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The *trans–cis* Isomerization Reaction Dynamics in Sensory Rhodopsin II by Femtosecond Time-Resolved Midinfrared Spectroscopy: Chromophore and Protein Dynamics

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Abstract: *Transient infrared (IR) vibrational spectroscopy at subpicosecond time resolution on sensory rhodopsin II from* Natronomonas pharaonis, *NpSRII, has been performed for the first time. The experiments yield three time constants for the description of the primary photoinduced reaction dynamics, i.e. 0.5, 3.7–4.4, and 11 ps. The data are consistent with a sequential reaction scheme, with the isomerization taking place within 0.5 ps, succeeded by an electronic ground state relaxation. The 11 ps component, observed at 1550 and 1530 cm^{-1}, is attributed to dynamics of protein vibrational bands, possibly amide II bands of the protein backbone, perturbed by the ultrafast retinal photoisomerization. Similar observations, yet not as strongly expressed, have been made earlier in bacteriorhodopsin and halorhodopsin.* © 2006 Wiley Periodicals, Inc. Biopolymers 82: 358–362, 2006

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INTRODUCTION

The archaebacterial retinal proteins bacteriorhodopsin (BR), halorhodopsin (HR), and sensory rhodopsin II (SRII) undergo an all-*trans* to 13-*cis* photoisomerization of the covalently bound retinal chromophore as the primary, photoinduced reaction. This elementary photoreaction drives various important biological processes such as proton- (BR) and chloride-pumping (HR) as well as photoreception (SR). Ultrafast infrared vibrational spectroscopy is well suited to provide molecular insights into processes such as structural changes of the chromophore, its vibrational relaxation, and the dynamics of the protein environment that are associated with the photoreaction. This knowledge is essential for a consistent picture of the primary reaction dynamics. In earlier publications we presented results on the ultrafast vibrational dynamics in BR[1] and HR.[2] It was shown that the isomerization as a structural change itself is clearly monitored by vibrational fingerprint bands. Thus, based on structural dynamics, it was demonstrated that the isomerization takes place within 0.5 ps in BR[1] and 2 ps in HR.[2]

In the present article, this series of experiments is extended for the first time to the photoreceptor sensory rhodopsin II (NpSRII) of *Natronomonas pharaonis*. The early states in the photocycle of NpSRII have been addressed previously by other methods. From femtosecond time-resolved absorption[3] and fluorescence[4] experiments, it was concluded that the excited electronic state decay leads to the isomerized product within 0.3–0.4 ps,[3] respectively 0.08–0.25 ps.[4] Further relaxation within 4–5 ps leads to the batho-product KNpSRII (λ_{max} at 510 nm),[3] which has a quantum yield of 0.5 (as determined for the later M-state)[5] and a lifetime of 1 μs.[6] Infrared vibrational difference spectra of KNpSRII have already been obtained by low-temperature and nanosecond time-resolved Fourier transform infrared (FTIR) studies.[7] The first femtosecond time-resolved IR experiments in limited spectral regions as presented here show directly by means of vibrational marker bands how the 13-*cis* configuration of the chromophore is formed within less than 500 fs, accompanied by slower relaxation steps of the chromopore and parts of the protein matrix.

MATERIALS AND METHODS

Material and Sample Preparation

NpSRII was expressed in *Escherichia coli* and purified as described.[8,9] The solubilized photoreceptor without the transducer was reconstituted into purple membrane lipids in accordance with standard procedures.[10] Starting with a suspension (buffer: 1 m*M* Tris, 15 m*M* KCl at pH 7.0), NpSRII films were prepared on CaF_2 windows of 1.5-in. diameter and sealed by a second, similar window. The films were hydrated sufficiently to ensure protein function but low enough to avoid unnecessary IR absorption losses by water. During the experiments, the sample was rotated and moved laterally to the focused exciting and probing laser beams in order to provide fresh sample conditions at each laser shot. On average, a sampled volume was reexcited (and probed) again only after about 2 s. To check for sample functionality and degradation, photocycle kinetics and steady state absorption spectra were measured before and after each experiment. All measurements were performed at room temperature.

