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Resonant Two-Photon Excitation Pathways During Retinal-Isomerization in Bacteriorhodopsin

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Abstract. Resonant two-photon excitation is observed in Bacteriorhodopsin using transient absorption experiments with hyperspectral probing (440 – 770 nm) at different excitation wavelengths. Signal contributions from ground as well as excited electronic states show distinct dependences on excitation energies and wavelengths during all timescales of population relaxation. An additional photoproduct is observed upon high-energy excitation with an absorption maximum red-shifted with respect to the known K-intermediate, exclusively formed under linear excitation conditions. Spectral signatures of this photoproduct persist on a timescale of tens of nanoseconds after excitation, comparable to the lifetime of the K-intermediate. The observed additional photoproduct is likely to be a precursor state of an eventually forming blue-shifted, thermally stable photoproduct observed under prolonged high-intensity illumination of BR samples.

1 Introduction

The photo-induced *trans-cis* isomerization of retinal in Bacteriorhodopsin (BR) is one of the most important model reactions in femtosecond spectroscopy of biological samples. Photo-excitation of BR-samples with visible light induces a sub-picosecond isomerization of retinal followed by dynamics that take place on pico- to millisecond timescale. Despite intensive experimental effort, open questions persist regarding especially contributions from resonant two-photon excitation pathways to the formation of stable photoproducts [1-2]. We report on femtosecond transient absorption experiments on the dynamics and the formation of photoproducts in BR in dependence of the excitation energy of pump-pulses over nearly three orders of magnitude (3 – 900 nJ) as well as the excitation wavelength. Our results suggest contributions from resonant two-photon excitation over a wide range of excitation wavelengths to the formation of photoproducts of different origin compared to the known K-photoproduct for excitation with high pulse energies (> 100 nJ).

2 Experimental Methods

Near transform-limited pump-pulses of 20 – 30 fs time-duration were generated in a non-collinear optical parametric amplifier at a rate of 1 kHz with spectra centered at 510 nm and 590 nm. White-light probe-pulses were generated by focusing ~1 μJ of the regenerative amplifier output (795 nm) in a 1.5 mm sapphire plate. The time-resolution of the setup was 50 – 80 fs, dependent on the detection wavelength. Hyperspectral (440 – 770 nm) pump-probe data on freshly prepared BR-suspensions in

aqueous HEPES-buffer (0.01 M) were collected with pump- and probe pulses having parallel polarization. Light-adaption of BR-samples was carried out before and during the experiments.

3 Results and Discussion

Femtosecond transient absorption experiments were carried out on a series of excitation energies ranging from the linear excitation regime (3 – 50 nJ) up to the strongly nonlinear excitation regime (above 100 nJ, see below) for excitation at 510 nm and 590 nm. The hyperspectral datasets were subjected to a global fitting analysis which yields at least four species-associated spectra (SAS) with approximate time constants of 125 fs, 500 fs, 1.5 – 2.5ps and > 250 ps. These correspond to the known time constants for BR's excited-state equilibration after excitation, excited-state decay (I-intermediate), thermal photo-product equilibration (J-intermediate) and later steps of photoproduct transformations (K-intermediate), respectively. For nonlinear excitation (> 100 nJ) an additional SAS-component is observed with a time constant of approximately 10 ps, which obtains similar amplitude as the SAS of the K-intermediate. Examples of SAS are depicted in Figure. 1.

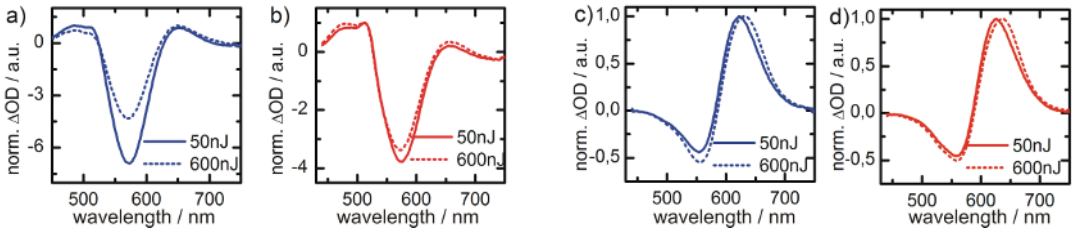

Fig. 1. Examples of species-associated spectra (SAS) for BR with time-constants of ~ 500fs (a) and b)) and > 250 ps (c) and d)) for excitation at 510 nm (blue) and 590 nm (red). For clarity, all SAS have been normalized to facilitate comparison.

