CHEMICAL PHYSICS OF BIOLOGICAL PROCESSES

Femtochemistry of Rhodopsins

M. A. Ostrovsky^{a,*} and V. A. Nadtochenko^b

^a Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, 119991 Russia

^b Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences, Moscow, 119991 Russia

*e-mail: ostrovsky3535@mail.ru

Received October 1, 2020; revised October 1, 2020; accepted October 20, 2020

Abstract—The review considers the spectral kinetic data obtained by us by femtosecond absorption laser spectroscopy for the photochromic reaction of retinal isomerization in animal rhodopsin (type II), namely, bovine visual rhodopsin and microbial rhodopsins (type I), such as *Exiguobacterium sibiricum* rhodopsin and *Halobacterium salinarum* bacteriorhodopsin. It is shown that the elementary act of the photoreaction of retinal isomerization in type I and type II rhodopsins can be interpreted as a transition through a conical intersection with retention of the coherence of the vibrational wave packets generated during excitation. The coherent nature of the reaction is most pronounced in visual rhodopsin as a result of the barrier-free movement along the excited surface of potential energy, which also leads to an extremely high rate of retinal isomerization compared to microbial rhodopsins. Differences in the dynamics of photochemical reactions of type I and type II rhodopsins can be related to both differences in the initial isomeric forms of their chromophores (all-*trans* and 11-*cis* retinal, respectively), as well as with the effect of the protein environment on the chromophore. Despite the practically identical values of the quantum yields of the direct photoreaction of visual rhodopsin and bacteriorhodopsin, the reverse photoreaction of visual rhodopsin is much less effective ($\phi = 0.15$) than in the case of bacteriorhodopsin ($\phi = 0.81$). It can be assumed that the photobiological mechanism for converting light into an information process in the evolutionarily younger visual rhodopsins (type II rhodopsins) should be more reliable than the mechanism for converting light into a photoenergetic process in the evolutionarily more ancient microbial rhodopsins (type I rhodopsins). The low value of the quantum yield of the reverse reaction of visual rhodopsin can be considered as an increase in the reliability of the forward reaction, which triggers the process of phototransduction.

Keywords: photochemistry, femtosecond absorption laser pump–probe spectroscopy, primary reactions, coherent reactions, microbial rhodopsins, retinal-containing proteins, visual rhodopsin, *Exiguobacterium sibiricum* rhodopsin, *Halobacterium salinarum* bacteriorhodopsin

DOI: 10.1134/S1990793121020226

INTRODUCTION

The light-sensitive proteins of rhodopsins are present in all the kingdoms of living organisms. They are divided into microbial rhodopsins (type I) and animal rhodopsins (type II). Most representatives of type I rhodopsins function as ion pumps, performing a photoenergetic function (the simplest photosynthesis); some of them, functioning as cationic or anionic channels, or binding to a carrier protein in response to a light signal, perform a photosensory function. The extensive family of microbial rhodopsins continues to grow. Thus, two new families have recently been discovered. The first one includes the so-called heliorhodopsins, the biological function of which is not yet clear [1]. The second one includes rhodopsins found in fungi, green algae, and unicellular collar flagellates (choanoflagellates), which exhibit enzymatic activity (see review [2]). These enzymatic rhodopsins have, in contrast to the classical rhodopsins, not seven alpha-helical transmembrane strands but eight or even nine.

For example, in rhodopsin phosphodiesterase, an additional eighth strand together with a long *N*-terminal polypeptide fragment is responsible for its enzymatic activity [3]. The structure of the conserved chromophore center in all microbial rhodopsins has much in common. As for type II animal rhodopsins, most of them function as G-protein-binding receptors, providing photoinformational functions, the main one being visual.

Thus, in the modern sense, the term rhodopsin applies to retinal-containing proteins of both microbial and animal origin. All of them, with the exception of the enzymatic microbial rhodopsins, have the same architecture of seven alpha-helices that cross the biological membrane. However, there is no amino acid sequence homology between microbial and animal rhodopsins (see review [4]).

The most conserved domain in each of the rhodopsin types is the chromophore center, which is somewhat different in microbial and animal rhodopsins. At

the same time, even minor changes in the structure of the chromophore center in the amino acid environment of retinal and in the isomeric form of retinal itself significantly change the spectral, photochemical, and a number of other functionally important properties of the molecule.