Transient Absorption Spectroscopy

The visible-pump/infrared-probe (VIS/IR) spectrometer has been described elsewhere.[2] In brief: Short excitation [ca. 190 fs full width at half maximum (FWHM)] at λ_{pu} = 505 nm and probe pulses (ca. 200 fs FWHM) between 1700 and 1000 cm^{-1} were generated by means of nonlinear optical devices, driven by laser pulses (160 fs FWHM, 775 nm, 0.6 kHz repetition rate) of a commercial titanium-sapphire laser amplifier system (CPA2001, Clark-MXR, Inc., Dexter, Michigan, USA) and an instrumental response function of typically 280 fs FWHM. Pump-induced absorbance differences $\Delta A(t,\lambda_{pr})$ were measured as a function of the delay time t between pump and probe pulse at specific probe wavelengths λ_{pr}. The quantitative evaluation was restricted to delay times $t > 150$ fs because the signals at earlier delay times are partly obscured by contributions of the free induction decay as induced by the IR pulse and perturbed by the excitation pulse. In all experiments $\Delta A(t,\lambda_{pr})$ was modeled according to

$$\Delta A(t,\lambda_{pr}) = A_0(\lambda_{pr}) + \sum_{1}^{N} A_i(\lambda_{pr}) \cdot e^{-t/\tau_i} \qquad (1)$$

with $A_0(\lambda_{pr})$ the pump-induced difference absorption spectrum after long delay times (80 ps in our experiments) and $A_i(\lambda_{pr})$ the decay (τ_i) associated spectra (DAS). Equation (1) was applied to absorbance transients at each individual probe wavelength as well as in a global analysis (GA) to whole sets of absorbance transients.

RESULTS AND DISCUSSION

Transient absorption experiments on NpSRII were performed in limited spectral regions, i.e. in the "fingerprint" region between 1180 and 1206 cm^{-1} with vibrational marker bands (C—C-stretching vibrations coupled to C—H-bending modes) that indicate chromophore configuration (13-*cis* vs. all-*trans*), and in the region between 1530 and 1572 cm^{-1} comprising ethylenic stretch vibrations (in-phase C=C-stretching vibrations) of the chromophore as well as major parts

of amide II absorption. The chromophore vibrational bands in BR, HR, and NpSRII show a high resemblance.[7,11] Thus, band assignments are made in analogy to the corresponding analysis in BR.[1]

Fingerprint Region: Chromophore Isomerization

Figure 1a shows IR difference spectra at various selected delay times. At around 1203 cm^{-1} an initial negative absorbance difference (bleach, diminished absorbance due to decreased NpSRII ground state population) decays partially within 40 ps, whereas at around 1190 cm^{-1} a positive absorbance change (excitation-induced formation of new vibrational bands) rises quickly within less than 4 ps. Representative kinetic traces are shown in Figure 1c together with simulations as the result of the global kinetic analysis in this spectral region. The global analysis yields time constants of 0.5 ± 0.1 and 4.4 ± 0.2 ps with corresponding DAS as depicted in Figure 1b. They show that the partial recovery of the negative signal at around 1203 cm^{-1} is composed of both the 0.5 and the 4.4 ps component whereas the rise of the positive signal (Figure 1a) occurs exclusively with the fast component (0.5 ps DAS in Figure 1b). Furthermore, the 4.4 ps DAS indicates that the intensity around 1190 cm^{-1} decays partially with the slow time constant. If, in analogy to BR,[12,13] the bands at ca. 1203 and at 1190–1198 cm^{-1} are assigned to the C—C stretch vibrations indicative for chromophore all-*trans* (1203 cm^{-1}), respectively 13-*cis* configuration (1190–1198 cm^{-1}), the observed kinetics clearly show that the *trans–cis* isomerization occurs very rapidly with about 500 fs. Moreover, they are consistent with a sequential reaction scheme, where the isomerization leads to fast 13-*cis* product formation and partial recovery of the all-*trans* configuration (according to the isomerization quantum yield of 0.5) and is then followed by a relaxation of the chromophore on the time scale of 4–5 ps. The latter step is indicated by the shape of the 4.4 ps DAS: The observed absorbance decrease in the product band region (below 1197 cm^{-1}) and concomitant absorbance increase in the educt band region can be caused by torsional relaxation of the chromophore and vibrational cooling, both leading to a wavenumber upshift of absorbance strength.

Note that the observed time constant of 0.5 ps must be considered as an upper limit for the isomerization for two reasons. First, no deconvolution of the experimental kinetic traces with the instrumental response function was performed (see above). Second, relaxation of vibrationally excited states is likely to contribute to the observed kinetics but cannot (within this data set) be separated from the structural dynamics, e.g. by a distinct DAS, leading to longer apparent time constants.

