BRIEF COMMUNICATION

KINETICS OF THE 580-NM ULTRAFAST BACTERIORHODOPSIN TRANSIENT

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ABSTRACT We have observed, by low-temperature picosecond spectroscopy, a photo-induced transient at 580 nm in light-adapted bacteriorhodopsin. The transient was characterized by bleaching in the 550–585-nm regions within 6 ps and recovery in approximately 20 ps. The spectral intensity of the transient is found to be enhanced at lower temperatures, and the lifetime slightly elongated.

At low oxygen tension or in the absence of a suitable source of metabolic carbon, *Halobacterium halobium* will synthesize a purple pigment, bacteriorhodopsin (bR_{570}), whose light-adapted form has a λ_{max} at 568 nm, believed to consist of all *trans* retinal (1). One difference between bacteriorhodopsin and rhodopsin is that the absorption of a photon initiates a cyclic reaction rather than the visual transduction process. Extremely weak fluorescence has been observed from bacteriorhodopsin at room temperature which is greatly enhanced at liquid nitrogen temperatures (2). The fluorescence is red-shifted from the absorption maximum, with reported lifetimes of 15 and 40 ps at 248 and 77°K (3,4). The first photoinduced intermediate, *K*, has an absorption spectrum further to the red, and is stable at low temperatures. At physiological temperature *K* is formed in less than 15 ps, with a lifetime of about 4 μs (5) A short-lived transient ($\sim$15 ps) has also been observed at room temperature. To gain additional information about the initial photoinduced reaction and the nature of this initial spectroscopic intermediate, we have measured the kinetics at low temperatures.

METHODS

Bacteriorhodopsin was prepared by the technique of Oesterhelt and Stoeckenius (6). It was suspended in a glycerol-water solution (60:40) with an optical density of 7/cm. It was then placed in a specially constructed copper block cell with 1.5 · mm optical path length attached to an Air Products helitrans Dewar flask (Air Products & Chemicals, Inc., Allentown, Pa.).

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The sample was light adapted by exposure to orange light ($\lambda < 560$ nm) for 30 min at room temperature. The sample was then quickly frozen in the dark to liquid nitrogen temperatures to assure that only the light-adapted form was present.

Picosecond absorption studies were carried out with a double beam spectrometer described previously (7). Optical density changes were observed at 6 ps, 1 ns, and 2 min after excitation. After each experiment, the photoproduct, *K*, was converted back to the starting pigment with a 3 mW He/Ne laser, and the absorbance checked for greater than 90% conversion. After about 20 laser shots, significant bleaching of the sample throughout its absorption spectrum could be observed, and the experiments were continued after a fresh solution was placed in the cell.

RESULTS AND DISCUSSION

Time-resolved optical density changes in the absorption spectrum were studied between 550 and 630 nm at 68°K. Two predominant changes were recorded: an increase in absorbance appeared within 6 ps at 620 and 630 nm, which remained unchanged 1 ns after excitation. Even 2 min after excitation, this absorbance was identical to that observed immediately after excitation; also between 550 and 585 nm, a large transient bleaching was seen within 6 ps, which recovered in about 20 ps. Data taken 70 ps, 1 ns, and 2 min after excitation show that the ultrafast bleaching observed had completely disappeared within 20 ps. Typical asymptotic optical density changes were 0.35 at 630 nm and 0.17 at 550 nm.

A possible assignment for the observed changes is that the 550–585 nm bleaching portrays the depopulation of the ground electronic state and its subsequent repopulation to 70% of the original value within 20 ps. This view is in agreement with previously reported data where the quantum yield for conversion of bR_{570} to *K* is ~0.3, leaving 70% for eventual return to the ground electronic state. The observed 20 ps for the decay of bleaching time reflects the decay lifetime of an upper singlet state populated with the 530-nm pulse.

The dynamics of bacteriorhodopsin at 68°K appear to be identical to those at room temperature (8). The rate of formation of the positive optical density changes that can be attributed to the formation of *K* appears to be greater than $\sim 10^{11}$ s^{-1}. This transient is far more prominent at the lower temperatures than at room temperature, being about a factor or two or three more intense. Due to its higher intensity at 68°K it was possible to observe the absorption increase not only at the isosbestic but also at wavelengths between 550 and 585 nm. The lifetime is also somewhat longer, i.e. 20 ps rather than 15 ps reported previously (Fig. 1). Although the time elongation is evident, we do not attach further meaning to it, because such a small time increment could easily be due to the larger transient changes seen at 68°K which would account for a more accurate determination of the decay time. Another effect that is certain upon cooling of the sample is an increase in the transient bleaching. Assuming that the fluorescence quantum yield (9) and fluoresence lifetime data (3,4) are correct, the transient cannot be identified with the fluorescence species, because the fluorescence undergoes a factor of 15 change in quantum yield and at least a factor of 3 change in

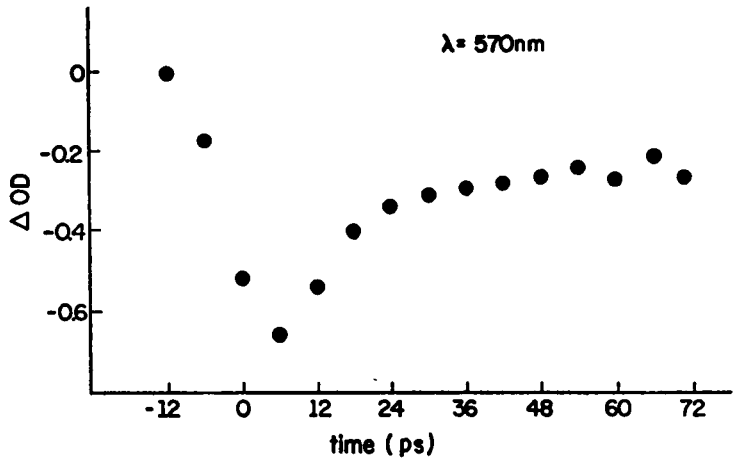

FIGURE 1 Histogram of the 570-nm transient observed at 68°K ($\tau \sim 20$ ps).

fluorescence lifetime, whereas the transient lifetime appears nearly unaffected. The fluorescing species must not be an intermediate in the photochemistry (10) but rather supports our excited state proposal, because even at low temperature 68°K bK_{690} is formed within 20 ps. Changes in the membrane, however, which result in an increase in fluorescence intensity might also be responsible for the increased size of the transient bleaching. The transient may be a precursor to bK_{590}, which is nearly identical to the red-shifted product, or a nonfluorescing excited state of the native pigment. Slight differences in the protein conformation which relax in 15–20 ps to the stable form might account for the observed spectrum. However, one would expect such a conformation change to be quite sensitive to temperature which is contrary to the data. Cooling to even lower temperatures might help clarify the assignment of the transient.

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