The photochemical reaction of rhodopsins is the isomerization of their chromophore group, retinal. In the case of visual rhodopsin, it is the isomerization of 11-*cis* into all-*trans* retinal; in the case of microbial rhodopsins, from all-*trans*- to 13-*cis* retinal. Rhodopsins, as light-sensitive molecules, represent an ideal model for studying and understanding the mechanism of the photochemical isomerization reaction in general.

After the discovery by R. Hubbard and G. Wald of the photoisomerization of the chromophore group of visual rhodopsin, from 11-*cis* retinal to all-*trans* form, as the primary reaction of vision [5], the mechanism of this unique photochemical reaction to this day is actively being studied. With regard to vision, it is important to emphasize that the absorption of just one quantum of light by one of about 10^9 rhodopsin molecules in the optic retinal cell, the rod, is sufficient to initiate an enzymatic amplification cascade and the generation of a photoreceptor, i.e., visual, signal.

The main questions were “What is the rate and quantum yield of *cis*–*trans* isomerization, and what is the mechanism of this reaction?” The answer to the first question has now been given. Isomerization occurs in femtosecond times [6], its efficiency is very high, and the quantum yield is 0.65 [7], while 58% of the absorbed energy is stored in the molecule. In recent years, great strides have been made in understanding the mechanism of this reaction. In brief, isomerization occurs through the conical intersection (CI) of potential energy surfaces (PES), which connects the excited state (S_1) of retinal with the first reaction product, photorhodopsin (Photo), in the ground state (S_0) [8, 9]. It has been shown that the *cis*–*trans* transition in visual rhodopsin occurs in a fantastically short time, 50–100 fs, and that this is a coherent reaction. It is becoming obvious that the highest speed and efficiency of this transition is provided by the interaction of the chromophore group with its nearest protein environment in the chromophore center of the rhodopsin molecule. The mechanism of this interaction remains to be clarified (see reviews [10, 11]). Indeed, such fundamental characteristics of the rhodopsin molecule as the position of the maximum in the absorption spectrum, the lifetime of the excited state, the quantum yield of isomerization, and vibrational coherence observed in the first photoproduct of the reaction directly depend on the interaction of retinal with its nearest protein environment in the close chromophore center.

In the visual rhodopsin molecule, the volume of the chromophore center is only $660 \text{ \AA}^3$, and the interaction surface of 11-*cis* retinal with the protein envi-

ronment is $\sim 230 \text{ \AA}^2$ [12]. This means that the close protein environment of 11-*cis* retinal not only does not hinder, but, on the contrary, actively promotes an ultrafast and efficient process of photoinduced change in the geometry of the chromophore group. It remains to be seen how exactly such a close protein environment contributes to the acceleration and enhancement of the efficiency of this photochemical reaction.

Comparison of the times of rhodopsins I and II reaching CI shows that for visual rhodopsin, both bovine [13–17] and octopus [18], it is several times shorter than for the microbial bacteriorhodopsin of archaeobacteria [19, 20]. This difference can be related to the different *cis*–*trans* and *trans*–*cis* transitions in the visual and microbial rhodopsins, respectively, and with the different structure of their chromophore centers. The first assumption is confirmed by the fact that in the gas phase the photoisomerization of 11-*cis* retinal to the all-*trans* form occurs almost eight times faster than the photoactivated transition from all-*trans* retinal to *cis*-isomers [21]. The protein environment of retinal, both in the visual rhodopsin molecule and in bacteriorhodopsin, accelerates this reaction several times. In any case, the higher rate of photoisomerization of 11-*cis* retinal in the visual rhodopsin molecule compared to the photoisomerization of *trans*-retinal in bacterial rhodopsin can be considered as evidence of the greater perfection of visual rhodopsin as evolutionarily younger and intended for the photoinformation process.

From the point of view of the evolution of retinal-containing proteins, it is of interest to compare the parameters of the photochemical reactions of microbial (type I) and visual (type II) rhodopsins. It gives the impression that, despite the external similarity, the origin of rhodopsins of types I and II is not the same. In addition to differences in the amino acid sequence of the rhodopsins, intermediate forms indicating their relationship have not yet been found. In other words, we are talking, apparently, about the convergent evolution of these two types of rhodopsins, which is related to their physiological functions. It is surprising that in the course of such a convergent (independent) evolution, both the topography and structure of the chromophore center turned out to be similar (for more details, see [22]). Comparison of the dynamics of photoisomerization of the retinal chromophore in rhodopsins of animal and microbial origin, including the comparison of their direct and reverse photoreactions, is of significant interest from the point of view of the molecular evolution of retinal-containing proteins.

