Quantitative model for the cooperative interaction of the bacteriorhodopsin molecules in purple membranes

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Abstract The trimeric, asymmetric and sequential model for the cooperative interaction of the bacteriorhodopsin molecules in purple membranes [Zs. Tokaji, Biophys. J. 65 (1993) 1130–1134] is being extended in the paper. Analyses of data from absorption kinetic measurements with preexcitation and green background illumination, and photoselection measurements on oriented samples confirm the main features of this cooperative interaction and support the validity of the extended model. This model includes the observed heterogeneity of the non-excited state of the bacteriorhodopsin molecules in purple membranes, and the agreement with the data suggests that molecules in any other state than the bacteriorhodopsin ground state can alter the photocycle of their neighbors. The presented results seem to contradict other models for the cooperation of the bacteriorhodopsin molecules.

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Key words: Asymmetry; Decay of the M intermediate; Heterogeneity; Sequentiality; Trimeric cooperativity

1. Introduction

The proton translocation mechanism of the light driven proton pump bacteriorhodopsin (BR) is not fully understood [1]. Many details are known about which residues participate in the proton translocation [2,3], but only in the past few years has it become obvious that the processes are coupled to each other in the neighboring BR molecules [4–10]. This interaction is called cooperativity, and it is probably an allosteric regulation in the purple membranes [6].

Recently, on the basis of absorption kinetic studies I suggested a mechanism for the cooperative interaction [6]. According to this theory the cooperative interaction among the BR molecules is trimeric, asymmetric and sequential. This means that a photocycling BR molecule may alter the photocycle of only one of its two neighbors in the same trimer, and this alteration may appear in only one of the two possible decay sequences of these two molecules.

In the meantime other models (containing different features) for the cooperative interaction of the BR molecules have also been suggested. These do not assume that the cooperation is asymmetric and sequential [8–10] (and one of them that it is trimeric [9]).

The statements of my model that the cooperativity is asymmetric and sequential were originally somewhat heuristic, as only indirect evidence had been collected that these features were in fact true.

Another problem of the original model was that it did not take into account that the states of the BR molecules in non-excited purple membranes are to some extent heterogeneous, i.e. in a certain concentration the BR molecules are not in the state denoted by BR, but rather they are in N- or O-like states [11,12].

It is interesting that according to the data published by Radionov and Kaulen [13] the extent of the heterogeneity of the non-excited sample is probably very similar in the D96N mutant, too. However, note that in this mutant – if the lifetimes of the different millisecond processes are separated from each other by addition of azide – a fast decaying form of N seems to correspond to M_s (or more precisely to the late form of M_s designated M_s' [7]). The relative weight of this form increases from 15 to 40–45% with increasing actinic light density (just as does M_s in native BR [5]). (The reason for the difference that the form corresponding to M_s (M_s') contains protonated Schiff base is probably the high protonating ability of azide.)

For these reasons it was necessary to collect evidence for the asymmetry and sequentiality of the cooperative interaction, and to extend the original model to become suitable for use in calculations and evaluations. The results are presented in this paper.

2. Materials and methods

The preparation of purple membranes and the absorption kinetic measuring system were the same as described previously [5]. The actinic beams were provided by an excimer laser pumped dye laser and in the double excitation experiments a nitrogen laser pumped dye laser (with coumarin 307, 505 nm) as second excitation. For continuous background illumination an Ar^+ laser (514 nm) was used. The energy densities of the lights were characterized by the generated fraction cycling in the sample [5].

The purple membranes were incorporated in a 1.5 mm thick slice of 10% polyacrylamide gel, with absorbance of less than 0.3 at 570 nm. The samples contained 30 mM universal buffer (citric acid, monopotassium sulfate, borate, diethyl-barbiturate) with 1 M NaCl.

