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Investigation of the chromophore binding cavity in the 11-*cis* acceptable microbial rhodopsin MR ☆

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ABSTRACT

Rhodopsins are photoactive molecules functioning as photo-energy or photo-signal converters with the chromophore retinal. Recently we characterized a unique microbial rhodopsin (middle rhodopsin, MR) which can also bind 11-*cis* retinal besides all-*trans* and 13-*cis* retinal at a particular ratio. In this study, we investigated the structural characteristics around the retinal binding cavity in MR. The results suggest that the space of the retinal binding site of MR is less restricted to the retinal chromophore and the presence of the 11-*cis* conformer is regulated by the residues located around the retinal. Furthermore, although the triple mutant of MR has identical residues with the well-studied microbial rhodopsin bacteriorhodopsin (BR) within 5 Å from the retinal, the absorption maximum and retinal composition of MR did not reach those of BR, indicating that some long-range effect(s) (>5 Å) is also important for the maintenance of the chemical properties of MR.

1. Introduction

Biological molecules have characteristic chemical structures which are constructed of nucleobases in the case of the DNA or of poly peptides in the case of proteins, and these molecules play central roles inside the cells as respective functional polymers. Some of the proteins bind cognate chromophores, and these photoactive proteins are widespread in many kinds of organisms, as photo-energy or photo-signal converters. Amongst them, the retinal (vitamin A-aldehyde) containing proteins, the rhodopsins, are able to work as such photo-energy converters by generating a light-driven electrogenic ion transport across the cell membrane which is utilized by the ATP synthesis, and, thus, generates biochemical energy from light. Other rhodopsins are photo-signal converters by initializing visual signaling cascades, phototaxis responses or photo-induced transcriptional regulations [1–5].

All rhodopsin molecules have seven transmembrane α-helices which bind the chromophore retinal, and they are classified into two groups, microbial (type-1) and animal (type-2) rhodopsins [1,2]. Type-1 rhodopsins can be found in many organisms in the microbial world, in prokaryotes (bacteria and archaea) as well as in eukaryotes (fungi and algae). They can function as light-driven ion transporters, the most studied of which is bacteriorhodopsin BR, or as photosensory receptors, like SRII, in these microorganisms [6]. Recently, we found a new type-1 rhodopsin molecule from *Haloquadratum walsbyi* (named middle rhodopsin, MR), possessing characteristic properties of both BR and SRII [7]. MR has been identified as a transition molecule in the evolution from a light-driven proton pumping rhodopsin [as is bacteriorhodopsin (BR)] to a photosensor for negative phototaxis [sensory rhodopsin (SRII)] [7]. On the other hand type-2 rhodopsins, such as the visual pigments, are G-Protein Coupled Receptors (GPCRs) that are widespread in vertebrates and in invertebrates, and that mediate a GDP–GTP exchange reaction [8,9]. All of the biological functions of rhodopsin molecules are triggered by light absorption. Many type-1 rhodopsins have all-*trans* retinal in the original state, and this retinal chromophore is isomerized to 13-*cis* by photoactivation [10]. However, there exist also those, like BR or ASR, which accommodate both all-*trans* and 13-*cis* retinal in their original state, and which can be interconverted by illumination [10,11]. In contrast, many type-2 rhodopsins have 11-*cis* retinal in their original state, which is isomerized to all-*trans* by photoactivation [8,9]. Thus the retinal compositions of rhodopsins are distinctly different as being all-*trans* (13-*cis*) for type-1 or 11-*cis* (all-*trans*) for type-2 (Fig. 1). Interestingly, MR has a unique character of binding a mixture of all-*trans*, 13-*cis* and 11-*cis* retinal isomers in the dark. The

Abbreviations: DDM, *n*-dodecyl-β-D-maltoside; BR, bacteriorhodopsin; *Hw*BR, BR from *Haloquadratum walsbyi*; PSB, Protonated Schiff Base.

