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Contents

Femtosecond Spectroscopy of the Primary Events of Bacterial Photosynthesis

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Recent investigations of bacterial photosynthesis have shown that the primary steps occur on the time scale of one picosecond /1-3/. These processes can only be studied by means of ultrafast optical techniques. The standard experimental methods yield absorption changes induced by a short excitation pulse measured as a function of time and wavelength. Experiments are now possible with the temporal resolution of better than 100 fs. They present interesting information on the early dynamics of the primary processes. Optical techniques alone provide insufficient understanding of the molecular microscopic processes of the primary steps. It is important to add information from other experiments, e.g. from structure analysis, resonance Raman scattering, molecular dynamics calculations or experiments on model compounds.

In this note we are concerned with time-resolved experiments of the primary steps of photosynthetic units such as bacteriorhodopsin and bacterial reaction centers. We combine the results of ultrafast spectroscopy with other available data in order to gain a better insight into the molecular processes of the primary events.

Experimental

The experiments are performed using amplified pulses (t_p=100 fs, repetition rate 7.5 kHz) from a colliding pulse mode-locked laser operating at 620 nm for the excitation of the samples. Probing pulses are obtained by continuum generation in a jet of ethylene glycol. A narrow fraction of the continuum ($\Delta\lambda \simeq$ 10-15 nm) was selected. The probe pulses monitor the absorbance changes of the sample as a function of time delay between exciting and probing pulses. In order to avoid high exposure the sample was kept in a spinning cuvette. In this way, it was ascertained that each photosynthetic unit absorbs one photon only every second. There was no indication of any photodecomposition of the samples at the excitation densities used in the experiments (less than 10% of the molecules absorb one photon per excitation pulse).

Bacteriorhodopsin

Bacteriorhodopsin (BR) is a membrane protein contained in the cell membrane of Halobacterium halobium. It acts as a light-driven proton pump building up a proton gradient across the cell membrane upon illumination.

The absorption properties of BR are determined by the only pigment molecule retinal. 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. During the photochemical cycle the absorption properties of BR change substantially. Intermediate states named J,K,L,M,... have been

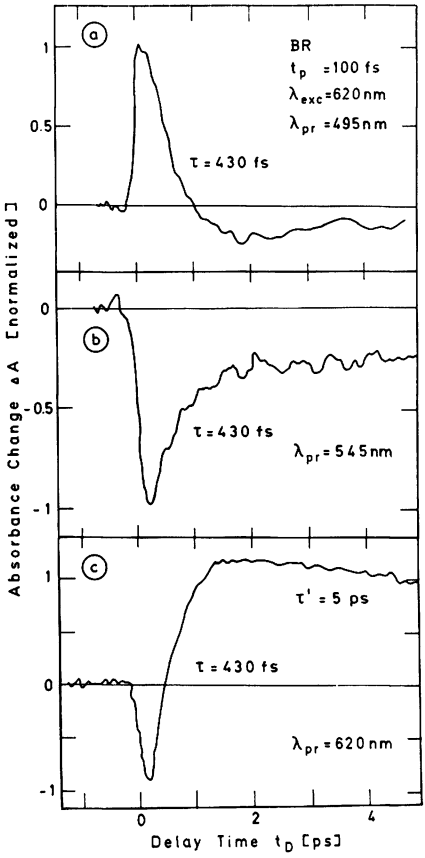

Fig.1 Bacteriorhodopsin: Absorption changes induced by 100 fs excitation pulses at 620 nm

identified. During the course of the cycle the retinal adopts the 13-cis configuration and the Schiff base loses its proton. The photochemical cycle is completed after ~ 10 ms. 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. It was not until recently, that a coherent picture of the primary molecular process was presented /1,4,5/.

Fig.1 shows absorption changes measured as a function of time delay after excitation of the BR molecules at 620 nm. When the molecules are probed at 490 nm, a strong induced absorption is found which builds up with the time resolution of the experiment of 100 fs. In a first relaxation process this absorption change decays with a time constant of 430 fs into a state of reduced absorption from which a slow 5 ps kinetic leads to a partial recovery of the absorption, which stays constant for times longer than 12 ps (not shown in Fig.1). The same time constants (< 100 fs, 430 fs, and 5 ps) are found at the other probing wavelengths of 545 nm and 620 nm in Fig.1b and c, respectively.

