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THE PRIMARY STEPS OF PHOTOSYNTHESIS IN BACTERIORHODOSPIN

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During the evolution of biological species on earth nature developed very elaborated systems, which convert the energy of sun light into chemical energy. Ideal systems for the study of photosynthesis are found in some bacteria, were the photosynthetic units can be separated from the rest of the bacterium and prepared as pure samples. Single, functionally active proteins are obtained, which allow to study the primary steps of photosynthesis by spectroscopic techniques.

In this paper we concentrate on bacteriorhodopsin, a special retinal containing photosynthetic system used in halobacterium halobium. Bacterio-rhodopsin (BR) acts as a light-driven proton pump generating a proton gradient across the cell membrane, which drives important physiological processes of the bacterium, e.g. the ATP synthesis. Here we study the primary photochemical reactions of bacteriorhodopsin, where the light energy is initially stored. These primary processes proceed on a very rapid time scale, and picosecond and femtosecond optical techniques are required.

Bacteriorhodopsin has a number of interesting properties/1/: it is a trans-membrane protein with known amino acid sequence. It uses the dye retinal as a functional pigment in the course of its photoreaction. The retinal molecule is bound via a protonated Schiff's base to lysine 217 of the aminoacid sequence. In bacteriorhodopsin the retinal can be chemically removed and replaced by the same or a chemically modified retinal. BR samples kept in the dark contain retinal molecules to 50% in the all-trans and to 50% in the 13-cis,15-syn configuration /2/. Only the all-trans retinal containing BR is able to pump protons, while the other BR molecules must be converted by light to the pump-active all-trans form. After strong illumination by light the BR sample contains 100% all-trans retinals (light-adapted bacteriorhodopsin BR_{la}) /3/.

Light-adapted bacteriorhodopsin:

In a number of publications the primary processes in light-adapted bacteriorhodopsin have been studied /4-6/. Here we give a brief summary of the results: The absorption of a photon by the retinal molecule brings the molecule to the potential surface of the excited electronic state (BRS_1) A first photochemical movement along

the reaction coordinates takes place. After the very short time of 430 fs the system returns to the ground-state potential surface and generates product state J, which exhibits a red-shifted absorption spectrum. With a slower time constant of approximately 3 ps /7/ the system again changes the spectral properties going to the product state K, which is stable for at least 1000 ps. During these steps the peak of the absorption spectrum of BR changes from 568 nm (ground-state BR) via 600 nm (J) to 590 nm (K) /6/. Experiments on BR preparations containing modified retinal molecules suggest that the very rapid primary reaction, which leads to the formation of the intermediate J, is an all-trans to 13-cis (presumably to 13-cis,14s-cis) isomerisation /8,9,14/. The changes of the absorption spectrum may be understood by movements of the protonated Schiff's base, away from a counter-ion during the isomerisation process /9/.

Bacteriorhodopsin containing 13-cis retinals:

Dark-adapted BR contains 50% 13-cis,15-syn retinals. It has been shown that upon excitation the cis retinal forms a red-shifted intermediate, which decays with 35 ms (cis cycle) /3/. Very recently, the formation of the red-shifted intermediate of dark-adapted BR was studied by Petrich et al. /10/, who found the same time constants for both light- and dark-adapted samples. Here we point to the differences between the cis and trans cycles by experiments on dark-adapted BR and BR containing a modified retinal. In the experiments on dark-adapted BR two reactions of the all-trans and 13-cis containing BR samples proceed in parallel and kinetics of the two species are superimposed. As a consequence the analysis of the experimental data is difficult. Nevertheless, interesting results are obtained at certain probing wavelengths. In order to obtain more convincing experimental data we studied a BR sample with increased 13-cis retinal concentration by femtosecond excite and probe measurements. The higher 13-cis concentration is obtained when the retinal molecule of native BR is replaced by a 13-demethyl retinal /11/. These samples ($BR_{13\text{-}dmr}$) contain 85% 13-cis retinals. We assume that the 13-cis form of both the $BR_{13\text{-}dmr}$ sample and the dark-adaped BR sample have very similar properties /12/. The results from the femtosecond time-resolved measurements on the $BR_{13\text{-}dmr}$ samples are shown in Fig.1 and 2 for four wavelengths of the probing light pulse (excitation wavelength λ = 620 nm). The experimental data are given by the circles, the solid lines are calculated fitting curves using exponential functions. Straight-forward is the analysis of the experimental data at 800 nm (Fig.1c). At this wavelength there is neither absorption of BR nor absorption of a ground-state photoproduct. The transmission changes found at this wavelength are due to the gain induced by the excited electronic state of the BR molecules. The observed curve is predominantly determined by one exponential with the time constant of 1.2 ps. We asigne this time constant to the lifetime of the excited electronic state of the 13-cis retinal molecules. In the fit of the experimental data of Fig.1c only a very weak component with a time constant of 0.4 ps was necessary to optimize the fit around time zero. The latter component with the relative amplitude of 15% is asigned to the remaining 15% of all-trans molecules in the sample. The other measurements (Fig.1a and

