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On the Role and Structural Outlook of Carboxy-Terminal Domain of Anabaena Sensory Rhodopsin

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Abstract

Anabaena Sensory Rhodopsin, ASR represents a small family of sensory rhodopsins among a vast family of microbial rhodopsins. In contrast to other microbial sensory rhodopsins, which are known to mediate photostimulated signal relay using cognate membrane transducer, ASR interacts to plausible soluble oligomeric transducer protein. Though the atomic resolution structure ASR is available, it only included only the transmembrane domain. Interestingly the available structure is remarkably different from other microbial sensory rhodopsins, with presence of water. Additionally, the sequence of missing cytoplasmic domain contains a series of positively charged Arginine residues in it. Authors have outlined the influence of this domain in addition to flexible structural outlook using Deep Mind's Nova Fold-2. Authors propose that charged amino acids are vital in ASR-transducer interaction. Subsequent attempts to explore the molecular basis of ASR and cognate transducer binding would be vital to understand the unique photo-mediated signal cascade in Anabaena PCC 7120.

Keywords: Microbial Rhodopsins; 7-TM [Transmembrane]; Retinal Chromophore; Anabaena PCC7120; Transducer; Photomediated Signaling

Abbreviations: ASR: Anabaena Sensory Rhodopsin; PSSP: Protein Secondary Structure Prediction.

Introduction

Microorganisms convert sunlight into biological signals or energy using microbial rhodopsins via intricate mechanism [1]. Vast family of such microbial rhodopsins are categorized as ion pumps with established model bacteriorhodopsin, proteorhodopsin etc [2-4].The seven transmembrane scaffolds of these membrane proteins are unique with presence of retinal chromophore [2-5]. The retinal chromophore is specific for the microbial family as all-trans while animal visual rhodopsin carry 11-cis retinal in ground state bound to lysine residue via a Schiff base [2-5]. Photomediated process either linked to energy conversion or initiation of intracellular signaling. Both roles are critical. In simplified notion, lower organisms utilize microbial for both functions, animals solely use a different family of rhodopsins, a unique subset of G-protein coupled receptor [6]. Among microbial rhodopsins, sensory rhodopsins are fewer compared to ion pumps and channelrhodopsins [4]. Well studied sensory rhodopsins from archaea are evident to mediate photo modulated response via interaction with their cognate transducer partner within the transmembrane. Such mechanism is quite distinct from mammalian visual rhodopsins, which primarily involve activation within the membrane and relay signal to cytoplasmic protein [4-6].

Anabaena sensory rhodopsin, ASR is a novel sensory microbial rhodopsin discovered in the freshwater cyanobacterium Anabaena sp. PCC7120 in contrast to the most found in a halophilic environment [7]. The gene encoding the membrane opsin protein of 261 residues, ASR and a smaller gene encoding a soluble protein of 125 residues, putative transducer are under the same promoter in a single operon [7].

ASR-Transducer Interaction

The proposed binding model of ASR-Transducer strongly supports the crucial role of the carboxyl terminus of sensory rhodopsin interacting with cytoplasmic transducer protein. Transducer forms stable tetramers in solution and isothermal microcalorimetry showed that the transducer tetramer binds to ASR with a stoichiometry of one ASRT tetramer per one ASR photoreceptor with a Kd of 8 μM in the highest affinity measurements [8]. The binding is specific but relatively weaker as probe by surface plasmon resonance study [7]. The isothermal titration calorimetry binding data with [8], and without carboxy terminus [unpublished data] along with receptor enriched membrane pull down assay outlined the critical role of carboxyl terminus in binding with cognate transducer [Trivedi, VD et al (unpublished data)]. Influence of carboxyl domain is evident as discussed under "role of carboxyl terminus" section.

Structural Insight

The crystal structure of ASR was the first reported for a eubacterial rhodopsin [9]. Overall topology is quite similar to those of the archaeal rhodopsins as depicted in Figure 1. The retinal binding is conserved. The retinal bound opsin absorbs at 549 nm, a pink colored photoreceptor. Unlike model ion pumps it carries serine residue as a strong indicative towards sensory role. The detailed biophysical characterization of ASR difference further outlined the role of carboxyl terminus [10]. However, the main divergence was observed on the cytoplasmic face of ASR [9], the expected interaction region with the putative soluble transducer ASRT [7,8]. The cytoplasmic face is structured with three ordered loops that are arranged very differently from their archaeal counterparts [9]. Another notable difference from the archaeal rhodopsins to ASR is that the interior region is far more hydrophilic, with numerous ordered water molecules forming hydrogen bonds with polar and charged residues [9]. Moreover, the atomic resolution structure of ASR does not account for much insight towards critical motif. For instance, the crystal structure of the ASR excludes the vital domain which is almost 12% of total sequence. The 35 amino acid carboxylic domain beyond 226, 227 through 261 [SRQTTGDRFAENTLQFVENITLFANSRRQQSRRRV] is missing in crystal structure.