The results presented above together with additional information from femtosecond experiments on deuterated BR /1/, from picosecond data on BR containing a sterically fixed retinal molecule /6/, and from resonance Raman

scattering /7/ yield the following microscopic picture of the primary molecular processes: Light promotes BR to a Franck-Condon state on the excited-state potential surface. From there a very fast (τ < 100 fs) molecular motion leads to the bottom of the S_1 potential surface. Internal conversion with τ' = 430 fs leads to the ground state intermediate J which contains the isomerized 13-cis retinal. Rearrangements of the protein surrounding causes the slower 5 ps kinetics leading to the intermediate K which is stable for the longer time of 300 ps.

Reaction Centers

Photosynthesis in green plants and in most bacterial systems uses chlorophyll or bacteriochlorophyll molecules arranged in so-called reaction centers (RC) where a primary charge separation is initiated. Reaction centers can be isolated from some bacteria. The reaction centers of Rhodopseudomonas viridis or Rhodopseudomonas spheroides contain six pigments absorbing in the visible and near infrared: 4 bacteriochlorophyll (BCl) and 2 bacteriopheophytin (BPh) of type b and a for R-viridis and R-spheroides, respectively. Two of the BCl form the excitonically coupled special pair, from which the charge separation originates. A major progress in the understanding of the molecular processes in the RC was recently achieved when the reaction centers of R-viridis were crystallized /8/ and when a structural analysis of these RC was completed /9/. The arrangement of pigments obtained by structure analysis allowed claculation of the excitonic coupling: it was shown that there is strong excitonic mixing between the various pigments of the RC, substantially influencing the absorption in the near infrared spectral region (λ > 750 nm) /10/.

It is the purpose of this chapter to compare the dynamic absorption changes in R-viridis - where the pigment arrangement is known - with those of R-spheroides. In addition, the data give information on excitation transfer between the pigments in the RC. Because of the strong excitonic interaction which influences the IR absorption bands we concentrate here on the visible absorption where the Q_x transitions are located. BCl absorbs around 600 nm, BPh around 540 nm and - in the RC of R-viridis - the special pair (P) exhibits a shoulder in the absorption around 620 nm.

Time-resolved absorption measurements are shown in Fig.2 for R-viridis (a,b) and R-spheroides (c,d) after excitation at 620 nm. First we probed around 545 nm at a frequency where the BPh absorbs. The data show at later delay times, i.e. for t_D > 200 fs a decay of the absorption with a time constant of 2.8 ps for both types of RC. The absorption decreases at 545 nm, where initially the BPh's absorb strongly suggesting that BPh is reduced to form BPh^-. Additional experiments at λ = 675 nm, a wavelength where BPh^- absorbs, support this interpretation. Figs. 2b and d show the rise of the absorption change on an expanded scale. In both cases the rise follows closely the integrated cross-correlation curve between exciting and probing pulses. We may deduce from these data that the absorption appears within our time resolution of 100 fs for the RC of R-viridis and R-spheroides. The molecular nature of this early state requires some consideration. It may simply be the excited electronic state P* of the special pair or it is a state P^{+-} which contains considerable contribution from a charge transfer state /2/. Both interpretations are possible within the scope of present knowledge.

The experimental findings of the two time constants of < 100 fs and 2.8 ps allow us to draw the following conclusions: After absorption of a photon at 620 nm the excitation is rapidly transferred, τ < 100 fs, to

