Laser Chem., 1999, Vol. 19, pp. 173–178
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FEMTOSECOND TIME RESOLVED INFRARED SPECTROSCOPY OF THE ETHYLENIC STRETCH VIBRATION DURING THE ALL-*TRANS* TO 13-*CIS* ISOMERIZATION OF BACTERIORHODOPSIN

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(Received 20 April 1997)

We report on room temperature infrared transient absorption experiments on Bacteriorhodopsin concerning the early picosecond dynamics of the all-*trans* to 13-*cis* isomerization of the retinal chromophore. The absorption band of the all-*trans* chromophore ethylenic stretch vibration bleaches concomitantly to photoexcitation whereas new absorption strength is created redshifted within less than 1 ps. the initial bleach recovers partly according to the non unity quantum yield of the photoisomerization within 2 ps. With a time constant of 3 ps the low energy absorption band is downshifted 5–10 cm^{-1}. Thus, vibrational relaxation and cooling processes appear to have ended within a few ps and on the time scale of 20 ps a stationary BR-K difference spectrum is obtained.

Keywords: Isomerization; transient infrared spectroscopy; bacteriorhodopsin; vibrational relaxation

1. INTRODUCTION

The dynamics of light induced photoreactions in proteins are object of intense research. We focus here on the trans-membrane protein Bacteriorhodopsin (BR) which serves as a light driven proton pump in

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Halobacteria. The pump mechanism is initiated by the all-*trans* to 13-*cis* isomerization of the covalently bound retinal chromophore. In an earlier study [1] the vibrational dynamics during the first picoseconds after photoexcitation was investigated by means of subpicosecond time resolved infrared (IR) absorption spectroscopy in the spectral region of the chromophoric C=NH stretch vibration. We have now extended this work into the region of the retinal ethylenic (C=C) stretch vibration at around 1530 cm^{-1}.

2. METHODS

The pump/probe setup is based on a 1 kHz CPM Titanium:Sapphire laser system. The pump pulses at 540 nm were extracted from a white light continuum, generated by the amplified pulses of about 180 fs FWHM at 790 nm. The infrared probe was taken from a continuous wave, liquid nitrogen cooled carbon monoxide (CO) laser, which can be tuned down to about 1450 cm^{-1}. Time resolution was introduced by optically gating the IR probe behind the sample with a weak pulse at 790 nm in a 0.5 mm $AgGaS_2$-crystal. The sample was a hydrated film of light adapted BR_{570} (the chromophore being in all-*trans* configuration) between two CaF_2 windows. The experiments were performed at room temperature.

3. RESULTS AND DISCUSSION

In Figure 1 the results are shown as a series of IR absorption transients in the region between 1536 and 1498 cm^{-1}. Displayed is the IR absorption difference signal obtained after photoexcitation of BR_{570} on top of the strong background signal due to the protein amide II absorption. The strong bleach at high wavenumbers cannot be time resolved. The partial recovery of the bleach occurs within about 2 ps although a faster component, *e.g.*, at 1526 and 1519 cm^{-1}, respectively, contributes. The recovery turns positive at 1519 cm^{-1}. At lower wavenumbers new absorption strength is created by the pump pulse. It rises with a time constant of about 0.7 ps and decays partially with a time constant of not much more than 3 ps. Within the experimental

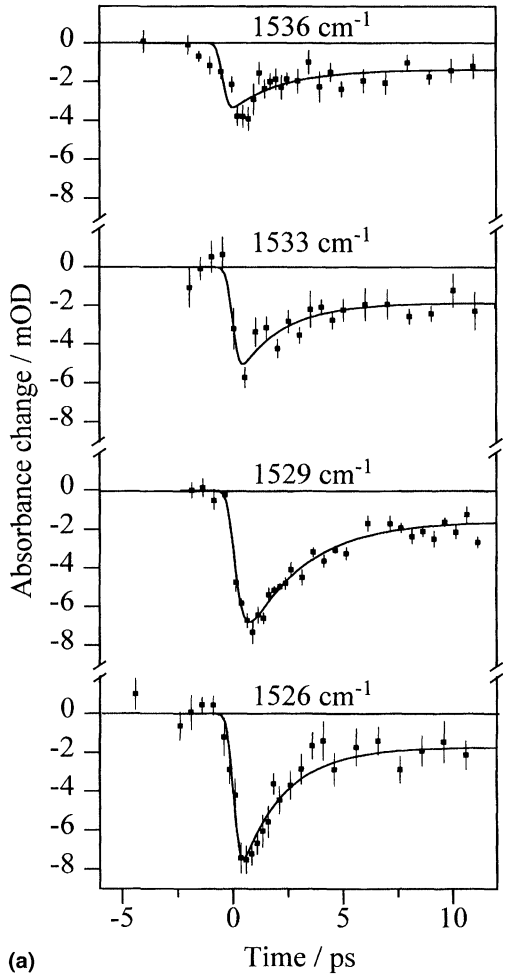

FIGURE 1a–c IR transients after photoexcitation of BR_{570} at 540 nm. Data were taken at room temperature.

error of the data, the amplitudes reached after a few picoseconds remain constant in all transients on a time scale up to 20 ps (data not shown).

