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Conical Intersection Participation in Femtosecond Dynamics of Visual Pigment Rhodopsin Chromophore *cis-trans* Photoisomerization

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The first and the only photochemical reaction in vision is the photoisomerization of 11-*cis*-retinal, the chromophore of the visual pigment rhodopsin, to the *trans* form. This reaction is one of the most rapid and effective responses in nature. In rhodopsin, it proceeds faster than for 200 fs [1–3], with a quantum yield of 0.65 [4], and is accompanied by a significant energy storage (~35 kcal/mol) [5]. It should be noted that, in a solution, free retinal undergoes photoisomerization within several picoseconds [6] with a quantum yield of 0.15 [7]. The acceleration of isomerization of 11-*cis*-retinal in the chromophore site of rhodopsin and the increase in the quantum yield of the protein are presumably associated with the influence of the protein environment on the chromophore.

The elementary primary act of retinal isomerization in rhodopsin is interpreted as a nonactivated energy passage through the conical intersection of the potential energy surfaces (PESs) of the electronically excited state S_1 and the ground state of the product S_0 [8, 9]. The observed low-frequency oscillations in the kinetic curves of difference absorption of photorhodopsin, the primary product of retinal photoisomerization in rhodopsin, suggest that the passage of the S_1/S_0 PES conical intersection is accompanied by the transfer of the coherent vibrational wave packet on the PES of the product [1, 3, 10].

A direct experimental detection of such an event as the passage through a conical intersection and the determination of the time required to reach it from the initial Franck–Condon (FC) state, which forms as a result of excitation with a femtosecond pulse, is a nontrivial task. Recently, a group of American [8] and Japan [11] authors obtained experimental evidence for the existence of a conical intersection in the reaction of retinal photoisomerization in rhodopsin. It was shown that the time required to reach the conical intersection point is about 80 fs for rhodopsin of both vertebrates [8] and invertebrates [11]. In contrast to the reaction of retinal photoisomerization in rhodopsin, is has recently been shown for retinal in solution that the conical intersection is reached within 2.9–3.8 ps [12]. The time required to reach the conical intersection [12] was determined by studying the kinetics of stimulated emission of retinal in solution.

In this work, the difference spectra and kinetics of the rhodopsin photoinduced absorption and the anisotropy of the rhodopsin photoinduced absorption at different delay times were studied by femtosecond polarization absorption laser spectroscopy method with a temporal resolution of 27 fs. The purpose of this work was to use an alternative approach to estimate the time to reach the conical intersection in the reaction of isomerization of 11-*cis*-retinal in rhodopsin and to perform a detailed analysis of the retinal excited states absorption and the rhodopsin primary photoproducts absorption at short delay times.

Rhodopsin extracts in a detergent (1.6% *n*-nonyl-β-*D*-heptylthioglucoside buffer, pH 6) were prepared as described in [3]. Immediately before the experiment, NH_2OH was added to a rhodopsin sample (c = 7 mg/mL; purity $A_{280}/A_{500} < 1.9$) to a final concentration of 0.1 M. All phases of the experiment were performed under a dim red light.

Experiments were performed with the use of a femtosecond time-resolved polarization laser absorption spectroscopy by using "pump-probe" technique described in [3, 13]. The excitation pulse (E = 70 nJ, $\Delta\tau$ = 27 fs, λ_{max} = 500 nm, and $\Delta\lambda_{1/2}$ = 15 nm) was focused to a spot 300 μm in diameter. White continuum pulse ($E \leq 10$ nJ, $\Delta\tau$ = 800 fs, and $\Delta\lambda$ = 400–900 nm),

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focused to a spot 100 μm in diameter, was used as a probe pulse. The polarization of the pump pulse was oriented at 0° (∥), 90° (⊥) and 54.7° (magic angle) with respect to the polarization of the probe pulse. Difference spectra were recorded with a time delay of the probe pulse from 0 to 10 ps. The absorption spectrum at each time delay was the average absorption spectrum of 50 momentum measurements. The correction of the zero time of the delay between the pump pulse and the corresponding spectral component λ of the probe pulse was performed as described in [14]. In addition, we removed the coherent artifact signal with use of the reference solution of detergent in the buffer [14]. Measurements were performed in a flow cell 0.5 mm thick. All experiments were performed at 22°C.

