SPECTROSCOPY OF BIOLOGICAL MOLECULES

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THE DYNAMICS OF THE FIRST STEPS OF THE PHOTOCYCLE OF BACTERIORHODOPSIN

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Photosynthesis in halobacteria is based on the ability of the protein bacteriorhodopsin (BR) to act as a light-driven proton pump /1/. The primary structure of bacteriorhodopsin is known: the polypeptide chain contains 248 amino acids and folds into seven α-helical segments /2/. The absorption properties of BR are determined by the conformation of the pigment molecule retinal and its interactions with the protein. Retinal is bound in BR via a Schiff base to one lysine of the polypeptide chain. In the light-adapted form of BR, the retinal molecule has the all-trans configuration and the Schiff base is protonated /3/. During the photochemical cycle the absorption properties of BR change substantially. Intermediate states named K, L, M, ... have been found. During the course of the cycle the retinal adapts the 13-cis configuration and the Schiff base looses its proton. The photochemical cycle is completed after ~ 10 ms, when the initial light-adapted form of BR is reached /2,4/. The first events of the photocycle of BR, where the optical energy is stored in the molecules, have been studied in a number of publications /5-12/. Unfortunately, the experimental results and the interpretation of the first molecular processes are not unambiguous.

In this Letter we present a summary of extensive experimental investigations using time resolved picosecond and femtosecond techniques. We applied advanced experimental techniques: the time resolution was improved down to 50 fs, a high-sensitivity detection system gave accurate data at a very low excitation of the specimen (always smaller than one photon per ten BR-molecules) and a variety of excitation and probing frequencies was applied /12,13/. Different BR samples were investigated: deuterated BR, non-deuterated BR, and BR containing the sterically fixed 9-12-phenylretinal /14/. The combination of all experimental results provides a new and consistent picture of the first events of the photocycle of bacteriorhodopsin.

Material and Methods

Bacteriorhodopsin (in the purple membrane) was prepared from Halobacterium halobium according to the procedure given in Ref.15. BR was measured at pH7 and room temperature. Deuterated samples were prepared (see Ref.13) by repeatedly suspending purple

membranes in D_2O, illuminating them strongly and centrifugating them. The exchange of the hydrogen by deuterium at the Schiff base nitrogen was confirmed by resonance Raman spectroscopy.

The principle of the time resolved experiments was as follows: Bacteriorhodopsin was excited by a first ultrashort light pulse which triggered the photochemical reaction. A second, properly delayed probing pulse monitored absorbance changes induced by the transient intermediates. Varying the time delay between excitation and probing pulses one was able to study the dynamics of the absorption changes. The experimental systems are described in detail in earlier publications /12,13/.

Results

Time resolved measurements of light-induced absorbance changes are shown in Fig. 1 and 2. In Fig. 1 the absorbance changes from the very first events are presented. They were measured by using ultrashort exciting and probing pulses of 160 fs ($1.6 \cdot 10^{-13}$ s) duration (λ = 620 nm). This measurement at the high time resolution of 50 fs exhibits interesting details at very early times. The data of Fig.2 were recorded using longer exciting and probing pulses of 4 ps duration. The very first steps around time zero are no longer seen . But the evolution of the system at later times up to 300 ps is clearly seen. The various absorbance changes observed on the

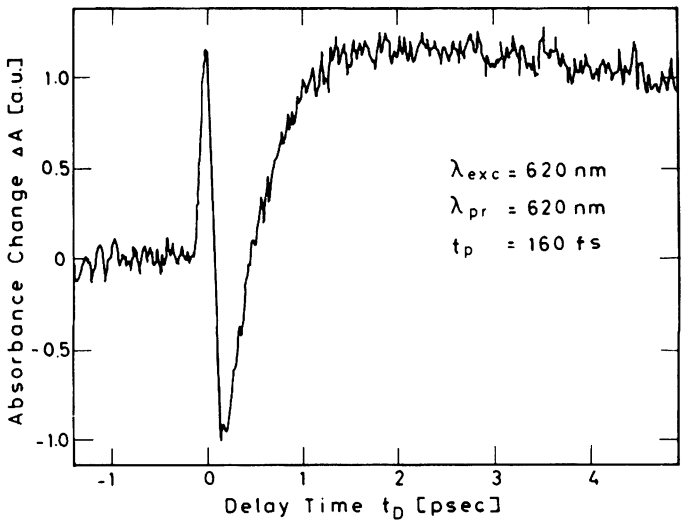

Fig.1
Transient absorbance changes in bacteriorhodopsin induced by an exciting pulse (λ = 620 nm, duration 160 fs) and probed at λ = 620 nm. The very rapid kinetics of 430 fs and 5 ps reflect the formation of the intermediate state J and K.