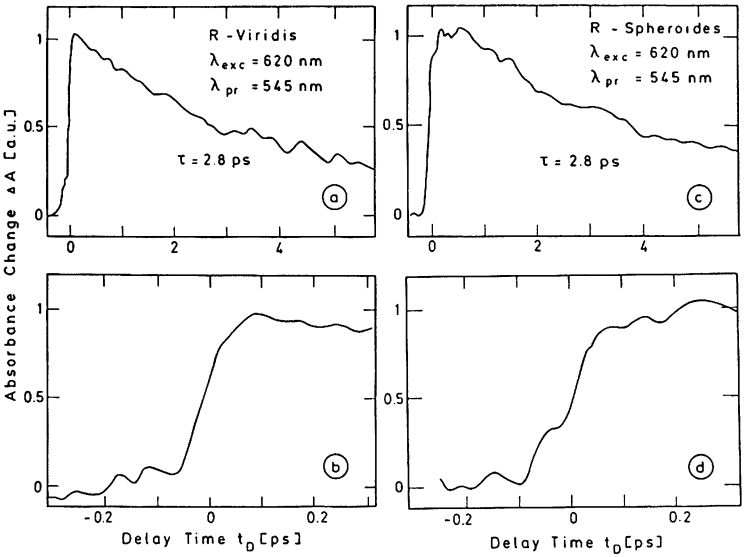

Fig.2 Reactions centers: Transient absorption changes measured at the probing wavelength 545 nm, where initially the BPh absorb. The same time dependence is found for the formation of BPh^- in the two reaction centers.

the special pair to form the primary state P* or P^{+-}. Subsequently, reduction of BPh to BPh^- occurs within 2.8 ps. Since both systems, the RC of R-viridis and of R-spheroides, behave in the same manner, we believe that their pigment arrangements and the interactions between the pigments are very similar.

Conclusions

The present investigations of the bacterial photosynthesis of two very different photosynthetic systems - bacteriorhodopsin and bacterial reaction centers -suggest very high reaction rates of the primary events. This common property can be well understood with the help of the reaction scheme shown in Fig.3. After optical excitation, fast molecular rearrangements occur on the excited-state potential surface. From there two pathways are possible: internal conversion to the initial ground

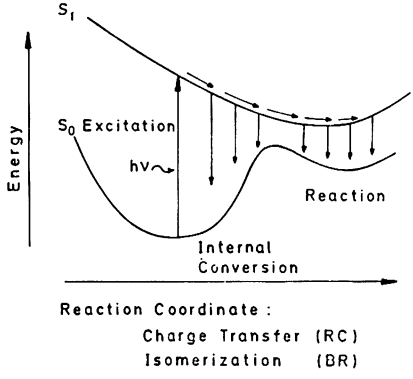

Fig.3 Schematic of the energy surfaces indicating the primary reactions in the investigated photosynthetic systems

state and the desired photochemical reaction. The internal conversion rates for most large pigment systems are very rapid. For this reason, the reactive channel must be faster in order to maintain reasonable quantum efficiencies in the first photosynthetic steps.

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1 M.C. Nuss, W. Zinth, W. Kaiser, E. Kölling, D. Oesterhelt, Chem. Phys. Lett. 117 (1985) 1
2 J.-L. Martin, J. Breton, A. J. Hoff, A. Migus, A. Antonetti, Proc. Nat. Acad. Sci. USA 83 (1986) 957
3 W. Zinth, M.C. Nuss, M.A. Franz, W. Kaiser, H. Michel, in "Antennas and Reaction Centers of Photosynthetic Bacteria", ed. M.E. Michel-Beyerle, Springer, Berlin 1985, p.286
4 H.J. Polland, M.A. Franz, W. Zinth, W. Kaiser, E. Kölling, D. Oesterhelt, Biophys. J. 49 (1986) 651; H.J. Polland, W. Zinth, W. Kaiser, in "Ultrafast Phenomena IV", ed. D.H. Auston, K.B. Eisenthal, Springer Series in Chem. Phys., Springer, Heidelberg 1984, p. 456
5 A.V. Sharkov, A.V. Pakulev, S.V. Chekalin, Y.A. Matveetz, Biochim. Biophys. Acta 808 (1985) 94
6 H.J. Polland, M.A. Franz, W. Zinth, W. Kaiser, E. Kölling, D. Oesterhelt, Biochim. Biophys. Acta 767 (1984) 635
7 M. Braiman, R. Mathies, Proc. Nat. Acad. Sci. USA 79 (1982) 403
8 H. Michel, J. Mol. Biol. 158 (1982) 567
9 J. Deisenhofer, O. Epp, K. Miki, R. Huber, H. Michel, J. Mol. Biol. 180 (1984) 385
10 W. Zinth, E.W. Knapp, S.F. Fischer, W. Kaiser, J. Deisenhofer, H. Michel, Chem. Phys. Lett. 119 (1985) 1