The differential absorption spectrum $\Delta A(\lambda, t) = A(\lambda, t) - A_0(\lambda)$ is the difference of the absorption spectrum of rhodopsin at a delay time t after the excitation pulse and the absorption spectrum of rhodopsin without excitation. The following components can be observed in the difference absorption spectrum: (1) $\Delta A > 0$, absorption from the excited state (excited state absorption, ESA) or from the reaction product state; (2) $\Delta A < 0$, bleaching (BL), a negative bleaching signal in the absorption band of rhodopsin, due to depletion of the population of the ground state; and (3) $\Delta A < 0$, stimulated emission (SE), a negative signal determined by stimulated emission from the electronically excited state. In case of a temporary overlap of the pump and probe pulses, a component determined by the coherence of the ground and excited electronic states appeared in the signal $\Delta A(\lambda, t)$. In our experimental conditions, at a pulse duration of 27 fs, the electronic coherence signal at a time delay of 30 fs and more can be neglected. The anisotropy of differential absorption $\Delta A(\lambda, t)$ was calculated as $r = \dfrac{\Delta A_{\parallel} - \Delta A_{\perp}}{\Delta A_{\parallel} + 2\Delta A_{\perp}}$. The absorption at the magic angle was defined as $\Delta A_{mag} = \dfrac{1}{3(\Delta A_{\parallel} + 2\Delta A_{\perp})}$. Absorption ΔA, measured in the orientation of pump and probe polarization at the magic angle 54.7°, does not depend on the angle θ between vectors of the dipole moment of the optical excitation transition μ_1 and the dipole moment of the optical probing transition μ_2. Therefore, possible effects of rotation of angle θ in the ΔA_{mag} signal are not manifested.

The anisotropy r of absorption signal corresponding to the transition between nondegenerate electronic states ΔA is expressed as $r = \dfrac{3\cos^2(\theta) - 1}{5}$. If the ΔA band is assigned to an individual electronic transition, the r value ranges from –0.2 to 0.4: at $\theta = 0°$, $r = 0.4$ and at $\theta = 90°$, $r = -0.2$. The differential absorption signal may be the result of superposition of several absorption bands assigned to different optical transitions i (e.g., ESA, BL, SE): $\Delta A(\lambda, t) = \Delta A_{ESA}(\lambda, t) + \Delta A_{BL}(\lambda, t) + \Delta A_{SE}(\lambda, t)$. Each transition is characterized by anisotropy r_i. If several bands overlap, the anisotropy r of signal $\Delta A(\lambda, t)$ is expressed as the mean of all individual optical transitions i: $r = \dfrac{\sum_i \Delta A^{(i)}_{mag} r_i}{\sum_i \Delta A^{(i)}_{mag}}$. In the case of superposition of absorption bands, anisotropy r can range from $-\infty$ to ∞. The analysis of anisotropy changes with time $r(t)$ gives an idea of the kinetics of electron–electron transitions. Within the Condon approximation, after the transition from μ_1 to a new state with moment μ_2, the $r(t)$ value should not depend on the nuclear coordinate.

The rotation of the molecular backbone of retinal at delay times on the order of hundreds of femtoseconds can be ignored, because the rotational diffusion of molecules with a hydrodynamic volume comparable with the volume of retinal in non-viscous solvents is determined by the correlation time on the order of tens of picoseconds; for retinal in the protein globule, it can be expected that the rotation time will be much longer. It can therefore be assumed that the changes in the anisotropy $r(t)$ of absorption $\Delta A(\lambda, t)$ at retinal isomerization on the femtosecond timescale are determined by a change in angle θ caused by the turn of the dipole moment upon the electron–electron transition in the conical intersection area but not by the rotation of angle θ caused by the turn of the molecular backbone.

