increasing the energy density of the short (10 ns) actinic laser pulse. Moreover, when a pre-exciting flash is applied to the BR sample, the absolute amplitude of the M_s is higher in the signal induced by a second flash, applied with a delay from 100 μs to 100 ms. These facts together prove that either the leftover BR ground-state population becomes different due to the pre-excitation, or there is a cooperative interaction between the BR molecules.

Bacteriorhodopsin; Cooperativity; Ground state heterogeneity; Photocycle; Yield of the M intermediate

1. INTRODUCTION

Bacteriorhodopsin (BR) is a light-driven proton pump (for a recent review see e.g. [1]). A comprehensive model of the BR photocycle, which would be able to describe it in a wide range of the environmental conditions, has not been established yet. One of the greatest problems concerning the BR photocycle is the origin of the biphasic decay of the M intermediate. Most of the recent studies try to solve this problem by introducing only branchings [2,3] and backreactions [4,5] into the photocycle, or suppose more than one thermally equilibrating BR ground-state with independent photocycles [6,7].

A cooperative mechanism in the production of the slowly decaying M has been suggested earlier [8], but in most of the recent studies this possibility has been neglected (or rejected, e.g. in [9]).

In this letter we demonstrate that, either cooperativity between the BR molecules, or heterogeneity of the ground-states induced by the exciting light (suggested in [11]) is indispensable for the description of the photocycle of BR.

2. MATERIALS AND METHODS

For absorption kinetics measurements, the BR sample was isolated according to standard procedures, and was incorporated in a 10% polyacrylamide gel (2 mm thickness, absorbance ca. 0.4 at 570 nm). The samples contained 100 mM universal buffer (citric acid, monopotassium sulfate, borate, diethyl-barbiturate) with the indicated pH. In control measurements carried out at pH 7 and pH 9.5 essentially the same results have been obtained. All measurements were performed on light adapted BR.

The actinic flashes were provided by a dye laser containing coumarin 307 (emission at 505 nm), which was pumped by an excimer and by a nitrogen laser in the case of the double excitations, and by the excimer laser in the case of the actinic light density dependence experiments. The energy density of the light pulses (half-durations: ca. 10 ns) was ca. 20 mJ/cm² and 2 mJ/cm² in the double excitation experiments, and 60 mJ/cm² in the actinic light density dependence measurements, as the highest value.

For the measuring beam a 75 W Xe arc lamp (PTI, PS 200X, USA) was used. Before and after the thermostatted sample monochromators (Oriel, 7240 series, USA) were used. The measuring beam was carefully reduced until it did not cause detectable photoconversion. The absorption changes were detected by a photomultiplier (Hamamatsu R928, Japan) and collected on a quasi-logarithmic time scale described in [12]. The time resolution was ca. 0.3 μs. The curves are usually the average of at least 50 repetitions (repetition rate was 0.3/s).

3. RESULTS AND DISCUSSION

3.1. *Light density dependence of the M_f/M_s ratio*

The ratio of the amplitudes of the rapidly and slowly decaying M intermediates (M_f/M_s) depends on the actinic light density, as can be seen at their characteristic wavelength of 412 nm; in Fig. 1a from the original data traces, and in Fig. 1b from the relative weight of the M_f to the total M yield, determined by least-square exponential fitting. The lifetimes were found to be constant versus the actinic light density (M_f: 3 ms, M_s: 40 ms). (A detailed study on the dependence of the BR photocycle on the exciting light energy density is submitted elsewhere.)

It is obvious, that neither a backreaction, nor a branching after the end of the 10-ns-long laser excitation can in itself be responsible for an actinic light

Correspondence address: Zs. Dancsházy, Institute of Biophysics, Biology Research Centre, Szeged, PO Box 521, H-6701, Hungary. Fax: (36) (62) 13 726

Abbreviations: BR, bacteriorhodopsin; M_f and M_s, rapidly and slowly decaying M intermediates; L_s, slowly decaying L intermediate

Published by Elsevier Science Publishers B.V.

