THE EFFECTS OF HEAVY WATER IN THE PROTEORHODOPSIN PHOTOCYCLE

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Abstract. The proton transporting photocycle of the proteorhodopsin at its normal pH (9.5) shows a marked deuterium effect. It was shown earlier that the intermediates N and PR' are responsible for the proton uptake and release. By proton-deuteron exchange the M_2-N and N-PR' transitions become 2–3 times slower. On the contrary, the early μs domain is less affected than the decay part of the photocycle. The effects measured on proteorhodopsin are very similar to those measured on bacteriorhodopsin.

Key words: bacteriorhodopsin, proteorhodopsin, deuterium, photocycle, absorption kinetics.

INTRODUCTION

The proton pumping retinal protein, the proteorhodopsin (PR) was identified in the γ-proteobacterium, a marine bacterioplankton. The protein was functionally expressed in *Escherichia coli* [1, 2]. As the pK_a of the proton acceptor group is high (7.1, compared to that of BR, which has 2.6), the proton transporting photocycle of the PR was studied at pH 9.5 [14].

Early in the BR research, the effect of D_2O on the photocurrent kinetics [5, 7] and on the absorption kinetic traces were measured [8], but only recently a thorough study was published [3], which related the changes in the kinetics of the intermediates to the exchange of the proton to deuterium, using the so-called proton inventory method [15]. Thus far no studies were done concerning the deuterium isotope effect on proteorhodopsin.

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MATERIALS AND METHODS

PR was expressed in *Escherichia coli* strain UT5600 and prepared as described before [2, 4]. The measurements were performed on membrane encased in polyacrylamide gel on samples having optical density around 0.5 at 570 nm. To ensure a good H_2O to D_2O exchange and the same measuring conditions, before the measurements all the samples were dried on P_2O_5 and re-soaked in 100 mM NaCl solution. The desired concentration of the solution was freshly prepared from dried NaCl and H_2O or D_2O, respectively. The pH was adjusted with HCl, DCl, NaOH and NaOD respectively. The pD of the heavy water solution was taken 0.4 units higher to the measured pH [3].

The gel samples were placed in a quartz-cuvette and that in a temperature controlled sample holder. The excitation was performed with frequency doubled Nd-YAG laser (Surelite I0, Continuum, Santa Clara, CA), of 1.5–2 mJ/cm^2 energy density at 532 nm. Perpendicular to the laser excitation a 55 W halogen lamp provided the measuring light through a heat filter. The laser flash induced an absorption change in the sample, which caused a change in the measuring light. Absorption kinetic signals after going through the monochromator and the photomultiplier were recorded with a transient recorder card (NI-DAQ PCI-5102, National Instruments, Austin, TX) with 16 MB memory (Fig. 1).

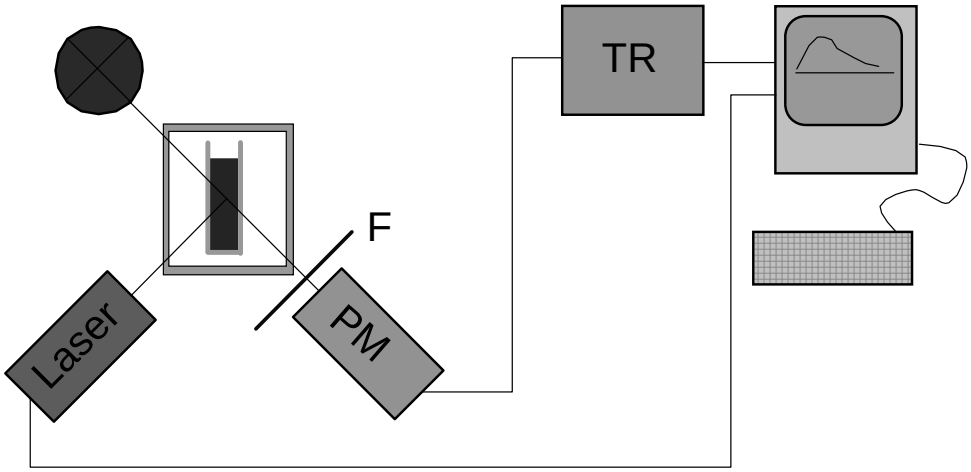

Fig. 1. The measuring setup for absorption kinetic.