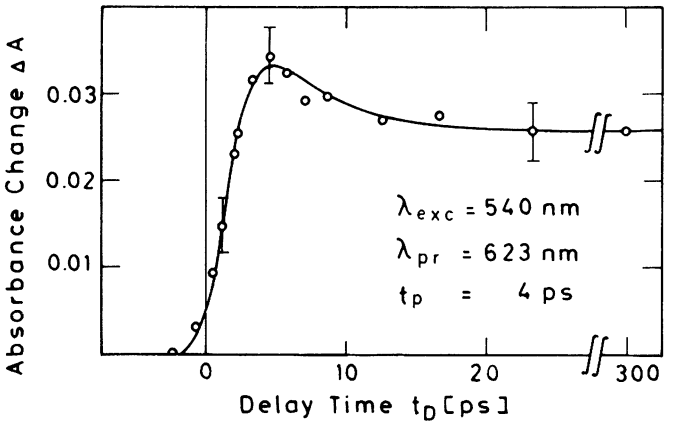

Fig.2
Transient absorbance changes in bacteriorhodopsin measured with pulses of 4 ps duration. Exciting pulse at λ = 540 nm and probing pulse at λ = 623 nm. The longer duration of the pulses smears out the information around time zero which can only be measured with shorter pulses (see Fig.1).

femtoseond and picosecond time scale (probing wavelengths λ = 620 nm) are as follows: (i) A strong peak of induced absorbance occurs at time zero (see Fig.1). We could show that this apparent absorbance increase is not related to a photochemical process; it is due to a nonlinear optical process resulting from the hexagonal arrangement of the protein in the purple membrane (second harmonic generation) /13/. (ii) After the initial peak the absorption decreases very rapidly due to the formation of the excited electronic state BRS1. (iii) With a time constant of 430±50 fs the absorption again increases (see Fig.1). With this time constant the strongly absorbing intermediate J is formed. (iv) The generation of the third intermediate K with the time constant of 5±2 ps (see Fig.2) causes the subsequent small relative absorbance decrease. (v) The absorbance change remains constant for the further observation time of 300 ps.

Picosecond investigations at a large number of other probing frequencies support the interpretation given above /12/. All data fit the model where the three intermediate state BRS1, J, and K are formed one after the other with the time constants $\tau_{BRS1\rightarrow J}$=0.43 ps and $\tau_{J\rightarrow K}$=5 ps. The picosecond investigations allow to determine the absorbance spectra of the intermediates shown in Fig.3. These spectra together with time resolved fluorescence measurements strongly support the interpretation that (i) BRS1 is an excited electronic state, whereas (ii) the (non-emitting) states J and K are electronic ground states.

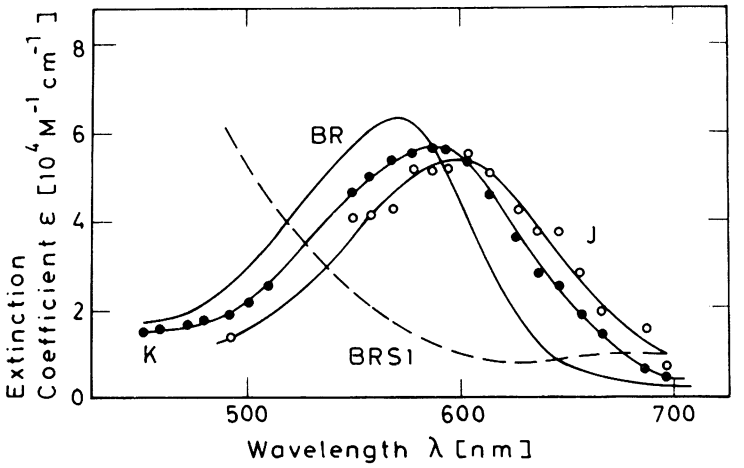

Fig.3
Absorption spectra of bacteriorhodopsin (BR) and of the intermediate states BRS1, J and K. Data are deduced from picosecond excite/probe experiments at a variety of probing wavelengths. An efficiency $\eta = 0.6$ for the formation of states J and K is assumed.