Figure 1 shows the difference spectra of photoinduced rhodopsin absorption ΔA_{mag} (*1*), the anisotropy r spectra (*2*), and the corresponding model linear curves (*3*). The spectrum $\Delta A_{mag}(t = 30$ fs) in the range of 400–600 nm is dominated by the ESA band with a peak at approximately 500 nm, which is determined by the excited-state absorption and photoexcitation energy transfer to the higher levels $S_1 \rightarrow S_n$ (Fig. 1a). This band overlaps with the bleaching band BL ($\Delta A_{BL} < 0$), since the band of rhodopsin absorption from the ground state $S_0 \rightarrow S_1$ is located in this area. The observed overall signal $\Delta A(\lambda, t) = \Delta A_{ESA}(\lambda, t) + \Delta A_{BL}(\lambda, t) > 0$, which indicates a higher section of the $S_1 \rightarrow S_n$ transition, which is responsible for ESA, as compared to the $S_0 \rightarrow S_1$ transition, associated with BL. In addition, it was be assumed that there is not one, but several ESA bands in this range. This assumption is confirmed by the complex structure of the spectrum, which additionally points out to overlapping of more than two absorption bands (Fig. 1a). The negative peak at approximately $530 < \lambda < 570$ nm, against the background of ESA absorption and the sharp emission in the anisotropy r spectrum in the same region $530 < \lambda < 570$ nm, indicates that the stimulated emission $\Delta A_{SE}(530 < \lambda < 570$ nm) contributes to the total signal and that the differential absorption is the sum of signals $\Delta A(530 < \lambda < 570$ nm) $= \Delta A_{ESA} + \Delta A_{BL} + \Delta A_{SE}$. The stimulated emission SE is also