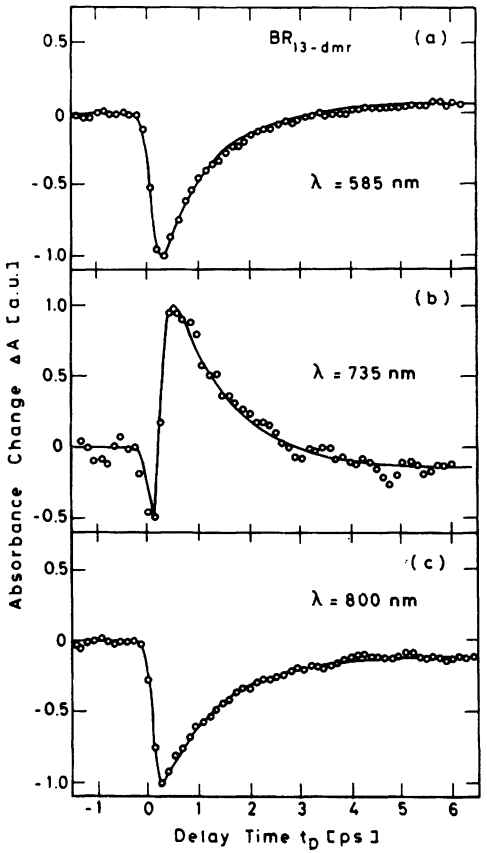

Fig.1 Absorption changes induced by femtosecond light pulses (λ_{ex} = 620 nm) in bacteriorhodopsin containing 13-demethylretinal at different probing wavelengths. Curve 1c shows a clear 1.2 ps kinetic assigned to the cis photocycle.

1b at λ = 585 nm and 735 nm, respectively) also display the fast 0.4 ps and the slower 1.2 ps kinetic together with a 3 ps kinetic (see below); e.g. at 585 nm the 0.4 ps all-trans kinetic shows up most clearly.

Of special interest is the question, if a J to K transition is also found in the cycle of the 13-cis containing BR molecules. Fig.2 shows the experimental results at the probing wavelength of λ = 660 nm. At this wavelenth the J to K transition is clearly seen in light-adapted BR samples. Also in the $BR_{13\text{-}dmr}$ samples the "J" to "K" transitions is evident. The 3 ps kinetic found here has the same amplitude as in the light-adapted BR samples, demonstrating that similar processes occur at that time in the cis and trans cycle.

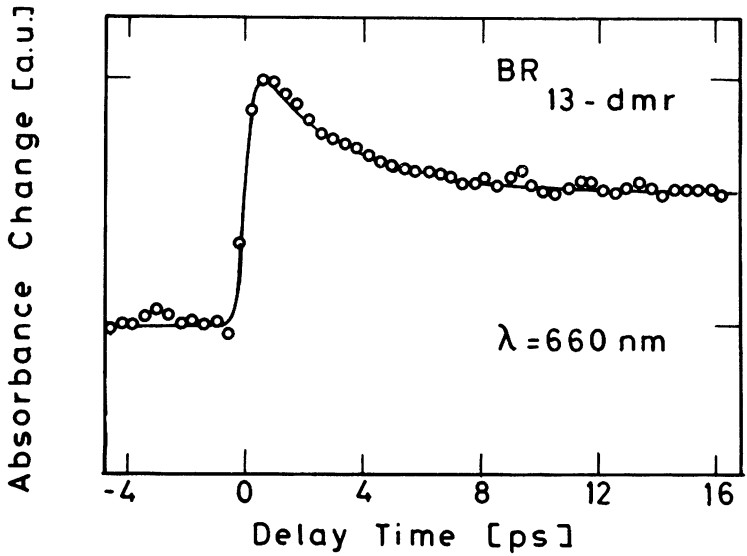

Fig.2 Time-resolved absorption measurement showing the existence of the "J" to "K" transition in the cis cycle.

We give a brief summary on other observations. The absorption spectra of the initial ground state are only slightly different in BR_{1a} and $BR_{13\text{-}dmr}$. The fluorescence spectra are similar. The product states K exhibit similar absorption spectra. Considerable differences are found for the absorption of the excited electronic state, which is much stronger for the 13-cis retinal than for the all-trans retinal samples in the near IR at 735 nm, while at 490 nm, in the blue, the 13-cis retinal containing BR samples absorb less.

A comparison of the experimental data of the primary processes of the trans and cis cycles of BR indicates:
i) the excited states of the two cycles have different absorption spectra and different decay kinetics,
ii) the final picosecond products show similar absorption properties,
iii) there is in both cycles a 3 ps ground-state kinetic relating two red-shifted products (J to K transition).