The data collected with a sampling rate of 50 ns were converted to a logarithmic time scale by averaging on logarithmically equidistant time intervals. Each measurement was the average of 100–200 signals. The absorption changes

recorded at several wavelengths contain the apparent rate constants. The fit of the absorption kinetic traces was done with the RATE program [9, 12].

During the fit with the RATE program, the spectral changes were transformed to time-dependent concentration changes of intermediates with the following equation:

$$\Delta A_{\lambda}(t) = \sum_{i} \Delta\varepsilon_{\lambda i} \cdot C_i(t) \tag{1}$$

where $\Delta A_{\lambda}(t)$ is the column matrix of absorption changes measured at different wavelengths, $\varepsilon_{\lambda i}$ is the matrix of extinction coefficients [6], and $C_i(t)$ is the column matrix made from the concentration of photocycle intermediates. The time-dependence of the concentration of intermediates can be calculated with the following equation:

$$\frac{\mathrm{d}C_i}{\mathrm{d}t} = \sum_{j=i+1} (k_{ji} \cdot C_j - k_{ij} \cdot C_i) \tag{2}$$

where k_{ij} and k_{ji} are the rate constants. The fit resulted microscopic kinetic parameters of a given photocycle.

Various sequential and parallel models were fitted to the absorption kinetic signals but only the model with sequential reactions was suitable for interpreting the experimental data. The only model that fulfilled all the criteria contains the sequential reactions:

$$\mathrm{K} \Leftrightarrow \mathrm{M_1} \Rightarrow \mathrm{M_2} \Leftrightarrow \mathrm{N} \Leftrightarrow \mathrm{PR'} \Rightarrow \mathrm{PR} \tag{3}$$

At low pH, the multiphase decay of the kinetic signal [10, 11] indicated the existence of a spectrally silent intermediate PR', with similar spectrum to that of PR, just as in the case of high pH.

RESULTS AND DISCUSSIONS

The BR at neutral pH exhibited a weak kinetic isotope effect in the early, μs time interval [13]. For all the others steps of the photocycle, the isotope effect becomes important, the largest change occurring for the L-M transition which is associated to the deprotonation of the Schiff-base and the proton release to the extracellular side.

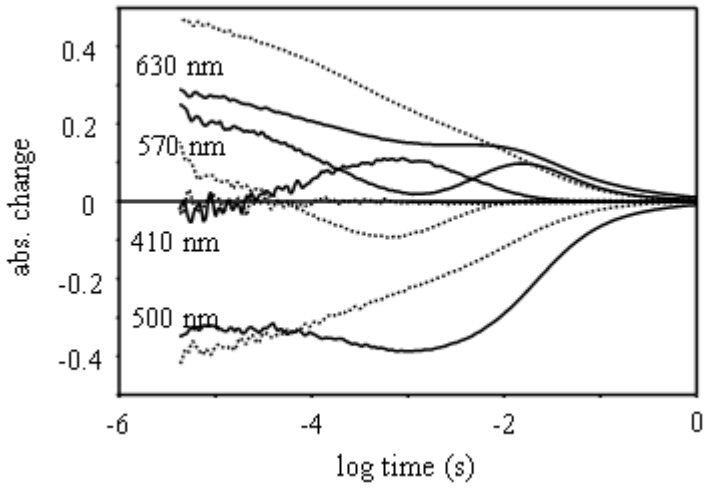

Fig. 2. Absorption kinetic signals measured at four wavelengths on proteorhodopsin. Measuring conditions: 100 mM NaCl dissolved in H_2O (broken line) or D_2O (continuous line) at pH (pD) 5.2. The pD was calculated as described in the Materials and Methods chapter.