COMPARISON OF MICROBIAL RHODOPSINS (TYPE I) AND ANIMAL RHODOPSINS (TYPE II)

We compared the spectral characteristics, isomeric composition of retinals, and rates and quantum yields of photochemical reactions of three different rhodop-

sins [14–17, 20, 23–27]. Two of them are representatives of type I microbial rhodopsins: bacteriorhodopsin (BR) of the halophilic archaebacterium *Halobacterium salinarum* and the recently discovered rhodopsin (ESR) from the soil psychrotrophic bacterium *Exiguobacterium sibiricum* found in the Siberian permafrost, which is about three million years old [28]. The third rhodopsin is the classic type II visual rhodopsin, namely, rhodopsin (Rh) of the *Bos taurus* bovine rods.

We studied photochromic reactions of type I (BR and ESR) and type II (Rh) rhodopsins in the femto- and picosecond time range by the femtosecond polarization absorption laser spectroscopy using the pump–probe and pump–pump–probe schemes. A detailed analysis of the time evolution of the differential spectra of these systems after a pulse (pulses) of femtosecond excitation is given in [14–17, 20, 23–27].

Femtosecond laser spectroscopy with a time resolution of 25 fs and probing in a wide spectral range (400–900 nm) made it possible to reveal the spectra of intermediate states that are formed during the primary reactions of the studied proteins and have much in common, and to determine the characteristic transition times between these states. Figure 1 shows the typical spectra ($\Delta A(\lambda)$) and kinetic curves ($\Delta A(t)$) of the differential photoinduced absorption of Rh, BR, and ESR. The differential absorption signal ($\Delta A(\lambda, t)$) consists of three components: an absorption signal with a positive sign $\Delta A(\lambda, t) > 0$, which is related to the transition from the electronically excited state (excited state absorption (ESA)) and/or from the ground state of the reaction product (ground state absorption (GSA)); a negative signal $\Delta A(\lambda, t) < 0$ for the bleaching (BL) of the absorption band of the ground state caused by depletion of the population of the ground state after excitation; and a negative signal $\Delta A(\lambda, t) < 0$ of stimulated emission (SE) from the electronically excited state. The spectra shown in Figs. 1a–1c contain all three spectral components: ΔA_{ESA} , ΔA_{BL} , and ΔA_{SE} .

The ΔA_{ESA} signal reflects the $S_n \leftarrow S_1$ absorption, which dominates in the short-wavelength spectral region: 410–490 (Rh) and 400–510 nm (BR and ESR). The ΔA_{SE} signal appears in the long-wavelength spectral regions of 610 to 710 (Rh) and 690 to 880 nm (BR and ESR). These signals originate from an excited state (Rh* in the case of Rh and intermediate I in the cases of BR and ESR) that is different from the Franck–Condon (FC) state, the formation of which is related to the special topology of S_1 -PES of 11-*cis* and all-*trans* retinal.

For all systems, in the spectral region of 550 to 650 nm, we can note a long-lived absorption band that appears as the ΔA_{ESA} and ΔA_{SE} signals disappear; consequently, this band was attributed to the absorption of the first products of the photoisomerization reaction of retinal in the ground state (ΔA_{GSA}). These products, photorhodopsin and bathorhodopsin (Batho) in the case of Rh ($\lambda_{\text{max}} \sim 570$ and 535 nm, respectively) and

intermediates J and K in the cases of BR ($\lambda_{\text{max}} \sim 625$ and 590 nm, respectively) and ESR ($\lambda_{\text{max}} \sim 590$ and 555 nm, respectively) successively replace each other. The Photo $\rightarrow$ Batho transition, as well as the J $\rightarrow$ K transition, is related to the vibrational relaxation of retinal and the completion of its isomerization. In differential spectra, this transition manifests itself as a short-wavelength shift of the absorption band of the reaction product observed in the picosecond time range (Fig. 1a, curves 4–6; Figs. 1b, 1c, curves 5, 6).

In the region of the stationary absorption of Rh, BR, and ESR ($\lambda_{\text{max}} = 498, 568$, and 528 nm, respectively), the ΔA_{BL} signal is observed; at early delay times, this signal is overlapped by the ΔA_{ESA} signal, then, as the excited state decays, reaches its maximum intensity by about 100–200 fs, and at later times decreases as a result of the appearance of the ΔA_{GSA} signal (Fig. 1d, curve 3; Figs. 1e, 1f, curves 3).