For the measurements of the asymmetry the purple membranes were oriented by electric field, and this state of the sample was fixed by polymerization of acrylamide [14]. The distance between the orienting electrodes was 1.5 mm. The film polarizers were used in the actinic beam in front of the sample, and in the measuring beam behind it.

The measured absorbance changes were evaluated by fitting exponential components as described previously [4,5]. For the description of the M decay (at 412 nm) two components were used the amplitude factors of which were considered to be proportional to the amounts of corresponding photocycle intermediates M_f and M_s, respectively (see [6]).

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Abbreviations: BR, bacteriorhodopsin; M_f and M_s, rapidly and slowly decaying components/forms of the M intermediate

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3. Results and discussion

3.1. The extended model for cooperativity

My original model for cooperativity [6] is asymmetric and sequential but contains no contribution from the heterogeneity of the sample. Thus the relative weight of M_s (the intermediate that is induced by cooperativity) should start from 0 at the low limit of actinic light densities. The experimental observation is that the relative weight of M_s is usually about 15% at this condition (see e.g. [5]), and from other studies it is known that the non-excited sample contains N- or O-like states (i.e. is heterogeneous) [11,12].

The extended model constructed here supposes that the molecules in these states interact with their neighbors as if they were photocycling. This means that a molecule on the suitable side upon excitation will turn over through the pathway ($M_s \rightarrow BR$) that is usually induced by its neighbor photocycling [7].

The other assumption is that all of the states of the BR molecule other than the BR ground state are equally efficient with respect to the alteration of its neighbor. This means that the extent of the changes in the kinetics does not depend on whether a part of the sample is in M, N, O, or in other states.

The third assumption is that the molecules in the non-ground state have a photocycle without any M-like intermediate, and thus produce no direct contribution to the M kinetics determined at 412 nm.

Let us suppose that the β fraction of the non-excited sample is not in the ground state (this can originate from the heterogeneity of the sample or additionally e.g. from the influence of a preexciting light pulse). The fraction cycling due to the probe flash is p.

The calculation is similar to the one carried out without heterogeneity [6], but more cases have to be considered as subcases of the states of the trimer with 1, 2 or 3 non-ground-state molecules.

With $(1-\beta)^3$ probability the trimer contains only ground-state molecules. For these trimers the number of molecules choosing M_s instead of M_f is $0.5P_d + 1.5P_t = 0.5*3p^2(1-p) + 1.5p^3$ (where P_d and P_t are the probabilities that the trimer contains two or three excited molecules, respectively) [6].

With a $3\beta(1-\beta)^2$ probability the trimer originally contains one non-ground-state molecule. For this case the substates are shown in Fig. 1.

With a $3\beta^2(1-\beta)$ probability the trimer contains two non-ground-state molecules, and the substates are shown in Fig. 2.

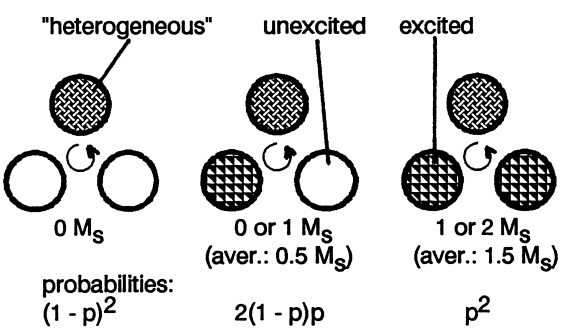

Fig. 1. The possible substates of a trimer which contained one non-ground-state ('heterogeneous') molecule before excitation.

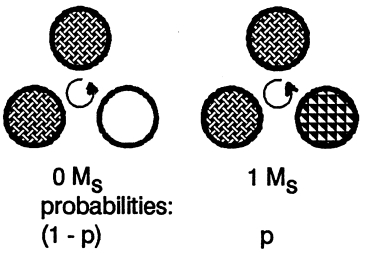

Fig. 2. The possible substates of a trimer which contained two non-ground-state ('heterogeneous') molecules before excitation.