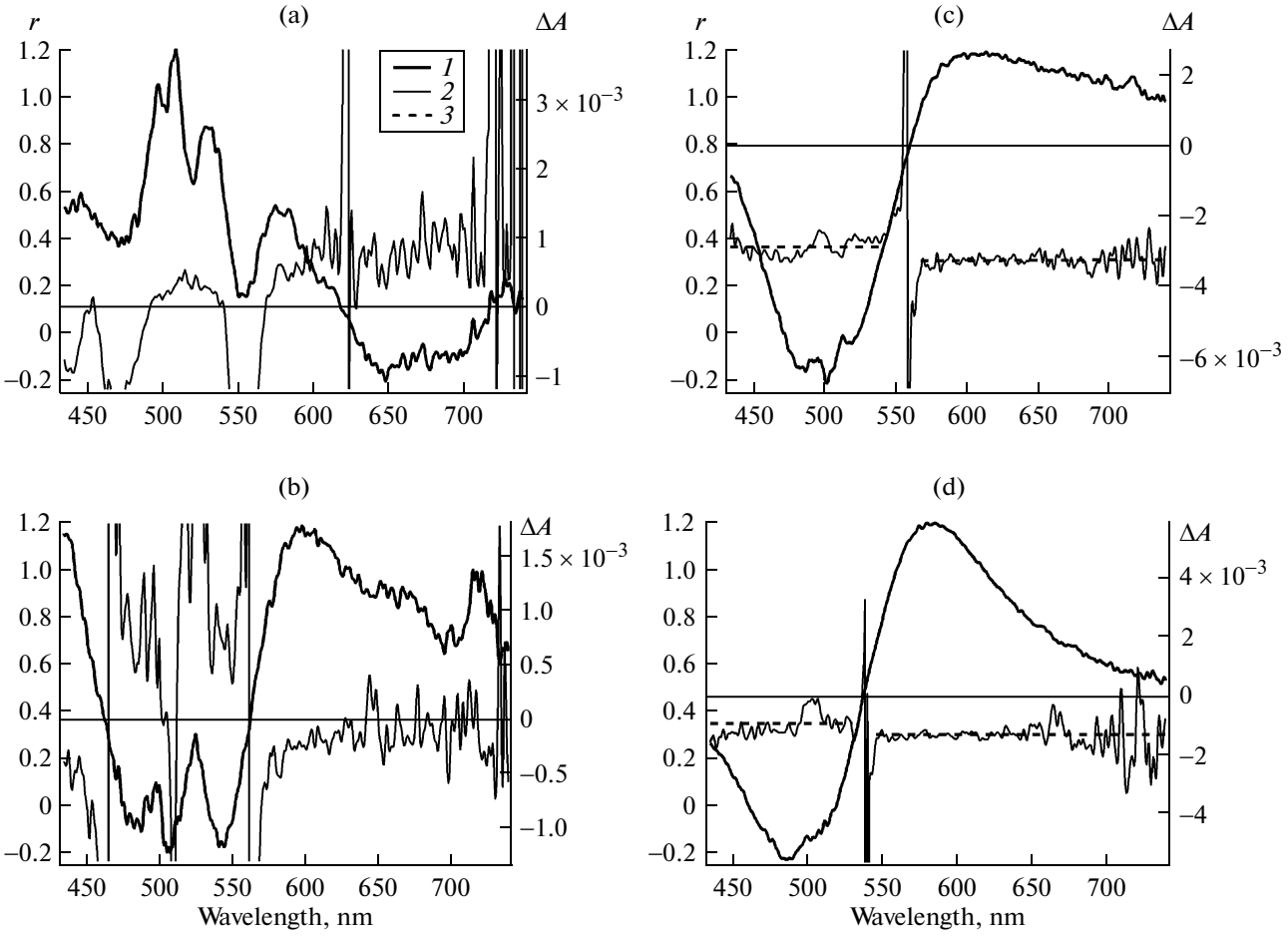

Fig. 1. Difference spectra of photoinduced rhodopsin absorption obtained as a result of excitation with 500-nm pulses at the magic angle (curves *1*, right axis) and the corresponding spectra of the anisotropy function (curves *2*, left axis), represented in the spectral range 435–740 nm at a probing delay time of (a) 30, (b) 60, (c) 100, and (d) 200 fs. The figure also shows the model linear curves obtained for the spectra of the anisotropy function in certain spectral regions ((c, d) curves *3*, left axis).

observed in the region $620 < \lambda < 700$ nm—a negative signal ΔA_{mag} ($t = 30$ fs, $620 < \lambda < 700$ nm). This negative signal cannot be attributed to BL, because $S_0 \rightarrow S_1$ absorption of rhodopsin is absent in this area. The anisotropy value in this SE band is $r(620 < \lambda < 700\ \text{nm}) = 0.34 \pm 0.08$ and, within the error, is close to 0.4 (i.e., to the anisotropy value of the theoretically expected anisotropy for the $S_1 \rightarrow S_0$ transition).

As seen in Fig. 1b, the ESA absorption $S_1 \rightarrow S_n$ in the spectrum $\Delta A_{mag}(400 < \lambda < 570$ nm) was significantly reduced by 60 fs of delay, and the BL band $\Delta A_{BL} < 0$ became dominant. However, the complex spectrum $r(400 < \lambda < 570$ nm) indicates that the signal ΔA_{mag} at a delay of 60 fs contained a significant contribution from the ESA absorption $S_1 \rightarrow S_n$.

Differential absorption spectra of rhodopsin (*1*), shown in Figs. 1c and 1d, refer to the delay time of 100 and 200 fs, respectively. These spectra contain the absorption band in the red region of the spectrum (550–750 nm) and the bleaching band in the short-range region of the spectrum (400–550 nm). Spectrum r takes the simple form—two values in the absorption and bleaching ranges, which are almost independent of the probing wavelength; in this situation, the ΔA_{mag} spectrum is determined by the absorption of the product and the bleaching of the ground state.

This qualitative analysis of the difference spectra at the initial delay time allowed us to conclude that the time of the system transition from the excited state to the first photoproduct, in which retinal is already in the *trans* configuration, is determined by the range of 60–100 fs and is about 80 ± 20 fs. In this time range, the complex anisotropy spectrum r is simplified and the bands corresponding to the stimulated emission SE disappears. In other words, this temporal parameter characterizes the process of the conical intersection crossing by the system and is consistent with the experimental results obtained in [8, 11] in analyzing the SE signal of the $S_1 \rightarrow S_0$ transition.