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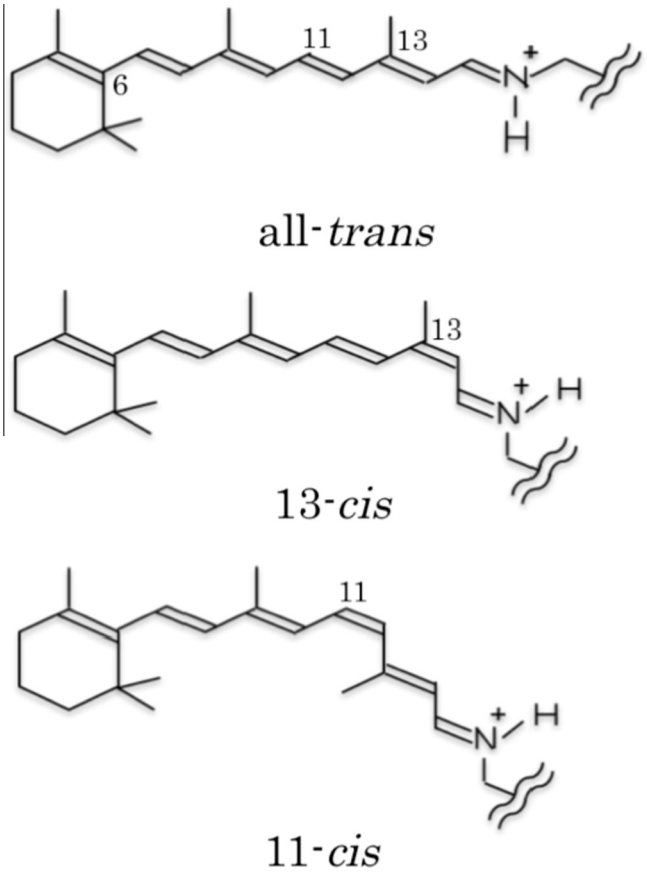

Fig. 1. Molecular structures of three types of retinal isomers: all-*trans*, 13-*cis* and 11-*cis*. In general, two of them, all-*trans* and 13-*cis* retinal, are utilized by microbial (type-1) rhodopsins, while the 11-*cis* and all-*trans* isomers are utilized by animal (type-2) rhodopsins.

absorption spectra and the photocycle of MR having all-*trans*, 13-*cis* or 11-*cis* have been determined by using high performance liquid chromatography (HPLC) and laser-flash spectroscopy [12]. Although, the crystal structure of MR is still unknown, the 11-*cis* configuration has a drastically different shape compared with all-*trans* retinal and would, therefore, suggest a large rearrangement in the peptide backbone. Interestingly, the retinal composition of MR is altered by illumination, shifting from all-*trans* to 11-*cis* retinal, suggesting the functional importance of the 11-*cis* and/or all-*trans* retinal chromophores [7]. This also indicates that MR might be a missing link in the evolution from type-1 rhodopsins (microorganisms) to type-2 rhodopsins (animals), because MR is the first microbial rhodopsin molecule discovered that has 11-*cis* retinal as a chromophore similar to type-2 rhodopsins.

The question is how to accommodate for this unique retinal composition? In this study, the bleaching and regeneration of the retinal chromophore of MR were performed, using various retinal isomers, and their time constants were compared. Additionally, the retinal composition of the wild-type MR was compared to that of various mutants in which up to three amino acid residues were replaced by their corresponding residues in BR. On the basis of these results and other findings for BR, SRI and SRII, the shape of the retinal binding cavity in MR is discussed.