When comparing the Rh, BR, and ESR systems at the qualitative level, it may be noted that the excited state relaxation and the formation of primary isomerization products occur faster in Rh than in BR and ESR, as can be seen from a comparison of the kinetic curves of the accumulation of isomerization products (Fig. 2).

The construction of model exponential curves for the experimental kinetic curves in a wide probing range [14, 15, 17, 20, 26] and the use of the CONTIN program for analysis [27], made it possible to characterize the times of the observed processes and clarify the schemes of the primary reactions of the studied proteins. Approximate schemes of the elementary process of retinal isomerization in visual and microbial rhodopsins, which are based on the analysis of the femtosecond data, are shown in Figs. 3 and 4.

As mentioned earlier, the elementary process of retinal isomerization in all the studied proteins proceeds in an excited state by passing S_1/S_0 -PES through CI, which leads to the formation of the first short-lived product with the isomerized retinal. The participation of CI also implies the transition of some of the excited molecules to the initial state with the nonisomerized retinal (Figs. 3, 4). This process takes place on the same time scale as the formation of the main isomerization product.

The photoisomerization reaction of retinal both in protein [14, 15, 17, 20, 29, 30] and in solution [31] proceeds in a coherent mode. Upon excitation, a femtosecond pulse forms coherent vibrational quantum wave packets in the FC state, and the coherence is maintained when passing through the CI. This is most clearly seen in the case of Rh. Oscillatory components can be identified on the kinetic curves reflecting the formation of the product Photo and the initial state (Fig. 1d, curves 3, 4; Fig. 2, curve J); which, apparently, is related to a barrier-free transition from the electronically excited state of retinal to isomerization

Fig. 1. Differential spectra of photoinduced absorption of (a) Rh, (b) BR, and (c) ESR recorded at delay times (ps): (1) -0.2 , (2) 0.03 , (3) 0.1 , (4) 0.2 , (5) 0.8 , and (6) 9 in the case of Rh; (1) -0.2 , (2) 0.05 , (3) 0.12 , (4) 0.5 , (5) 1 , and (6) 10 in the case of BR; (1) -0.2 , (2) 0.12 , (3) 0.2 , (4) 0.5 , (5) 2 , and (6) 10 in the case of ESR. Kinetic curves of photoinduced absorption of (d) Rh, (e) BR, and (f) ESR recorded at probing wavelengths (nm): (1) 410 , (2) 450 , (3) 480 , (4) 580 , (5) 620 , and (6) 700 in the case of Rh; (1) 470 , (2) 500 , (3) 590 , (4) 640 , (5) 730 , and (6) 850 in the case of BR; (1) 470 , (2) 500 , (3) 575 , (4) 605 , (5) 730 , and (6) 850 in the case of ESR. Kinetic curves are presented on a linear scale of the delay time up to 1 (2) ps and then on a logarithmic scale of the delay time for Rh (BR and ESR).

products (Fig. 3a). In the case of microbial rhodopsins, in particular BR and ESR, the structure of all-*trans* retinal and its interaction with the surrounding amino acid residues determine the presence of a small barrier on the S_1 -PES, due to which the wave packet is delayed on the way to the CI (Fig. 3b). The BR and ESR photoreaction products are formed more slowly than Rh and without pronounced coherent effects (Fig. 2). Nevertheless, the use of short exciting pulses (~ 10 fs) during initiation of the BR photoreaction makes it possible to observe oscillations with a wide frequency range both in signals from the excited state (intermediate I) and in the first photoproduct (intermediate J) [32], which indicates a coherent BR photoreaction mode, as in the case of Rh.

When analyzing the time-resolved data of the rhodopsins studied by us, the following times of the observed processes were obtained. The relaxation time of the initial Franck–Condon state is estimated at

~ 30 – 50 fs for all systems. The time of passing through CI for Rh is estimated at ~ 50 fs by the disappearance of the ΔA_{ESA} signal, after which the primary isomerization product (Photo) containing all-*trans* retinal with a highly twisted and strained structure and an excess of vibrational energy is formed. The vibrational relaxation of retinal occurs in two stages with characteristic times of 60 and 2.1 ps. The first stage is probably related to the redistribution of vibrational energy within retinal, which manifests itself in the photoinduced signals as a short-wavelength shift of the broad absorption band of Photo to ~ 580 nm at delay times of 100 to 200 fs (Fig. 1a, curves 3, 4). The second stage probably reflects the transfer of excess vibrational energy from retinal to the protein environment and manifests itself as a further shift of the ΔA_{GSA} (Photo) band by about 10 nm during the formation of the next product Batho. At this stage, the *cis* $\rightarrow$ *trans* isomerization of retinal is completed.