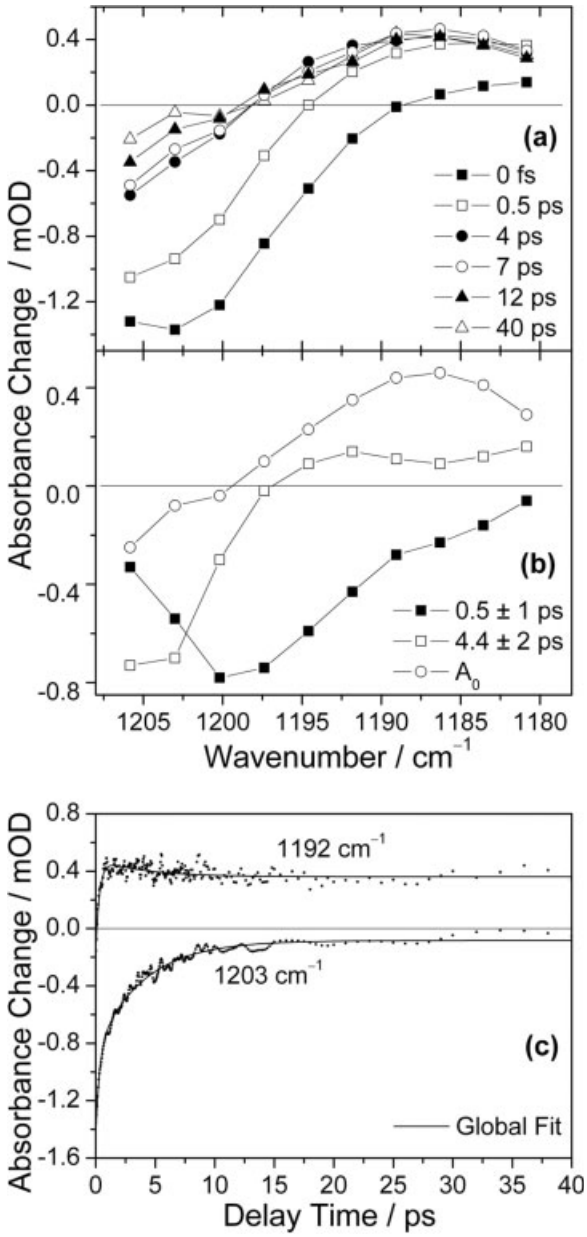

FIGURE 1 Fingerprint region. (a) IR difference spectra after excitation at 505 nm at various delay times. (b) Decay-associated spectra (DAS) with time constants of 0.5 ± 0.1 and 4.4 ± 0.2 ps. A_0: DAS associated with an infinite lifetime. (c) IR absorbance transients at 1203 and 1192 cm^{-1} (for presentation purposes, smoothed over three points) together with a biexponential fit as the result of the global analysis.

Ethylenic Stretch Region

Figure 2a shows IR difference spectra in the region between 1530 and 1572 cm^{-1} at various selected