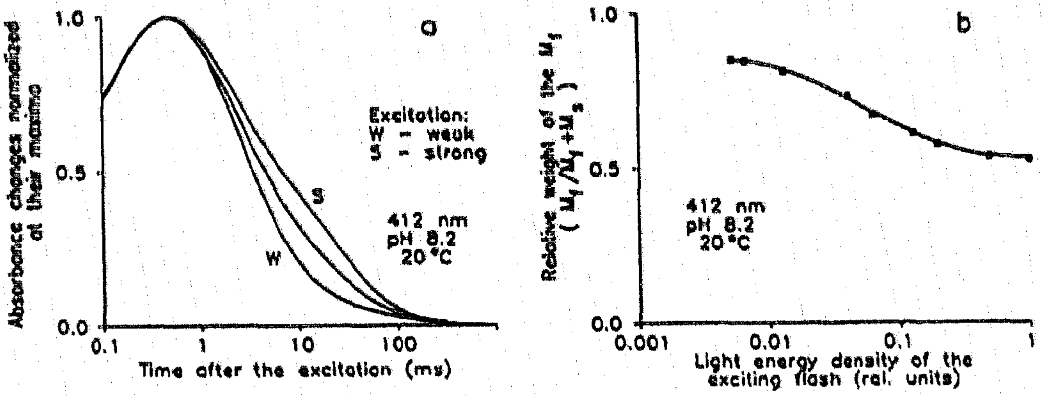

Fig. 1. Actinic light density dependence of the M kinetics; (a) original data traces, (b) results of the exponential fittings.

density-dependent shift in the ratios of the M intermediates. (The rate constants, which describe these processes are not expected to depend on the fraction cycling.)

In this way only the following possibilities remain to describe the actinic light density dependence of the M intermediates: (a) at least two ground-states of BR exist (with different quantum efficiencies/extinction coefficients). One of them leads to the M_f, and another one to the M_s; (Heterogeneity of the BR ground-states). (b) Those molecules, which had been excited, but their photocycles were aborted (e.g. due to the less than unity quantum efficiency), form another, different ground-state, whose excitation by the same actinic light pulse leads to M_s instead of M_f; (Light-induced heterogeneity of the BR ground-states, as suggested e.g. in [11].) (c) the reexcitation of an early photocycle intermediate (K or KL) could lead to M_s formation as well; and (d) cooperativity exists between the BR molecules arranged in the purple membrane sheets, as suggested e.g. in [8].

3.2. *Pre-excitation induced overproduction of the M_s*

When the sample is pre-excited by a short, nearly saturating laser pulse, at a certain time (ca. 100 μs–100 ms) before the beginning of the flash-photolysis measurement, the amplitude of the absorption kinetic signal can only be expected to decrease, due to the lower population of the ground-state(s) in the sample. However, though the total amplitude of the signal decreases, there is a time range when the signal following the pre-excitation exceeds its original value, as can be seen at 412 nm in Fig. 2. (The signal originating from the pre-excitation, which produced ca. 25% fraction cycling, was subtracted from the traces.) When the pre-exciting flash was applied less then 100 μs before the actinic pulse the relative weight of the M_s was also increased in the signal. However as the signal was complicated with the excitation of the L intermediates, it was not studied in detail.

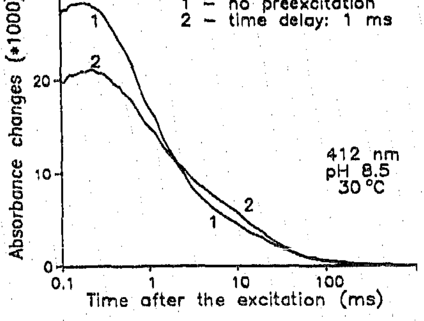

Fig. 2. Influence of the pre-excitation on the M kinetics.

The absorption kinetics (taken versus the delay time between the two flashes) were evaluated by the fitting of exponentials. The change in the amplitudes and the independence of the lifetimes are shown in Fig. 3. It is obvious that the yield of M_s exceeds its original value in a wide time range of the pre-excitation, indicating, that possibility (a) is insufficient as the sole reason (both ground-states should have been depleted to some extent by the preexcitation). Moreover, possibility (c) can be excluded, because in the case of a few milliseconds delay between the two flashes the early intermediates have decayed, so they cannot be reexcited.

The kinetic behavior of the overproduction of the M_s can be described as follows.

i. The perturbation can be induced at least within 10 ns, as can be observed in the experiments of the flash energy density dependence (Fig. 1), when the 'two excitations' take place during this short period.

ii. The extent of the perturbation remains practically constant upto 10 ms (Fig. 3a). It is important to note that M_f completely decays by that time, which can be seen from the original absorbance trace measured at 412 nm (Fig. 3a, dotted line), and which is also obvious from its lifetime (1 ms in Fig. 3b).

iii. The perturbation of the BR photocycle disappears with a time constant of ca. 80 ms (Fig. 3a). This value is significantly slower than the lifetime of M_s (20 ms), and is approx. equal to the life time of the N (the