The lifetime of the excited state in BR and ESR is much longer than in Rh. The dynamics of the formation and decay of intermediate **I** is resolved in time [27]. When formed with a characteristic time of 40 to 50 fs, it undergoes vibrational relaxation, the time of which was estimated at 130 (BR) and 160 fs (ESR), after which the retinal isomerization begins, which proceeds noticeably slower than in Rh. Thus, the characteristic time of formation of the first product with isomerized 13-*cis* retinal, intermediate **J**, is 480 (BR) and 690 fs (ESR). Intermediate **K**, as in the case of Rh, is formed in the picosecond time range in 1.8 (BR) and 6.3 ps (ESR).

Another feature of primary reactions is the presence of heterogeneity of the excited state in the case of microbial rhodopsins, which many researchers related to the heterogeneity of the initial state of these proteins. This leads to photoactivation of several parallel processes, some of which pass through the so-called nonreactive excited states that do not lead to retinal isomerization (Fig. 4). For BR and ESR, one such nonreactive state was revealed, the lifetime and contribution of which to the overall decay process were estimated at 2.4 ps/6% (BR) and 5 ps/19% (ESR) (Fig. 4, reactions (2) and (3), respectively). For Rh, the presence of a nonreactive excited state is also assumed judging by the dynamics of the disappearance of fluorescence, which is characterized by two times in the femto- and picosecond ranges [33]. The lifetime and contribution of this state were estimated by us at 2.4 ps/4% (Fig. 4, reaction (1)).

The ratio of the isomerization and relaxation processes to the initial state determines the quantum yield of the 11-*cis* $\rightarrow$ all-*trans* and all-*trans* $\rightarrow$ 13-*cis* photoisomerization of retinal in Rh and microbial rhodopsins, respectively. The quantum yields of this reaction are quite high and amount to $\phi(\text{Rh} \rightarrow \text{Batho}) = 0.65$ [7] and $\phi(\text{BR} \rightarrow \text{K}) = 0.64$ [34, 35].

Thus, despite the general scheme that can be used to describe the photoreaction of all the studied rhodopsins (Fig. 4), the Rh photoreaction has a significant difference from microbial rhodopsins. This is a barrier-free transition through CI, which is carried out at a high rate and a high degree of coherence of the reaction vibrational modes.

It can be concluded that the dynamics of the photochemical reaction of type I and II rhodopsins is directly related to the isomeric form of their chromophores. Thus, the photoreaction of 11-*cis* retinal in the gas phase, when there is no influence of the environment, proceeds without a barrier in the femtosecond time range (400 fs), while the photoreaction of all-*trans* retinal, due to a small barrier on S_1 -PES, occurs in the picosecond time range (3 ps) [21]. In the chromophore center of the protein part of the molecule, the interaction of retinal with the nearest amino acid residues significantly accelerates the photoisomerization reaction and excludes the formation of alternative

Fig. 2. Nonnormalized kinetic curves for the formation of isomerization products (1) Rh, (2) BR, and (3) ESR, and the corresponding model exponential curves (1'–3') presented at probing wavelengths of 570, 640, and 605 nm, respectively. The kinetic curves are presented in linear (–0.2 to 2 ps) and logarithmic (2–9 ps) delay time scales.

products. The protein environment of retinal also changes the CI structure, greatly increasing the probability of rhodopsin transition into a photoreaction product, compared to the return to the initial state, as was shown by the QM/MM methods using Rh as an example [36].

As can be seen from Fig. 3, the representation of the elementary act of the isomerization process as the transition through the CI suggests the possibility of reverse photoreactions during retinal isomerization: all-*trans* (Batho) $\rightarrow$ 11-*cis* (Rh) and 13-*cis* (intermediate **K**) $\rightarrow$ all-*trans* (BR). We demonstrated the possibility of such transitions initiated in the early picosecond time range, and also estimated their quantum yield [17, 20, 23]. The three-pulse femtosecond absorption laser spectroscopy experiments were carried out for this purpose. The first actinic pulse with the absorption wavelength of retinal in the corresponding rhodopsin triggered the isomerization reaction. After the formation of the isomerization product (Batho in the case of Rh and intermediate **K** in the case of BR), a second pump pulse that was spectrally tuned to the absorption wavelength of this state was applied. This second pulse initiated a reverse photoreaction. The third, spectrally broad pulse of the white continuum made it possible to measure the amount of retinal that passed into the initial state and, thus, to

Fig. 3. Structure of the potential energy surfaces of type II rhodopsins by the example of (a) Rh and type I rhodopsins by the example of (b) BR, which shows the reaction pathway of their direct and reverse photoreactions in the femto- and picosecond time ranges. The structures of 11-*cis*, 13-*cis*, and all-*trans* retinals in the composition of (a) Rh and (b) BR molecules are also shown.