In the case of PR at pH = 5.2, the shift of the absorption kinetic signals, by changing the protons to deuterons, also shows kinetic isotope effect (Fig. 2). Similarly to BR, the early μs time domain is less affected than the ms and decay part of the photocycle. Unfortunately, in the case of deuterated sample it was impossible to perform a good fit to the model in order to obtain the concentrations of the intermediates at pH 5.2. But, as the absorption signal at 410 nm indicates, M intermediate can be found in the deuterated sample at this pH (Fig. 2). In the PR photocycle there is no L intermediate [4, 14]. In the case of PR samples at the normal pH of 9.5, the decay of the K intermediate leads to an early appearance of the M. The early part of the K decay (Fig. 3) belongs to the K to M_1 transition, which becomes only about 1.4 times slower by the proton-deuteron exchange. Interestingly, the deprotonation step in PR is much less sensitive to the isotope effect compared to BR. The Schiff-base deprotonation could be followed by a local rearrangement of the protein, around the isomerized retinal, resulting a small charge shift [14], similar to that in BR but the rate limiting step is the deprotonation of the Schiff-base The second part of the K decay is determined by the M_1 to M_2 transition and seems almost independent of isotope effect. Based on the BR analogy this step could be the accessibility switch [14].

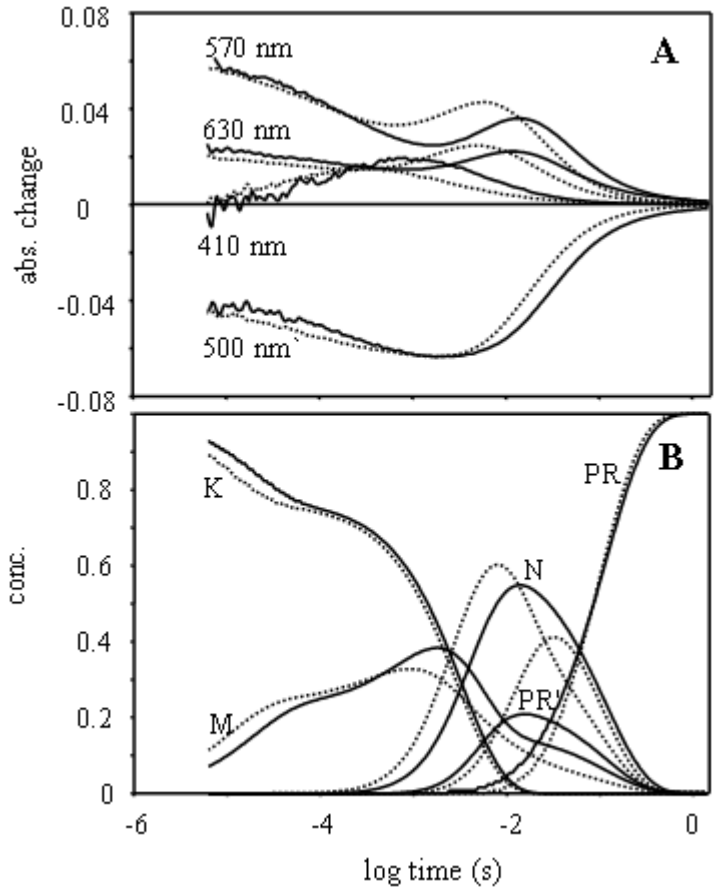

Fig. 3. Absorption kinetic signals measured at four wavelengths on proteorhodopsin. Measuring conditions: 100 mM NaCl dissolved in H_2O (broken line) or D_2O (continuous line) at pH (pD) 9.5 (A) and the time-dependent concentrations in the fitted photocycle model (B).

From an earlier study is known that in PR the proton uptake precedes the release step and it is considered to coincide with the M_2 to N transition [4]. The intermediates N and PR' are responsible for the proton uptake and release and are affected by the isotope exchange (Fig. 3B). The M_2 to N and N to PR' transitions become 2–3 times slower by proton-deuteron exchange. The PR' to PR transition, which is the last step in the photocycle, looks unaffected. This indicates that during this transition the rate limiting process does not require major proton motion or H bond rearrangements.

CONCLUSIONS

We carried out absorption kinetic measurements to study the proton dependent steps of the transport process of PR photocycle as compared to that for BR in H_2O and D_2O. In BR at normal pH all photocycle steps exhibited a strong isotope effect. In a similar manner, PR at both its normal pH (9.5) and at pH 5.2 shows an important deuterium effect as well. Besides, no M intermediate is found for the deuterated sample at pH 5.2. The effects measured on PR were comparable to those measured on BR.

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