These findings are explained within the frame of a recently published model of the trans-cis cycles /10,12/. In Fig.3a, the retinal lysine, part of BR, is shown schematically. Also shown are four residues A_1 to A_4 (presumably from aspartic acids /13,14/), which are within reach of the NH group of the Schiff's base during the isomerisation motions of the retinal molecules (circles shown in Fig.3b-e) . It was suggested in recent publications /10,12/ that during the primary processes in the cis and trans cycle the NH group is brought into contact with the residue A_3H, see Fig.3b and c (starting from residue A_1- and A_2- in the trans and cis cycle, respectively). This interpretation is strongly supported by our experimental data (point

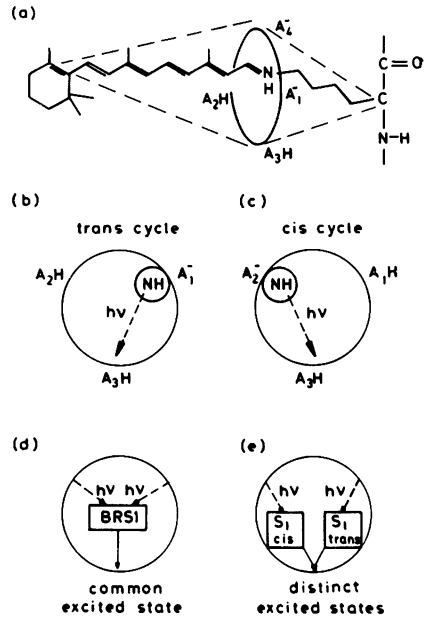

Fig.3 Model for the primary reactions in the cis and trans photocycle of bacteriorhodopsin. (a) Schematic of the retinal and residues A_1 to A_4 in the neighborhood of the Schiff's base. (b) and (c) Formation of the final picosecond photoproduct during the trans and the cis cycle, respectively, by the transfer of the NH-group during the isomerisation motion. (d) and (e) Model for the transfer pathways. Only the transfer via two distinct excited states (e) is in agreement with the experimental data.

2 and 3). Additional information on the transfer path of the primary reaction originates from our results on the properties of the excited electronic states. The different absorption properties together with the difference in the decay kinetics indicate that the transfer to the ground-state product does not go via one excited state BRS_1, common for the cis and trans cycle (as would be suggested by model of Fig.3d). It rather shows that two distinct excited states, S_1-cis and S_1-trans are involved in the course of the two primary photoreactions (model shown in Fig.3e). While the reaction path ways are different in the cis and trans photocycle, the primary reactions in both cases are rapid photoisomerisations of the retinal molecule.

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1. J.K. Lanyi, in Bioenergetics, ed. L. Ernster, Elsevier, Amsterdam 1984, p. 315
2. G.S. Harbison, S.O. Smith, J.A. Pardoen, C. Winkel, J.Lugtenburg, J. Herzfeld, R.A. Mathies, R.G. Griffin, Proc. Natl.Acad. Sci. US 81, 1706 (1984)
3. W. Sperling, P. Carl, C.N. Rafferty, N. Dencher, Biophys. Struct. Mech. 3, 79 (1977)
4. M.C. Nuss, W. Zinth, W. Kaiser, E. Kölling, D. Oesterhelt, Chem. Phys. Lett.1, 117 (1985)
5. A.V. Sharkov, A.V. Pakulev, S.V. Chekalin, Y.A. Matveetz, Biochim. Biophys. Acta 808, 94 (1985)
6. H.J. Polland, M.A. Franz, W. Zinth, W. Kaiser, E. Kölling, D. Oesterhelt, Biophys. J. 49, 651 (1986)
7. In Ref.6 we published a time constant of ≈5 ps for the J to K transition. More recent femtosecond experiments indicate a somewhat shorter time constant of 3±1 ps.
8. H.J. Polland, M.A. Franz, W. Zinth, W. Kaiser, E. Kölling, D. Oesterhelt, Biochim. Biophys. Acta 767, 635 (1984)
9. P. Tavan, K. Schulten, D. Oesterhelt, Biophys. J. 47, 415 (1985)
10. J.W. Petrich, J. Breton, J.L. Martin, A. Antonetti, Chem. Phys. Lett. 137, 369 (1987)
11. W. Gärtner, P. Towner, H. Hopf, D. Oesterhelt, Biochem. 22, 2637 (1983)
12. H.W. Trissl, W. Gärtner, Biochem. 26, 751 (1987)
13. M. Engelhard, K. Gerwert, B. Hess, W. Kreutz, F. Siebert, Biochem. 24, 400 (1985)
14. K.Gerwert and F.Siebert The EMBO Journal 5, 805 (1986)