Finally with a β^3 probability the trimer contains three non-ground-state molecules, and thus no signal and no M_s are generated.

Summing up the probabilities of all the substates, and dividing this expression by the signal amplitude, which is $3p(1-\beta)$ we get the relatively simple relationship

$$M_s/M = \beta + 0.5p(1-\beta) \quad (1)$$

where $M=M_f+M_s$, the number of molecules passing through the M state(s).

By comparing Eq. 1 to the experimental data, β is equal to the Y-intercept of the dependence of the relative weight of M_s versus the fraction cycling in single flash experiments. It is about 15% in our conditions, and it is the result of the heterogeneity of the non-excited sample.

Eq. 1 can also be used for the interpretation of absorption kinetic experiments with continuous background illumination, or with a preexciting flash. In this case we have to substitute

$$\beta = \beta_o + (1-\beta_o)p_e \quad (2)$$

where we now denote the intrinsic heterogeneity of the sample as β_o, and p_e denotes the probability that a molecule is excited into the photocycle by preexcitation or by continuous background (pre-)illumination.

3.2. Sequentiality and heterogeneity

The suggested sequential feature of the cooperativity means that the interaction appears or not depending on the decay sequence of the excited molecules. (At this moment it is unclear which (relatively early) transition of the photocycle is important in this respect. It may possibly be the $L \rightarrow M$ transition.)

If sequentiality holds true a preexciting laser flash with a sufficiently long delay time between this and the second probing flash should be twice as efficient as a single flash with the same energy. The reason is that if all of the photocycling molecules are generated by a single flash the probability that the suitable decay sequence of two neighboring photocycling molecules appears is 0.5. If the delay time is long enough the newly excited molecule can only be the second, thus if it is a suitable neighbor of an already photocycling molecule the probability of alteration of its photocycle (i.e. decay through M_s instead of M_f) is not 0.5, but 1.

The result of the absorption kinetic measurement with preexciting laser flash is shown in Fig. 3A. From this it is obvious that the expected effect appears: the slope of the line at preexcitation is almost twice that at single flashes.