Fig. 4. Schemes of the elementary photoisomerization reaction in (1) type II rhodopsin Rh and type I rhodopsins: (2) BR and (3) ESR. The reactive (r subscript) and nonreactive (nr subscript) pathways of the excited state decay are shown. The lifetimes of intermediate states are given in parentheses.

estimate the quantum yields in Rh and BR: in the case of visual rhodopsin, $\phi(\text{Batho} \rightarrow \text{Rh}) = 0.15$ [17, 20]; and in the case of bacteriorhodopsin, $\phi(\text{K} \rightarrow \text{BR}) = 0.81$ [20].

How can one explain the significantly lower efficiency of the reverse photoreaction of visual rhodopsin compared to bacterial, given that the quantum yields of the direct photoreaction in them practically

coincide, despite the differences in the S_1 -PES of the excited state of these proteins? One possible explanation is that isomerization of all-*trans* retinal to 11-*cis* is more difficult than the 11-*cis* $\rightarrow$ all-*trans* retinal isomerization. Moreover, the quantum yield of the all-*trans* $\rightarrow$ 13-*cis* retinal transition is higher than that of the all-*trans* $\rightarrow$ 11-*cis* retinal, as shown in [37]. However, in Rh, the formation of 13-*cis* retinal as a result of a reverse photoreaction is unlikely, since the Rh chromophore center is able to perceive 11-*cis* retinal as a chromophore group. In other words, the efficiency of the reverse photochemical reaction of Rh and BR is also related to both the characteristics of all-*trans* and 13-*cis* retinals, respectively, and the influence of the protein environment. The lower efficiency of the reverse photoreaction of Rh, thus increasing the reliability of the direct photoreaction, can be considered as one of the arguments in favor of the selection of the 11-*cis* isomer as the chromophore group of all visual pigments of invertebrates and vertebrates in the course of convergent evolution.

Another advantage of the 11-*cis* isomeric form of retinal as a chromophore group of animal rhodopsins as G-protein-binding receptors is that 11-*cis* retinal is a potent antagonist ligand. Due to this, being in the 11-*cis* form, it prevents the dark activation of the molecule. This is a fundamentally important condition: to maintain the low thermal “dark noise” of the photoreceptor cell, which is necessary for its operation in low-light conditions. At the same time, photoisomerized all-*trans* retinal acts in the long-lived photolysis product of rhodopsin, metarhodopsin II, as a potent agonist that promotes the initiation of the phototransduction process. It seems obvious that the photobiological mechanism of light conversion into an information process in evolutionarily younger visual rhodopsins (type II rhodopsins) should be more reliable than the mechanism of light conversion into a photoenergetic process in evolutionarily more ancient microbial rhodopsins (type I rhodopsins).

CONCLUSIONS

Femtosecond laser spectroscopy makes it possible to understand, to a certain extent, the role of protein in the control of photoisomerization of the rhodopsin chromophore, retinal. The asymmetric environment of retinal in the reactive state of opsin facilitates reaching the CI region quickly and the passage of this region in a coherent mode with the formation of the primary isomerization product. In contrast, in the gas phase, this process occurs much more slowly and with a lower quantum yield [21, 36]. In the case of microbial rhodopsins, the heterogeneity of the dynamics of the excited state indicates a special role of the protein environment of retinal in the course of primary reactions, which is detected by the femtosecond spectroscopy methods. This heterogeneity is apparently due to the heterogeneity of the initial state of the protein,

which is determined by the amino acid environment of the retinal. This is likely to be the reason for several parallel photoactivated processes, some of which pass through the so-called nonreactive excited states that do not lead to retinal isomerization. Differences in the dynamics of primary photochemical reactions of types I and II rhodopsins can be explained by different initial isomeric forms of their chromophores (all-*trans* and 11-*cis* retinals, respectively), as well as by the influence of the protein environment on the chromophore, which can be heterogeneous.

