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Femtosecond spectroscopic study of photochromic reactions of bacteriorhodopsin and visual rhodopsin

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ABSTRACT

Photochromic ultrafast reactions of bacteriorhodopsin (*H. salinarum*) and bovine rhodopsin were conducted with a femtosecond two-pump probe pulse setup with the time resolution of 20–25 fs. The dynamics of the forward and reverse photochemical reactions for both retinal-containing proteins was compared. It is demonstrated that when retinal-containing proteins are excited by femtosecond pulses, dynamics pattern of the vibrational coherent wave packets in the course of the reaction is different for bacteriorhodopsin and visual rhodopsin. As shown in these studies, the low-frequencies that form a wave packets experimentally observed in the dynamics of primary products formation as a result of retinal photoisomerization have different intensities and are clearer for bovine rhodopsin. Photo-reversible reactions for both retinal proteins were performed from the stage of the relatively stable photointermediates that appear within 3–5 ps after the light pulse impact. It is demonstrated that the efficiency of the reverse phototransition ***K***-form → bacteriorhodopsin is almost five-fold higher than that of the ***Batho***-intermediate → visual rhodopsin phototransition. The results obtained indicate that in the course of evolution the intramolecular mechanism of the chromophore-protein interaction in visual rhodopsin becomes more perfect and specific. The decrease in the probability of the reverse chromophore photoisomerization (all-*trans* → 11-*cis* retinal) in primary photo-induced rhodopsin products causes an increase in the efficiency of the photoreception process.

1. Introduction

Rhodopsins are retinal-containing transmembrane proteins that are capable of converting light energy for the implementation of biological functions of living organisms. Rhodopsins are classified into two groups – microbial rhodopsins (also known as Type 1 rhodopsins) and metazoan rhodopsins (also known as Type 2 rhodopsins) [1]. Microbial rhodopsins are involved in light-driven ion transport and sensory functions and present in many disparate eukaryotes and bacteria [2–4]. Metazoan rhodopsins mainly function as visual receptors [4,5].

Despite the differences in the mechanisms of the primary phototransformation processes, the microbial and metazoan rhodopsins are similar in structure and photochemical properties. They are a seven-transmembrane alpha-helix apoproteins, where retinal as a chromophore is covalently bound to a lysine in the seventh helix of the apoprotein [1,4]. It should be noted that in the case of microbial rhodopsins, a chromophore-binding domain contains all-*trans* retinal as a chromophore group, and in the case of metazoan rhodopsins, there is a 11-*cis* retinal.

All rhodopsins possess unique photochemical properties. Their photochemical reactions are among the fastest reactions in nature. An elementary act of chromophore photoisomerization in these proteins is measured in the femtosecond range [6–14] and exhibits a coherent character [8,13,15–19]. Moreover, the quantum yields of chromophore photoisomerization in microbial and metazoan rhodopsins and the temporal parameters of key intermediate formations as well are quite similar [11–13,20–26]. For example, the photoisomerization quantum yield is 0.64 for bacteriorhodopsin [20,21] and 0.65 for visual rhodopsin [22]. The formation time for the first relatively stable product for these retinal-containing proteins (***K*** intermediate for bacteriorhodopsin and bathorhodopsin for visual rhodopsin) is approximately 3 ps. Furthermore, in both the bacterial and photoreceptor cells, the physiological response to the light stimulus occurs in the millisecond time scale. For example, bacteriorhodopsin is deprotonated in the ***M*** intermediate and reprotonated upon formation of the ***N*** intermediate in a millisecond

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time scale [23,24]. In the course of visual rhodopsin phototransformation in the millisecond time scale, metarhodopsin II is formed, which is an agonist-bound active receptor state that is capable of G-protein transducin activation [25,26].

Along with the common photochemical properties of microbial and metazoan rhodopsins, there are a number of differences in the course of the retinal photoisomerization process in these retinal-containing proteins. In this regard, the photochemical properties of bacteriorhodopsin and bovine rhodopsin were studied most deeply among microbial and metazoan rhodopsins, respectively.

Bacteriorhodopsin is one of the Type 1 rhodopsins, which performs a photovoltaic energetic function, with targeted ion transport through the cellular membrane [27]. In bacteriorhodopsin, *trans*-retinal isomerizes into its 13-*cis* form under light. Bacteriorhodopsin phototransformation includes the formation of different intermediates within different time scale: ***I*** within 100 fs, ***J*** within 500 fs and ***K*** within 3 ps. The latter present the relatively stable intermediate formed, ***I*** consists of an excited state of bacteriorhodopsin and ***J*** is already present even at ground state [28]. For bacteriorhodopsin, a 3-state model was suggested [29–32] that postulated a small potential barrier on the S_1 potential energy surface along the isomerization coordinate (Fig. 1A). The coherent pattern of the retinal photoisomerization reaction has been demonstrated in coherent control experiments [18], and the dynamics of the coherent wave packet was observed in the emission band area of 800 nm [17]. The trends in the dynamics observed by tuning of the excitation wavelength allow an assignment of the wave packet dynamics to ground- and excited-state potential energy surfaces [33]. Coherent activation of hydrogen-out-of-plane wagging and backbone torsional modes concomitant with a deactivation of the reactive relaxation pathway were observed [34].