The Molecular Mechanism

Knowing the dynamics of the first events in native BR we want to discuss the related molecular processes: Measurements with deuterated and non-deuterated BR samples are shown in Fig.4 (for wavelengths see Fig.1). They were recorded by femtosecond light pulses. Fig.4a and Fig.4b give identical results i.e. there is no indication of a deuterium effect in the formation of intermediate J. In numerous picosecond experiments we have shown that also the formation of state K does not depend on deuteration. Our data are in contradiction to previous publications, where high excitation levels were used /8,16/. The absence of a deuterium effect on the femtosecond and picosecond dynamics of bacteriorhodopsin is a strong indication that a transfer of the proton at the retinal Schiff base linkage is not the primary photochemical process in BR.

On the other hand, the strong similarity of the absorption spectra of intermediates J and K, where the retinal is isomerized to the 13-cis configuration ,indicate that J already contains 13-cis retinal. That interpretation is supported by time resolved measurements with BR containing the sterically fixed

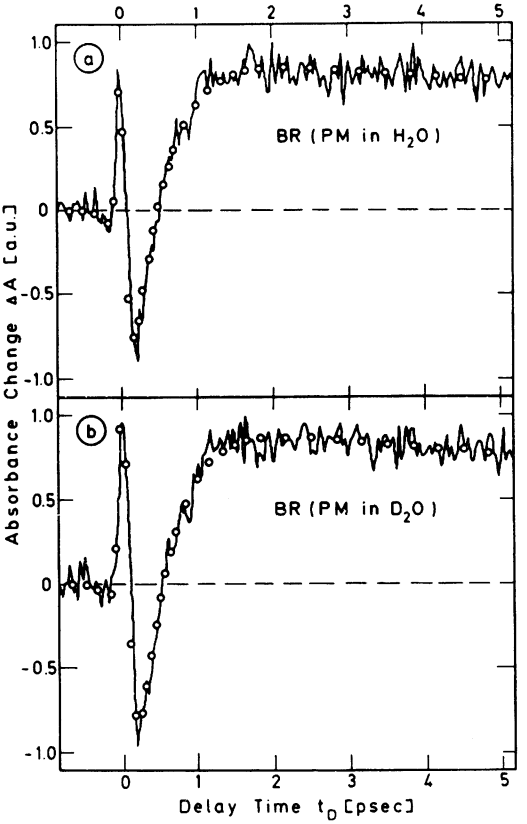

Fig.4
Transient absorbance changes measured with femtosecond light pulses. Excitation and probing wavelengthes 620 nm.
a) native BR (purple membranes suspended in H_2O)
b) deuterated BR (purple membranes suspended in D_2O, where hydrogen at the Schiff base was exchanged by a deuterium).
There is no deuterium effect in the first photochemical steps.

9-12-phenylretinal /14/. Here the excited electronic state has an increased lifetime of 10 ps and no photoproducts are formed. The lack of a ground state photoproduct in the system where isomerisation is blocked strongly supports the notion that isomerisation is the first molecular mechanism of the photocycle of bacteriorhodopsin.