The evolutionary origin of types I and II rhodopsins remains a matter of debate. It is believed that type I rhodopsins (for example, BR) appeared simultaneously with the emergence of the Earth's biosphere about 3 billion years ago, and type II rhodopsins, G-protein-binding receptors, about 1 billion years ago (for more details, see [22]). Most likely, they could arise independently, as a result of convergent evolution. In the course of this independent evolution, the chromophore centers, the intramolecular mechanism of retinal interaction with the nearest protein environment, and the isomeric form of a chromophore group have become somewhat different. In visual rhodopsin, this is manifested in the faster transition of retinal from an electronically excited state to the photoisomerization products and, with a lower probability, in a reverse photoreaction. For visual rhodopsins as photoinformational proteins in comparison with microbial ones as mainly photoenergetic proteins, such differences may have a functional meaning, increasing the reliability and efficiency of the direct reaction of 11-*cis* retinal isomerization triggering the process of phototransduction.

FUNDING

The study was financially supported by the Ministry of Science and Higher Education of the Russian Federation (agreement no. 075-15-2020-795, internal no. 13.1902.21.0027).

OPEN ACCESS

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

REFERENCES

1. S. Tahara, M. Singh, H. Kuramochi, et al., *J. Phys. Chem. B* **123**, 2507 (2019).
<https://doi.org/10.1021/acs.jpcc.9b00887>
2. S. Mukherjee, P. Hegemann, and M. Broser, *Curr. Opin. Struct. Biol.* **57**, 118 (2019).
<https://doi.org/10.1016/j.sbi.2019.02.003>
3. T. Ikuta, W. Shihoya, M. E. Sugiura, et al., *BioRxiv* preprint (2020).
<https://doi.org/10.1101/2020.04.14.04064>
4. O. P. Ernst, D. T. Lodowski, M. Elstner, et al., *Chem. Rev.* **114**, 126 (2014).
<https://doi.org/10.1021/cr4003769>
5. R. Hubbard and G. Wald, *J. Gen. Physiol.* **36**, 269 (1952).
<https://doi.org/10.1085/jgp.36.2.269>
6. R. W. Schoenlein, L. A. Peteanu, R. A. Mathies, et al., *Science, New Ser.* **254**, 412 (1991).
<https://doi.org/10.1126/science.1925597>
7. J. E. Kim, M. J. Tauber, and R. A. Mathies, *Biochemistry* **40**, 13774 (2001).
<https://doi.org/10.1021/bi0116137>
8. M. Garavelli, T. Vreven, P. Celani, et al., *J. Am. Chem. Soc.* **120**, 1285 (1998).
<https://doi.org/10.1021/ja972695i>
9. S. Hahn and G. Stock, *J. Phys. Chem. B* **104**, 1146 (2000).
<https://doi.org/10.1021/jp992939g>
10. M. A. Ostrovskii and T. B. Feldman, *Russ. Chem. Rev.* **81**, 1071 (2012).
11. R. A. Mathies, *Nat. Chem.* **7**, 945 (2015).
<https://doi.org/10.1038/nchem.2406>
12. J. Li, P. C. Edwards, and M. Burghammer, *J. Mol. Biol.* **343**, 1409 (2004).
<https://doi.org/10.1016/j.jmb.2004.08.090>
13. D. Polli, P. Altoè, O. Weingart, et al., *Nature (London, U.K.)* **467**, 440 (2010).
<https://doi.org/10.1038/nature09346>
14. O. A. Smitienko, I. V. Shelaev, F. E. Gostev, T. B. Fel'dman, V. A. Nadochenko, O. M. Sarkisov, and M. A. Ostrovsky, *Dokl. Biochem. Biophys.* **421**, 194 (2008).
15. O. A. Smitienko, M. N. Mozgovaya, I. V. Shelaev, F. E. Gostev, T. B. Feldman, V. A. Nadochenko, O. M. Sarkisov, and M. A. Ostrovsky, *Biochemistry (Moscow)* **75**, 25 (2010).
16. V. A. Nadochenko, O. A. Smitienko, T. B. Fel'dman, M. N. Mozgovaya, I. V. Shelaev, F. E. Gostev, O. M. Sarkisov, and M. A. Ostrovsky, *Dokl. Biochem. Biophys.* **446**, 242 (2012).