For the SAS connected with the excited state lifetime (~ 500 fs, Figure 1 a) and b)) a systematic increase of the time constant is observed with excitation energy: a ~ 400 fs time constant is found below 50 nJ excitation which increased to > 500 fs for above 100 nJ, independent of the excitation wavelength. In addition, we observe a relative reduction of the bleach-component (~ 570 nm) in this SAS with increasing excitation energy for 510 nm excitation (Figure 1 a)), which is less pronounced for 590 nm excitation (Figure 1 b)). For the long-lived SAS (> 250 ps, photoproduct), a spectral red-shift of the signal-maximum (> 600 nm, photoproduct) upon high-energy excitation is observed (> 100 nJ, see below). Also, the ratio between the amplitude of the maxima and minima in the long-lived SAS is sensitive to the excitation energy (Figure 1 c) and d)). These findings strongly indicate that increasing excitation energies on red and blue sides of the ground state absorption influence the dynamics and the photoproduct-distribution in BR.

In order to clarify the involvement of processes other than the K-photoproduct formation, a systematic screening of excitation energies was performed. In Figure 2 a) we show signal-intensities of initial ground-state bleach (at ~100 fs delay), and photoproduct signals at 510 nm. A similar dependence is also observed for 590 nm excitation (not shown). The photoproduct absorption was reconstructed from the raw-data by assuming the measured ground-state absorption as a bleach component [1]. Clearly, both contributions show different dependences on the excitation energy with earlier and nearly complete saturation of the photoproduct signal compared to the bleach signal. This observation is accompanied with a strong alteration of the transient difference-spectrum at 250 ps delay for high-energy excitation (Figure 2 b)): The absorption in the red wing (> 600 nm) of the photoproduct band is drastically enhanced with increasing excitation energy and a convergence of the band-maximum to about 645 nm is clearly evident. This shift persists up to tens of nanoseconds (not shown). In contrast, only a mild shift (< 10 nm) is observed for the ground state bleach. Similar effects are observed for 590 nm excitation (not shown).

At low-energy excitation (< 50 nJ) the transient difference-spectra at 250 ps delay closely resemble earlier reports.[1] This indicates that within the linear excitation regime, only the K-intermediate is formed. The strong deviation of photoproduct-spectra at low (< 50 nJ) and high (> 100 nJ) energy excitation cannot be explained by the formation of a single photoproduct and indicates that additional photoproducts are formed at high excitation-energy. The broadening of the photoproduct-spectra towards longer wavelengths shows that the absorption maxima of non-stationary additional products are red-shifted with respect to the K-absorption maximum.

In order to elucidate the evolution of the additional transient photoproducts on long timescales, fs-irradiation experiments with high-energy excitation (~ 800 nJ) were performed on a timescale of ~ 10 h. A clear reduction in optical density and a blue-shift of the stationary absorption band of the sample is observed together with an increased absorption between 300 – 500 nm (Figure 2 c)). This suggests that the red-absorbing intermediate found on a pico- to nanosecond timescale is not a stationary product and that additional evolution occurs on longer timescales (> 100 ns). Hence, the red-absorbing photoproduct can not be the same as two-photon induced photoproducts such as the Laser-induced blue-membrane- (LIBM) [3] or the F_{620}-state [4], which are observed for high-energy nanosecond excitation. Our results thus suggest that additional two-photon induced photoproducts can be generated by sub-30 fs excitation directly from the Franck-Condon point and that the LIBM and F_{620} photoproducts [3-4] are generated from later species in the photocycle.

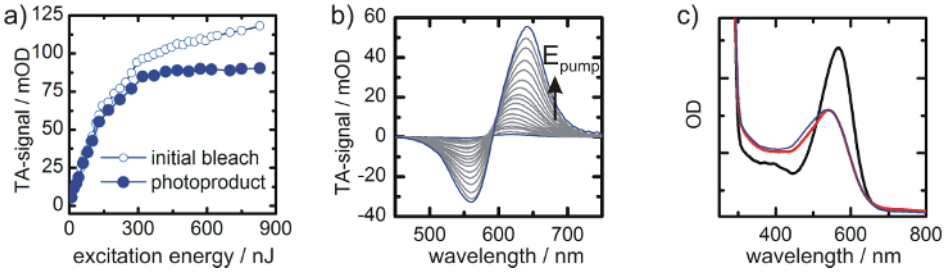

Fig. 2. a) Amplitude of ground-state bleach (open circles) and photoproduct-signal (filled circles) against excitation energy (510 nm excitation). The photoproduct-signal was scaled to the same linear dependence as the bleach signal between 0 and 50 nJ excitation energy. b) Spectral shift of the photoproduct band in T = 250 ps difference spectra for high-energy excitation (510 nm excitation). c) Change in stationary absorption spectra for long-time irradiation (~10) with high-energy excitation (800 nJ, red and blue) compared to the initial BR spectrum.

4 Conclusion

Hyperspectral femtosecond transient absorption signals and dynamics of Bacteriorhodopsin are dependent on the excitation-energy and excitation wavelength. Ground state bleach and photoproduct-spectra exhibit different saturation behavior with increasing energy of excitation-pulses. The transient difference spectra are altered upon high-energy excitation on blue and red sides of the ground state absorption, due to resonant two-photon excitation. Our findings give evidence for active reaction channels for two-photon excitation from the Franck-Condon point.

5 References

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