Figure 2 shows the kinetic curves $\Delta A_{mag}(t)$ (*1*), $\Delta A_{II}(t)$ (*2*), and $\Delta A_{\perp}(t)$ (*3*), as well as the kinetics of the

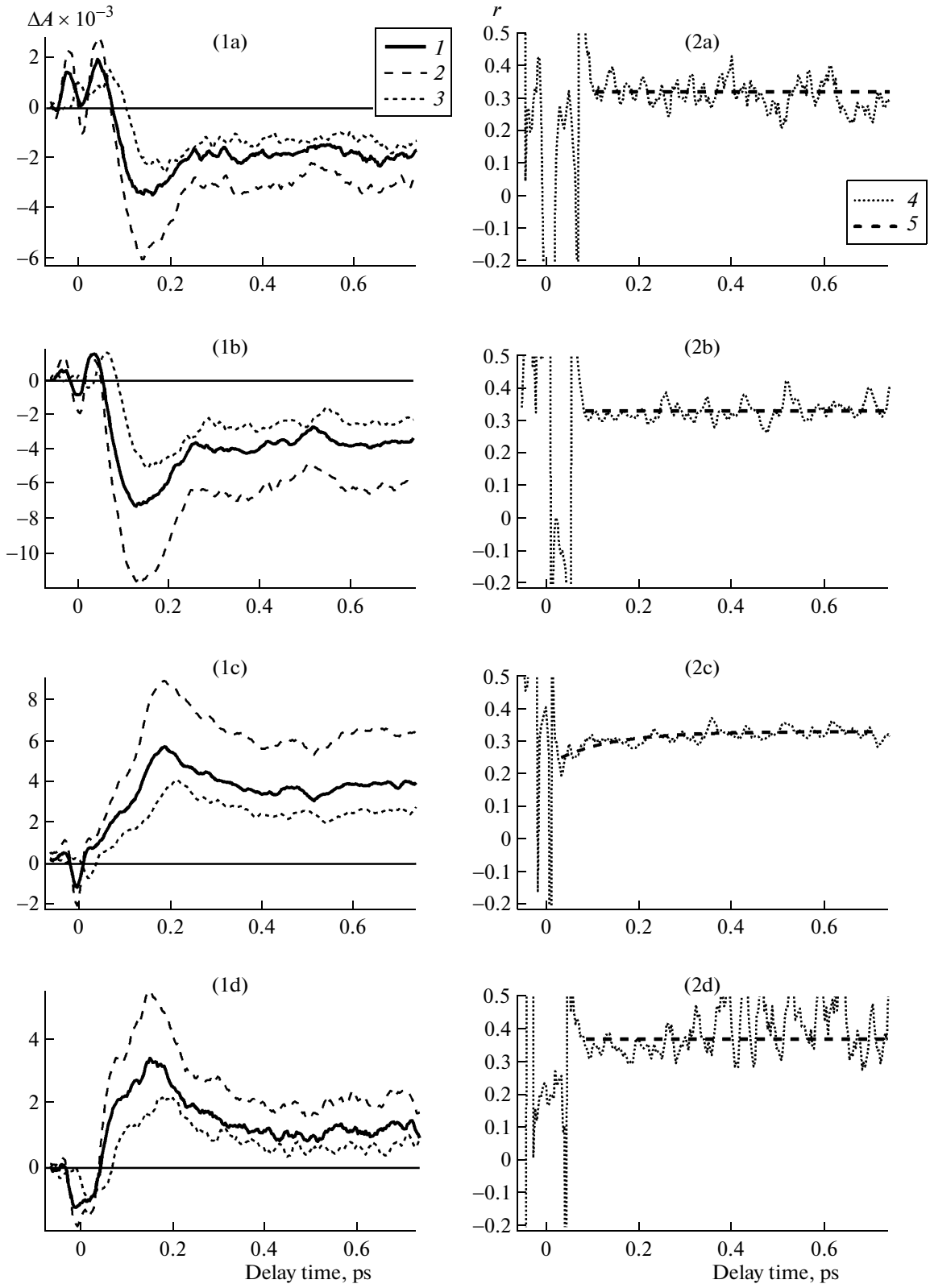

Fig. 2. Kinetic curves of differential photoinduced rhodopsin absorption ΔA_{mag} (curves *1*), ΔA_{II} (curves *2*), and $\Delta A_{\perp}$ (curves *3*) (1a–1d) and time-resolved anisotropy functions (2a–2d, curves *4*), represented in the time range −50–740 fs at probing wavelengths of (1a, 2a) 450, (1b, 2b) 480, (1c, 2c) 600, and (1d, 2d) 650 nm. The figure also shows the model curves obtained for the anisotropy functions in certain time ranges (2a–2d, curves *5*).