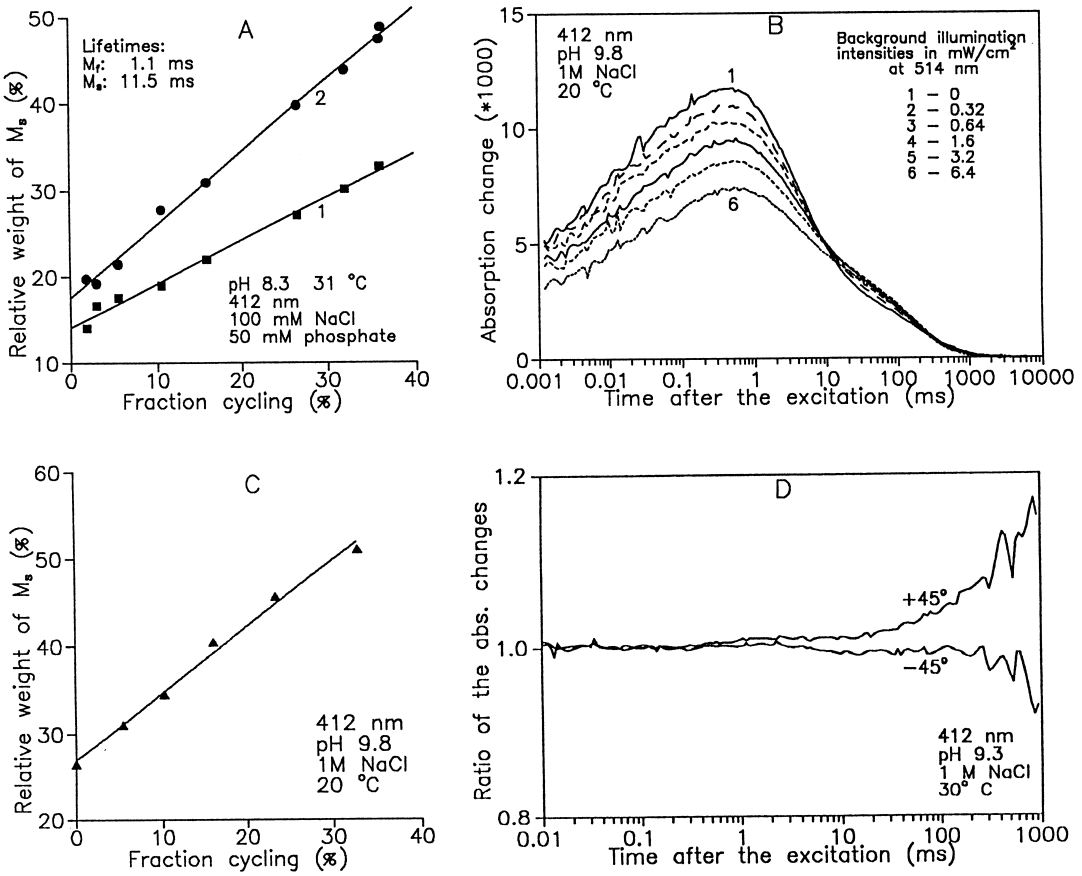

Fig. 3. A: Relative weight of the slow component of the M intermediate decay (M_s) versus the fraction cycling caused by single flash itself (1) and caused by a preexciting flash (2). The latter effect was measured in the kinetics due to a second (probe) flash. The delay time was 200 μs. B: The change in the M kinetics in the presence of green background illumination. C: The relative weight of the slow component of the M decay versus the fraction cycling generated by a green backround illumination. D: The ratio of the absorbance changes at 412 nm in an oriented BR sample before and after reversing, if the angle between the polarizations of the actinic and measuring beams is +45° or −45°, respectively. The ratios start to differ from 1 only in the millisecond time range.

From Eqs. 1 and 2 it is easy to calculate the expected slope for the relative weight of M_s versus the fraction cycling generated by a single flash (p):

$$0.5(1-\beta) \quad (3)$$

and the same for a preexciting laser flash (q):

$$(1-\beta_o)(1-0.5p) \quad (4)$$

From Fig. 3A, line 1 $\beta_o = 14\%$, and from the difference in the Y-intercepts of lines 1 and 2, $p = 0.09$ for the second (probe) flash.

With these data for the preexcitation case shown in Fig. 3A, line 2 the expected slope is 0.82, while the experimental data show a slope of 0.86. So the agreement is good.

For the single flash case Eq. 3 suggests a slope of 0.43, while experimentally a slope of 0.5 is observed (line 1). So the agreement is somewhat poorer.

However, if we take into account the possible imprecisions of the determination of the cycling fraction, and thus compare the expected ratios of the slopes of the two lines in Fig. 3A, we get good agreement between the theory and the measurements. The agreement is precise within 10%: The theoretically expected and the experimentally determined ratios of the slopes are 1.72, and 1.91, respectively.

It is worth noting that the determination of the cycling fraction demanded no additional experiments, because it can be measured directly in the double excitation experiments by the extent of the decrease in the signal amplitude due to preexcitation [15].

At high pH a more precise experiment can be carried out on the supposition that all of the non-ground-state molecules cooperate equally efficiently with their neighbors. At high pH a relatively large non-ground-state fraction can be built up by a not too strong continuous, green background illumination, due to the sufficiently large average turnover time of the photocycling molecules.

The absorption changes at high pH in the presence of different intensities of a green (514 nm) background illumination are shown in Fig. 3B. In this case, with increasing intensity of the green light, the peak amplitude of the signal decreases due to the build-up of a non-ground-state fraction, which does not form M intermediates upon excitation, and the amplitude of the slow phase of the M decay increases relatively due to cooperativity [16].