Visual rhodopsin is one of the Type 2 rhodopsins (G-protein-coupled receptor, GPCR), and it provides light perception as an information carrier [4]. In visual rhodopsin, 11-*cis* retinal photoisomerizes into its *trans*-form. As a result, by the 200 fs, the primary ground-state photoproduct is formed, which then relaxes to the bathorhodopsin (***Batho***) intermediate during 1.5–3 ps [16,35]. For visual rhodopsin, a 2-state model with a barrierless S_1 potential energy surface is suggested (Fig. 1B) [8,36]. In the case of visual rhodopsin, the dynamics of the coherent wave packet that is formed as a result of the femtosecond pulse absorption can be observed in the absorption signals of the ground-state reaction products, which have a time of coherence relaxation of approximately 1 ps [8,15,16].

The evolutionary relationship between microbial and metazoan rhodopsins is difficult to decide, because they show no clearly detectable identity at sequence level [37]. Herewith, the question is whether these two protein families diverge from an ancient common ancestral protein or have they converged on the same protein fold from independent origins, has been a subject of controversy for over 40 years [38]. Despite this fact, from the evolution point of view, comparative studies dealing with the differences and similarities are needed at this stage to understand the evolution of the photochemical properties of microbial rhodopsin (classified as old) and metazoan rhodopsin (classified as younger). There are a number of theoretical and experimental data sets that involve the comparative analysis of photochemical reactions of microbial and metazoan rhodopsins [9,19,39–44]. For example, some theoretical studies reported the differences in the wave packet dynamics that is formed during the time of the retinal photoisomerization reactions in visual rhodopsin and bacteriorhodopsin [39,40]. In visual rhodopsin, all the trajectories decays synchronize within the first 100 fs to the ground state [39], whereas the decay events in bacteriorhodopsin are widely distributed in time, i.e., between 50 and 500 fs [40]. The authors have suggested that the synchronous nature of the reaction dynamics in rhodopsin originates from weak perturbations of the protein surroundings and from dynamic regulation of volume-conserving motion of the chromophore [39]. Theoretical study [44] has shown that, by using light-responsive computer models of a eubacterial sensory rhodopsin and of a vertebrate visual rhodopsin, it is possible to identify a distinctive electronic character of the 11-*cis* chromophore that could have become an effective target for natural selection. In the

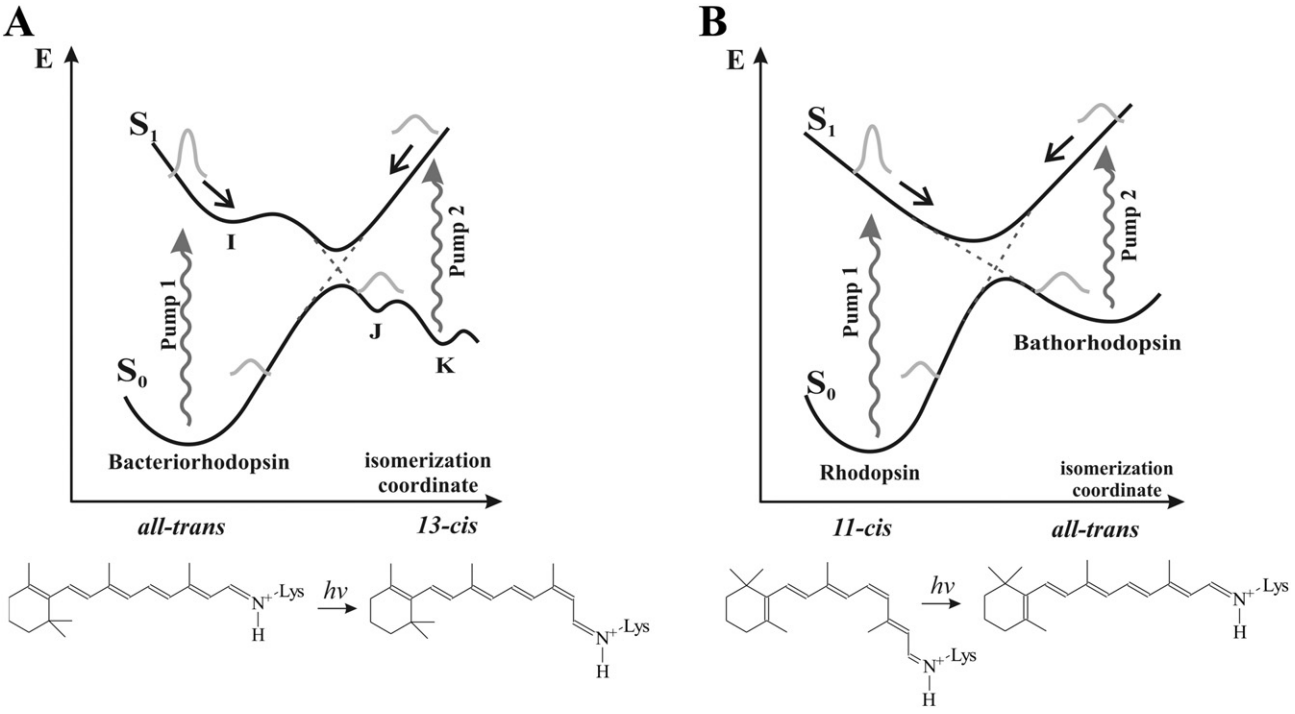

Fig. 1. Hypothetical schemes of potential energy surfaces participating in primary forward (*Pump 1*) and reverse (*Pump 2*) photochemical reactions of bacteriorhodopsin (A) and visual rhodopsin (B). Bacteriorhodopsin: 3-state model with a small potential barrier on the S_1 potential energy surface along the isomerization coordinate; visual rhodopsin: 2-state model with a barrierless S_1 potential energy surface.