Protein movement within the bacteriorhodopsin photocycle detected by beam deflection spectroscopy

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Protein movement within the bacteriorhodopsin photocycle detected by beam deflection spectroscopy

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Abstract: Photothermal beam deflection of the bacteriorhodopsin photocycle in the time range from 1 to 200 μs resolved a new process with a time constant of ca. 20 μs at 20 °C in addition to the decays of the K and L intermediates with time constants of 1.3 and 90 μs. The latter time constants are identical to those measured by flash photolysis with optical detection on the same samples. The additional process may be attributed to a protein conformational change concomitant with the proton transfer (I).

1. INTRODUCTION

Photothermal methods have been shown as useful in order to investigate conformational changes of photoreactive compounds [1]. As an extension of our studies with laser-induced optoacoustic spectroscopy (LIOAS) [2], we now apply photothermal beam deflection (PBD) to the bacteriorhodopsin system, since decays on a longer time scale than those reached by LIOAS can be studied by following the refractive index change in solution [3]. This makes it possible to obtain information about enthalpy and volume changes taking place during the steps involved in the proton transfer.

2. RESULTS AND DISCUSSION

Laser-induced photothermal beam deflection (PBD) with pulsed (8 ns) excitation and time-resolved detection was used to study bacteriorhodopsin (BR) photocycle kinetics in water. The perturbation of the refractive index caused in the medium and induced within about 100 ns was monitored by the deflection of a not-absorbed, CW probe beam (parallel to the pump beam), from a laser diode at 825 nm. For time intervals up to 200 μs after the pulse, heat conduction can be neglected and, therefore, photocycle kinetics in this range can be evaluated from the signal without deconvolution.

Nanosecond flash photolysis with optical detection of bacteriorhodopsin (BR) was carried out in order to identify spectrally discerned intermediates which result from changes of the conformation and protonation state of the retinal chromophore and closely adjacent amino acids. Measurements at different absorption maxima resolved the decay kinetics of the K and L intermediates with lifetimes of ca. 1.4 and 80 μs at 20 °C, respectively. Each decay was sufficiently well fitted with a monoexponential decay function. The equipment and data handling used were as previously described [4].

(I) The results presented in this contribution are part of the paper by the same authors due to appear in Biochim. Biophys. Acta (1994).
PBD investigation of the same solutions in the time range from ca. 1 \( \mu s \) to 200 \( \mu s \) revealed a new process with a time constant of ca. 20 \( \mu s \) in addition to the K and L decay kinetics which are the same as determined by flash photolysis.

PBD measurements in \( \text{D}_2\text{O} \) showed a much slower L decay than that in \( \text{H}_2\text{O} \). This is in accordance with the fact that the proton transfer takes place during L decay.

In case transients are photoproduced in a solution, there are three possible contributions to the amplitude of the decay components in PBD, i.e., radiationless deactivation of the transients, molecular movements, and the presence of a population lens [5].

Temperature dependent measurements from 0 to 35 °C have been carried out with both detection methods (optical and PBD) to determine the activation energies and preexponential factors of the different processes. PBD measurements at ca. 0 °C, where heat release does not contribute to the signal [6], revealed a significant contribution of molecular volume changes to the beam deflection signal in the case of the shortest and longest components. These volume changes are either due to conformational changes and/or protein solvent interactions.

Although we preliminary ascribe the 20 \( \mu s \) process to a protein conformational change between the L and the following M state, it remains to be measured how much of this component, as well as of the other two, is due to the population lens.

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References