anisotropy function $r(t)$ for the characteristic probing wavelengths (*4*) and the model curves corresponding to this function (*5*). Positive absorption $\Delta A > 0$, associated with the dominance of ESA from the electronically excited state $S_1 \rightarrow S_n$, disappeared by the time 73 fs (Fig. 2, 1a) and 60 fs (Fig. 2, 1b), and the isosbestic point for $\Delta A_{mag}(t) = 0$ in the differential signal was reached. In the stimulated emission signal SE ($\Delta A < 0$) of the $S_1 \rightarrow S_0$ transition at a wavelength of 650 nm (Fig. 2, 1d), the isosbestic point for $\Delta A_{mag}(t) = 0$ was reached at $t = 47$ fs, for $\Delta A_{\parallel}(t) = 0$ at $t = 47$ fs, and for $\Delta A_{\perp}(t) = 0$ at $t = 70$ fs. Until the isobestic points on the kinetic curves were reached, the anisotropy $r(t)$ underwent significant changes over time. These rapid changes could be associated with reaching the conical intersection by the system. The passage of the isobestic points $\Delta A_{mag}(t) = 0$ by the kinetic curve is reflected in the curves $r(t)$ as singularity (Fig. 2, 2a–2d). After the passage of the isosbestic point on the kinetic curves, a typical picture was an almost exponential growth to the constant value $r(t)$, which remained unchanged up to 10 ps. A characteristic example is curve (*4*), which was obtained at a probing wavelength of 600 nm (Fig. 2, 2d). After the time $t = 30$ fs, at which the anisotropy value was 0.26 ± 0.01, it increased to 0.33 ± 0.01 at 100–120 fs and remained constant for 10 ps. With allowance for the fact that the delay time after photoexcitation was 80 fs (the moment when the system crossed the conical intersection region), then a change in the $r(t)$ value at later delay times can be regarded as a sign of the exit of the system from the conical intersection. Within the Condon approximation, the $r(t)$ value does not depend on the nuclear coordinate. This rule holds for the kinetic curves recorded at time points after approximately 100–120 fs. As can be seen from Fig. 2, significant changes in the kinetic curves $\Delta A_{mag}(t > 200$ fs) are correlated to an almost constant $r(t)$ value in this time range. The observed changes in anisotropy with time $r(80 < t < 120$ fs) can be qualitatively explained assuming that, after the system leaves the conical intersection, the Born–Oppenheimer approximation and the Condon principle are breached—the dipole moment of the electron-vibrational transition and, as a consequence, angle θ and anisotropy $r(t)$ depend on the nuclear degrees of freedom. Since it can be accepted that the absorption in the range from 600 to 700 nm is determined by the optical transition of only the primary isomerization product, the r value can be used to determine the turning angle of the dipole moment of the transition product relative to the dipole moment of rhodopsin (~20°). In [15], the anisotropy was measured at a wavelength of 580 nm and amounted to $r(t > 200$ fs$) = 0.34 \pm 0.01$, which is consistent with the data presented in this paper. The authors of [15] did not analyze the issue of the time of the conical intersection crossing.

Note that the anisotropy value in the bleaching band region was $r(t = 200$ fs$) = 0.35 \pm 0.03$ (Fig. 1d), 435–540 nm), which is slightly lower than 0.4, which is characteristic of an individual bleaching band. Probably, this was due to the overlap of the bleaching band of rhodopsin and the absorption band of the first photoproduct.

Thus, the results obtained in this study show that the conical intersection point in the reaction of photoisomerization of 11-*cis*-retinal in rhodopsin is reached within a time of about 80 ± 20 fs. Qualitative analysis of the anisotropy spectra at time delay 100 and 200 fs showed that the anisotropy value almost did not depend on the probing wavelength in the absorption region of reaction products and in the bleaching region of the ground state of rhodopsin after the passage of the conical intersection by the system (Fig. 1c, 1d) at time delays of 100 a nd 200 fs. This suggests that, by this time approximately 100 fs, the system passes from the excited state to the ground state of the first photoproduct, photorhodopsin, which is completely formed by 200 fs, and the difference spectrum is determined by two bands—the absorption band and the bleaching band.

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