2. Experimental

2.1. Sample preparation

The expression plasmid for wild-type MR was constructed as previously described [7]. The single, double and triple mutant genes were constructed by PCR using the QuikChange site-directed mutagenesis method (Stratagene, La Jolla, CA) as described previ-

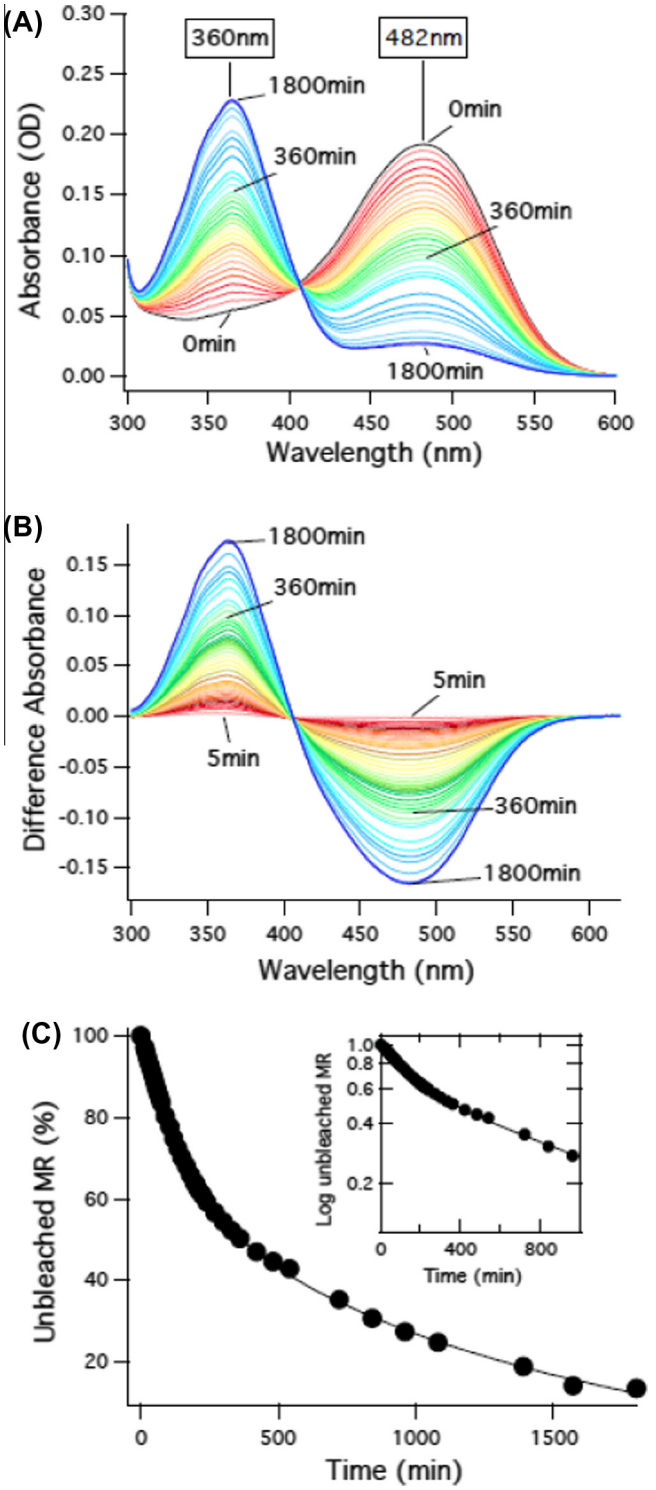

Fig. 2. (A) Reactivity of MR with hydroxylamine in the dark. The spectra were recorded at a number of time points after the addition of hydroxylamine (50 mM). The sample of 5 μM concentration was suspended in a buffer containing 50 mM Tris–HCl (pH 7.0), 1 M NaCl, and 0.05% DDM. The temperature was kept constant at 298 K. The peak of 360 nm represents the absorption of the retinal oxime. The peak at 482 nm corresponds to the absorption maximum of MR. (B) Difference spectra at each time point taking the spectra recorded at 0 min as a reference. (C) Bleaching of MR in the dark. The difference absorption at 482 nm are plotted against their respective times. The data were fitted with a double-exponential equation to estimate the rate constants.