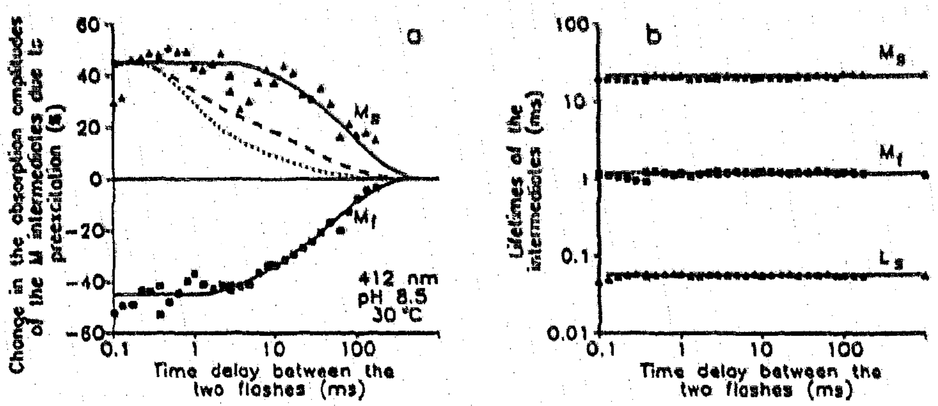

Fig. 3. Influence of the pre-excitation on the amplitudes (a) and lifetimes (b) of the M intermediates. The original absorbance amplitudes were: M_f, 0.017; M_s, 0.005. For illustration the absorption changes at 412 nm (""""") and at 570 nm ((- - -), inverted) induced by the pre-exciting flash are shown. For these two normalized original data traces, the vertical axis is the absorbance change, while the horizontal one is the time elapsed since the pre-exciting flash.

slowest step of the BR photocycle [6,10] determined from the absorbance changes measured at 570 nm (Fig. 3a, dashed line).

These indicate for possibility (b), that the processes leading to the light-induced ground-state should be very fast, and the equilibrium of the ground-states may be described by a long lifetime, or if cooperativity (possibility (d)) takes place, the interaction between the neighboring BR molecules may disappear only by the completion of their photocycles.

Optical thickness of the sample as the reason for the increase in the yield of the M_s was excluded in control experiments, in which the absorbance of the sample was reduced to 0.04.

An alternative explanation to the problem of the M_s was proposed in [10], suggesting that N, the product of the M_f, functions as another ground state, and leads to the M_s by photo-excitation. This possibility is disproved, because at 100 μs delay time, the M_f produced by the pre-excitation has not decayed yet, but the yield of the M_s by the second flash has already been increased.

In conclusion we have only two ways to describe the excitation-dependent yield of the M_s. Possibility (b) (light induced heterogeneity) leads to a model which is essentially the same as proposed in [11]. The other one, (d) supposes cooperative interaction between the BR molecules. Although this latter possibility was usually neglected, the existence of such an interaction has been demonstrated recently [13].

REFERENCES

[1] Oesterhelt, D. and Tittor, J. (1989) Trends Biochem. Sci. 14, 57-61.

[2] Groma, G.I. and Dancsházy, Zs. (1986) Biophys. J. 50, 357-366.

[3] Butt, H.J., Fendler, K., Dér, A. and Bamberg, E. (1989) Biophys. J. 56, 851-859.

[4] Chernavskii, D.S., Chizov, I.V., Lozier, R.H. Murina, T.M., Prokhorov, A.M. and Zubov, B.V. (1989) Photochem. Photobiol. 49, 649-653.

[5] Ames, J.B. and Mathies, R.A. (1990) Biochemistry 29, 7181-7190.

[6] Dancsházy, Zs., Govindjee, R. and Ebrey, T.G. (1988) Proc. Natl. Acad. Sci. USA 85, 6358-6361.

[7] Diller, R. and Stockburger, M. (1988) Biochemistry 27, 7641-7651.

[8] Ohno, K., Takeuchi, Y. and Yoshida, M. (1981) Photochem. Photobiol. 33, 573-578.

[9] Drachev, L.A., Kaulen, A.D., Skulachev, V.P. and Zorina, V.V. (1986) FEBS Lett. 209, 316-320.

[10] Kouyama, T., Nasuda-Kouyama, A., Ikegami, A., Mathew, M.K. and Stoeckenius, W. (1988) Biochemistry 27, 5855-5863.

[11] Birge, R.R., Cooper, T.M., Lawrence, A.F., Masthay, M.B., Vasilakis, C., Zhang, C.F. and Zidovetzki, R. (1989) J. Am. Chem. Soc. 111, 4063-4074.

[12] Gargyan, J., Kuschmitz, D., Schlüter, H., Klein, P. and Imre, Z. (1982) Asp. Molec. Bioenerg. 363, 543.

[13] Marinetti, T. (1988) Biophys. J. 54, 197-204.