The lifetimes of the two components of the M decay were found to be constant. The relative weights of M_s (at different intensities of the green background illumination) are shown in Fig. 3C versus the fraction cycling.

This dependence is a straight line. According to the calcu-

lations the value of the *Y*-intercept should originate from heterogeneity (14%) of the ground state, plus 12% from the influence of the probe flash which generated a 25% fraction cycling without background illumination. The sum of these values (26%) is in good agreement with the measured value of the *Y*-intercept of 27%.

The expected slope for the line of Fig. 3C on the basis of Eq. 4 is 0.75, which is in a very good agreement with the measured value (0.76).

The very good agreement of the data with the calculations shows that the constructed theory is probably not wrong, including the assumption that any non-ground-state molecule is equally suitable for altering the function of its photocycling neighbor.

3.3. Deformation and asymmetry

On the basis of the explanation developed by myself for the description of the cooperative interaction of the BR molecules in the purple membranes the cooperative interaction should act through a conformational coupling, and this coupling should be asymmetric [6]. This means that of two excited BR molecules of the same trimer only one may alter the photocycle of the other, and reversely not. In special conditions this effect should be observable as an asymmetry of an oriented sample.

In order to produce such conditions first the purple membranes were oriented (in an electric field) and fixed in a gel. The membranes, the planes of which were parallel to the plane of the gel slice, were excited and measured by linearly polarized lights, propagating in the direction of the normals of the membranes.

In this case it is not too difficult to see that if the angle between the polarizations of the actinic and measuring beams is 45° (or similar), the signal measured in + and −45° should be different due to the asymmetry. The ratio of the absorption changes measured in these two cases is shown in Fig. 3D. Here the reverse cases were produced by rotating the cuvette by 180° around its vertical axis. The + and − angles refer to the angles between the polarizations of the actinic and measuring beams in a coordinate system fixed to the laboratory.

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References

[1] Ebrey, T.G. (1993) in: Thermodynamics of Membranes, Receptors and Channels (Jackson, M., Ed.), pp. 353–387, CRC Press, New York.
[2] Lanyi, J.K. (1993) Biochim. Biophys. Acta 1183, 241–261.
[3] Lanyi, J.K. and Váró, G. (1995) Israel J. Chem. 35, 365–385.
[4] Tokaji, Zs. and Dancsházy, Zs. (1991) FEBS Lett. 281, 170–172.
[5] Dancsházy, Zs. and Tokaji, Zs. (1993) Biophys. J. 65, 823–831.
[6] Tokaji, Zs. (1993) Biophys. J. 65, 1130–1134.
[7] Tokaji, Zs. (1995) FEBS Lett. 357, 156–160.
[8] Shrager, R.I., Hendler, R.W. and Bose, S. (1995) Eur. J. Biochem. 229, 589–595.
[9] Váró, G., Needleman, R. and Lanyi, J.K. (1996) Biophys. J. 70, 461–467.
[10] Komrakov, A.Yu. and Kaulen, A.D. (1995) Biophys. Chem. 56, 113–119.
[11] Fukuda, K. and Kouyama, T. (1992) Biochemistry 31, 11740–11747.
[12] Brown, L.S., Zimányi, L., Needleman, R., Ottolenghi, M. and Lanyi, J.K. (1993) Biochemistry 32, 7679–7685.
[13] Radionov, A.N. and Kaulen, A.D. (1995) FEBS Lett. 377, 330–332.
[14] Dér, A., Hargittai, P. and Simon, J. (1985) J. Biochem. Biophys. Methods 10, 295–300.
[15] Tokaji, Zs. and Dancsházy, Zs. (1992) FEBS Lett. 311, 267–270.
[16] Tokaji, Zs. and Dancsházy, Zs. (1992) in: Structures and Functions of Retinal Proteins (Rigaud, J.L., Ed.), Vol. 221, pp. 175-178, John Libbey Eurotext, London.