According to the currently accepted picture of electronic dynamics of the BR photoisomerization obtained from optical transient absorption experiments [2, 3], the electronic ground state is reached

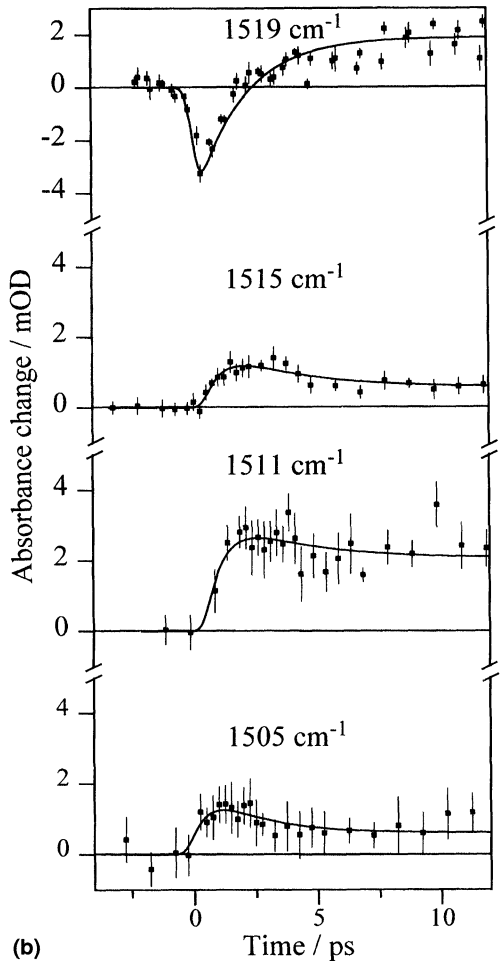

FIGURE 1 (Continued).

within 0.5 – 0.7 ps as an optically red shifted intermediate J. After about 3 ps the state K is formed, slightly blue shifted with respect to J and stationary on the time scale of tens of picoseconds. It is still an open question as to what degree the formation of the K state is determined by vibrational cooling or conformational relaxation after the fast isomerization in the sterically fairly rigid protein pocket [4, 5]. The results are interpreted as follows: Under photoexcitation the

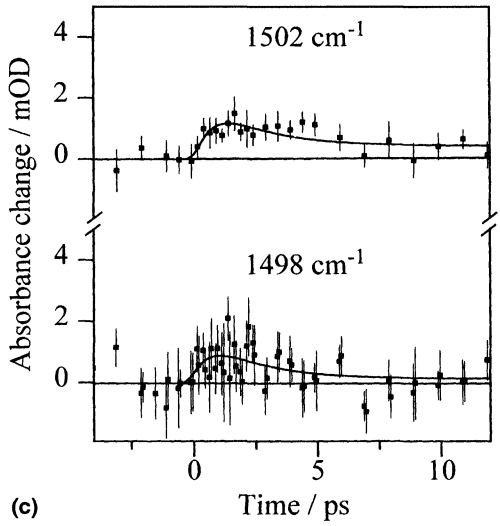

FIGURE 1 (Continued).

C=C stretch vibration is shifted to much lower wavenumbers [6] on the time scale of the pump pulse. Thereby the bleach occurs instantaneously. According to the BR photoisomerization quantum yield of 0.6 [7], a fraction of 0.4 of the excited molecules takes the reaction path back to the BR_{570} ground state leading to the partial recovery at around $1529\,cm^{-1}$. The slight downshift of the positive band at about $1505\,cm^{-1}$ within 3 ps (cp. Fig. 2) is identified with the J to K transition, although the recovery of BR_{570} as well must contribute to the optical transients. The shifting band is the signature of a vibrationally hot band but chromophoric conformational relaxation as a cause for the shift cannot be excluded without a more detailed analysis. The difference spectrum after 12 ps compares well with BR–K difference spectra obtained by low temperature FTIR spectroscopy (*e.g.* [8]). As can be seen from Figure 2 the amount of recovery of the bleach is not in accordance with the fraction 0.4, expected from the quantum yield of 0.6. Taking also into account the slight blue shift of the negative band with time and the faster component of the bleach recovery the strong bleach might be due to a stimulated emission signal in the IR. Its time course could yield information about the transition from S_1 to S_0 and the time the excited chromophore needs to become thermalized.

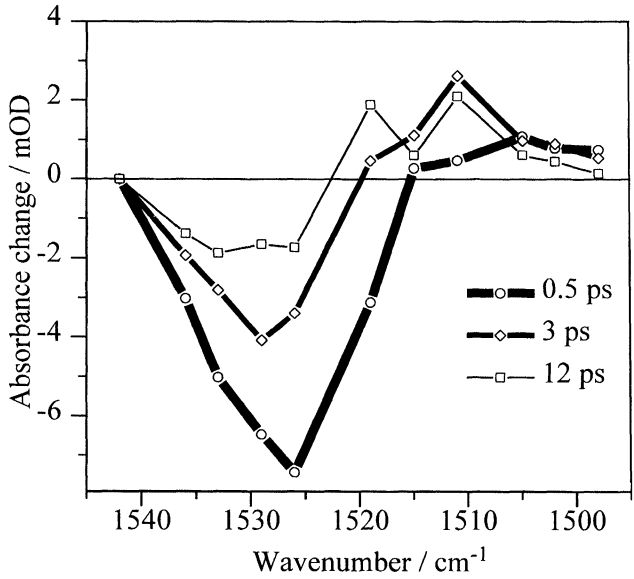

FIGURE 2 IR difference spectra, derived from the transients shown in Figure 1 at delay times of 0.5, 3 and 12 ps, respectively. The amplitudes of the data points at 1542 cm^{-1} are zero within the experimental error (transient data not shown).

Acknowledgement

We thank Prof. Dr. D. Stehlik for encouraging support and Prof. Dr. M. Heyn for providing the BR sample. This research was supported by the Deutsche Forschungsgemeinschaft.

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