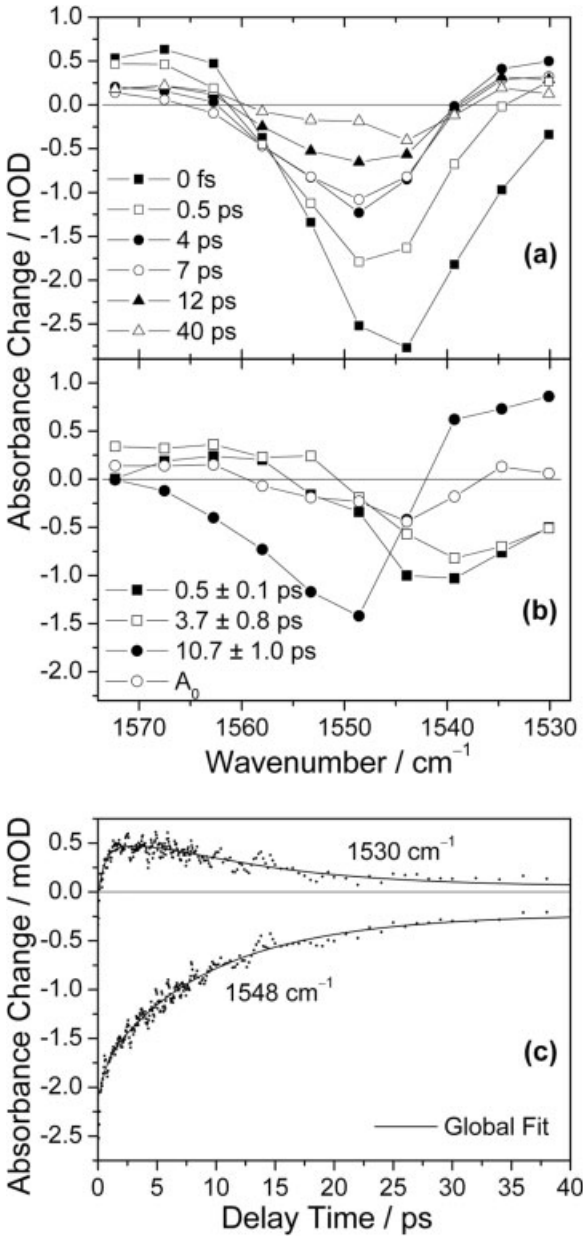

FIGURE 2 Ethylenic stretch region. (a) IR difference spectra after excitation at 505 nm at various delay times. (b) DAS with time constants of 0.5 ± 0.1, 3.7 ± 0.8, and 10.7 ± 1.0 ps. A_0: DAS associated with an infinite lifetime. (c) IR absorbance transients at 1548 and 1530 cm^{-1} (for presentation purposes, smoothed over three points) together with a triexponential fit as the result of the global analysis.

delay times, kinetic traces (Figure 2c), and the result of the global analysis in this region (Figure 2b). Three DAS are obtained with time constants of 0.5 ± 0.1, 3.7 ± 0.8, and 10.7 ± 1.0 ps. The first two time constants compare well with those obtained in the fingerprint region. The corresponding two DAS (Figure 2b) exhibit a common minimum at 1540–1545 cm^{-1}. Although their strong spectral overlap and the limited spectral region hampers a clearer identification of the various molecular processes, the observations made are consistent with those in the fingerprint region. Accordingly, the two DAS describe the kinetics of the ethylenic stretch vibration of the chromophore at ca. 1545 cm^{-1} in the unphotolyzed ground state of NpSRII (1548 cm^{-1}, as determined by resonance Raman experiments[11]). Upon photoisomerization with 0.5 ps, a corresponding product band rises at about the same spectral position. Applying the linear $\nu_{C=C}/\lambda_{max}$ dependency found for retinal proteins,[14–16] this observation is consistent with the formation of an electronic ground state species with an absorption maximum at about the same position as that of NpSRII at $\lambda_{max} = 498$ nm.[3,6] The amplitude of the 3.7 ps DAS around 1540 cm^{-1} and above indicates a subsequent relaxation process of the initially reached electronic ground state, leading to a further cancellation (partial absorbance decrease) of the all-*trans* ethylenic stretch absorbance.

Protein Contributions

Surprisingly, the global analysis of the region between 1530 and 1572 cm^{-1} yields a pronounced additional kinetic component of 10.7 ± 1.0 ps superimposed on the ethylenic stretch dynamics. The corresponding DAS is shown in Figure 2b with peaks at 1550 and 1530 cm^{-1}. Kinetic traces show a fast rise of the negative, respectively positive, signal (with contributions of the ethylenic stretch bands), which then decays partially with 10.7 ps. Based on the following arguments, the 10.7 ps DAS can be related to protein dynamics as induced by the rapid isomerization: (1) The spectral region is characteristic for amide II vibrational bands of the protein backbone. (2) This time constant has not been observed in earlier transient absorption[3] or fluorescence[4] experiments on NpSRII. In contrast to IR spectroscopy, these methods address exclusively electronic states of the chromophore and are only indirectly susceptible to changes of protein vibrational modes. (3) Analogue IR absorbance changes have been observed in nanosecond time-resolved and in low-temperature experiments on NpSRII addressing the K intermediate and assigned likewise.[7] (4) Similar kinetic and spectral features have as well been observed in femtosecond time-resolved IR experiments on BR[2] and HR.[2] However, their kinetics could not be identified in the global analysis but only in the kinetic analysis of the absorbance changes at single wavenumbers (1554 and 1535 cm^{-1}).

The similarity concerning spectral position and observed time constant among the three retinal proteins BR, HR, and NpSRII suggests that the time course of the underlying process is independent of the varying chromophore isomerization dynamics in these systems, but an intrinsic property of at least parts of the protein matrix that respond to the perturbation as exerted by the rapid photoreaction. Note that this process appears much more pronounced in NpSRII compared to BR and HR. This observation could be explained by the biological function of NpSRII as photoreceptor, where conformational changes of the protein are likely to be essential. It seems remarkable, though, that these protein conformational changes appear not only in the nanosecond time range[7] but already on the picosecond time scale.

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