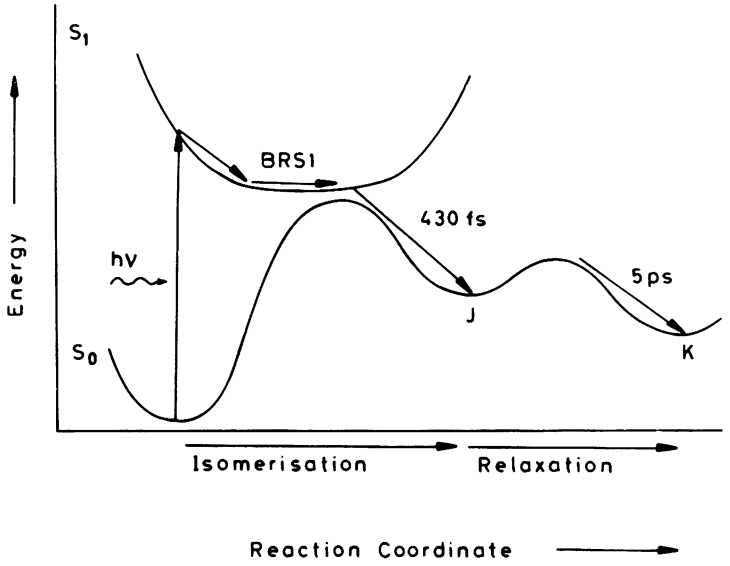

Fig. 5
Energy surface diagram illustrating the primary steps of the photocycle in bacteriorhodopsin with all-trans to 13-cis isomerisation occuring in the very first step forming state J within 430 fs.

Conclusions

The first molecular events are summarized in the schematic energy surface diagram of Fig.5. The absorbed photon promotes BR to the excited state surface S_1. Here a first rearrangement of the molecule leads to the bottom of the S_1 surface (state BRS1). From here partition to the original ground state BR and to the isomerized 13-cis ground state J takes place within 430 fs. Apparently there is no energy barrier against the formation of J. The J to K transition is a ground-state process where a rearrangement of the protein surrounding and relaxation processes cause the absorption changes.

The experiments reported here indicate that the primary process in the photocycle of bacteriorhodopsin is a very rapid photo-isomerisation which occurs within 430 fs.

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References

1. D. Oesterhelt and W. Stoeckenius, Proc. Natl. Acad. Sci. USA, 70, 2853 (1973).
2. For a review see: J.K. Lanyi in Bacteriorhodopsin and related light-energy converters in bioenergetics, ed. by L. Ernster, Elsevier, Amsterdam, New York (1984) p. 315.
3. S.O. Smith, A.B. Myers, J.A. Pardoen, C. Winkel, P.P.J. Mulder J. Lugtenburg and R. Mathies, Proc. Natl. Acad. Sci. USA 81, 2055, (1984).
4. M. Braiman and R. Mathies, Proc. Natl. Acad. Sci. USA, 79, 403 (1982).
5. K.J. Kaufmann, P.M. Rentzepis, W. Stoeckenius and A. Lewis, Biophys. Res. Commun. 68, 1109, (1976).
6. R.R. Alfano, W. Yu, R. Govindje, B. Becher and T.G. Ebrey Biophys.J. 16, 541, (1976).
7. K.J. Kaufmann, V. Sundstroem, T. Yamane and P.M. Rentzepis, Biophys. J. 22, 121, (1978).
8. M.L. Applebury, K.S. Peters and P.M. Rentzepis Biophys. J. 23, 375, (1978)
9. E.P. Ippen, C.V. Shank and A. Lewis, Science 200, 1279, (1978).
10. Y. Shichida, S. Matuoka, Y. Hidaka and T. Yoshizawa , Biochim. Biophs. Acta 723, 240, (1983).
11. A.V. Sharkov, Y.A. Matveetz, S.V. Chekalin, A.V. Konyashchenko, O.M. Brekhov and B.Y. Rootsky, Photochem. Photobiol. 38, 108, (1983).
12. H.J. Polland, M.A. Franz, W. Zinth, W. Kaiser, E. Kölling, and D. Oesterhelt, Biophys. J. (1985).
13. M.C. Nuss, W. Zinth, W. Kaiser, E. Kölling and D. Oesterhelt, Chem. Phys. Lett. (1985)
14. H.J. Polland, M.A. Franz, W. Zinth, W. Kaiser, E. Kölling and D. Oesterhelt, Biochem. Biophys. Acta 767, 635, (1984).
15. D. Oesterhelt and W. Stoeckenius, Methods Enzym. 31A, 667, (1974).
16. M.C. Downer, M. Islam, C.V. Shank, A. Harootunian and A. Lewis in Ultrafast Phenomena IV, ed. D.H. Auston and K.B. Eisenthal, Springer Verlag, Berlin, Heidelberg (1984) p. 500.