17. O. Smitienko, V. Nadochenko, T. Feldman, et al., *Molecules* **19**, 18351 (2014).
<https://doi.org/10.3390/molecules191118351>
18. A. Yabushita, T. Kobayashi, and M. Tsuda, *J. Phys. Chem. B* **116**, 1920 (2012).
<https://doi.org/10.1021/jp209356s>
19. T. Kobayashi, T. Saito, and H. Ohtani, *Nature (London, U.K.)* **414** (6863), 531 (2001).
<https://doi.org/10.1038/35107042>
20. T. B. Feldman, O. A. Smitienko, I. V. Shelaev, et al., *J. Photochem., Photobiol. B* **164**, 296 (2016).
<https://doi.org/10.1016/j.jphotobiol.2016.09.041>
21. H. V. Kiefer, E. Gruber, J. Langeland, et al., *Nat. Commun.* **10**, 1210 (2019).
<https://doi.org/10.1038/s41467-019-09225-7>
22. M. A. Ostrovskii, *Paleontol. Zh.* **51** (5), 103 (2017).
23. M. N. Mozgovaya, O. A. Smitienko, I. V. Shelaev, F. E. Gostev, T. B. Feldman, V. A. Nadochenko, O. M. Sarkisov, and M. A. Ostrovsky, *Dokl. Biochem. Biophys.* **435**, 302 (2010).
24. I. V. Shelaev, M. N. Mozgovaya, O. A. Smitienko, F. E. Gostev, T. B. Fel'dman, V. A. Nadochenko, O. M. Sarkisov, and M. A. Ostrovskii, *Russ. J. Phys. Chem. B* **8**, 510 (2014).
25. O. Smitienko, V. Nadochenko, T. Feldman, et al., in *Proceedings of the MSSMBS-2014 and DSCMBS-2014 International Workshops on Molecular Simulation Studies in Material and Biological Research* (Nova Science, New York, 2015), p. 29.
26. O. A. Smitienko, O. V. Nekrasova, A. V. Kudryavtsev, M. A. Yakovleva, I. V. Shelaev, F. E. Gostev, D. A. Dolgikh, I. B. Kolchugina, V. A. Nadochenko, M. P. Kirpichnikov, T. B. Feldman, and M. A. Ostrovsky, *Biochemistry (Moscow)* **82**, 490 (2017).
27. O. A. Smitienko, T. B. Feldman, L. E. Petrovskaya, O. V. Nekrasova, M. A. Yakovleva, I. V. Shelaev, F. E. Gostev, D. A. Cherepanov, I. B. Kolchugina, D. A. Dolgikh, V. A. Nadochenko, M. P. Kirpichnikov, and M. A. Ostrovsky, *J. Phys. Chem. B* **125**, 995 (2021).
28. D. F. Rodrigues, N. Ivanova, Z. He, et al., *BMC Genom.* **9**, 547 (2008).
<https://doi.org/10.1186/1471-2164-9-547>
29. Q. Wang, R. W. Shoenlein, L. A. Peteanu, et al., *Science (Washington, DC, U. S.)* **266** (5184), 422 (1994).
<https://doi.org/10.1126/science.7939680>
30. P. J. M. Johnson, A. Halpin, T. Morizumi, et al., *Nat. Chem.* **7**, 980 (2015).
<https://doi.org/10.1038/NCHEM.2398>
31. B. Hou, N. Friedman, M. Ottolenghi, et al., *Chem. Phys. Lett.* **381**, 549 (2003).
<https://doi.org/10.1016/j.cplett.2003.10.038>
32. P. J. M. Johnson, A. Halpin, T. Morizumi, et al., *Phys. Chem. Chem. Phys.* **16**, 21310 (2014).
<https://doi.org/10.1039/C4CP01826E>
33. H. Kandori, Y. Furutani, S. Nishimura, et al., *Chem. Phys. Lett.* **334**, 271 (2001).
[https://doi.org/10.1016/S0009-2614\(00\)01457-3](https://doi.org/10.1016/S0009-2614(00)01457-3)
34. R. Govindjee, S. P. Balashov, and T. G. Ebrey, *Biophys. J.* **58**, 597 (1990).
[https://doi.org/10.1016/s0006-3495\(90\)82403-6](https://doi.org/10.1016/s0006-3495(90)82403-6)
35. J. Tittor and D. Oesterheld, *FEBS Lett.* **263**, 269 (1990).
[https://doi.org/10.1016/0014-5793\(90\)81390-A](https://doi.org/10.1016/0014-5793(90)81390-A)
36. P. B. Coto, A. Strambi, and M. Olivucci, *Chem. Phys.* **347**, 483 (2008).
<https://doi.org/10.1016/j.chemphys.2008.03.035>
37. R. S. H. Liu and G. S. Hammond, *Photochem. Photobiol. Sci.* **2**, 835 (2003).
<https://doi.org/10.1039/B304027E>

Translated by G. Levit