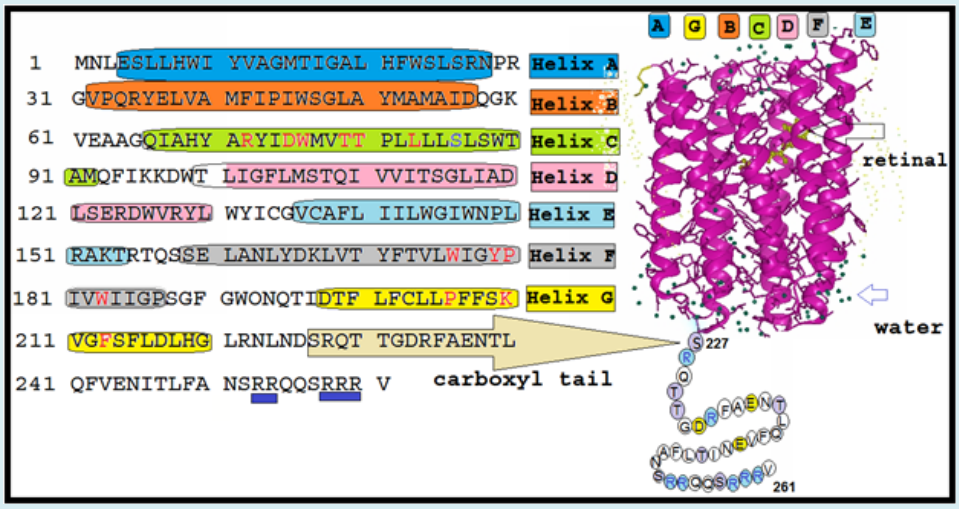

Figure 1: Anabaena Sensory Rhodopsin, ASR sequence and structural insight: [Left] with key residues. The seven transmembrane helices are color coded with retinal binding residues found in helices C, F and G are highlighted in red, and the non-ionizable residue [serine] found in sensory rhodopsins is blue. The positively charged motif at the C-terminus of ASR that is proposed to be the site of a protein: protein interaction is underlined as a blue box. The 7TM layout of A to G helix is outlined in color coded. [Right] Depiction of PDB 1XIO: [primarily within the 7TM region]. The atomic structure presented above excludes lipids, as reported in 1XIO [9]. The chromophore [all-trans retinal] is shown in yellow while the presence of water in structure is indicative of a novel hydrophilic core is shown by green dot. Sequence of the unresolved cytoplasmic domain is shown as hydroxyl containing residues, acidic and series of positively charged Arginine residue in unique color circles. The 35 amino acid cytoplasmic domain is highly rich in charged and polar residues and is likely to be involved with transducer interaction.

Role of Carboxy-Terminus

Influence on Photo cycle and chromic adaptation: The retinal chromophore upon photoisomerization from the all-trans to 13-cis form of microbial rhodopsins triggers a cyclic series of photochemical reactions (the photocycle). The photocycle of ASR is impacted by presence of transducer protein [7,10,11]. The photocycle of ASR is very slow compared to ion pumps [7]. The retinal isomerization is similar to model bacteriorhodopsin, but the hydrogen-bonding network around the Schiff base and cytoplasmic region is different [10,11]. Interestingly the light dark adaptation in ASR is opposite and influenced by transducer binding and/or presence of full length compared to 7TM truncated ASR [11-13]. These results suggest that the structural changes of ASR in the cytoplasmic domain play important roles in the activation of the transducer protein, and photochromic reaction is optimized for its sensor function [10].

With structural outlook and detailed biophysical characterization, ASR represented a dark state with primarily all-trans configuration [9,11]. A light adapted, primarily 13-cis state is blue shifted in spectrum with efficient reversible light induced interconversion between 13-cis and all trans unphotolyzed states of chromophore [9,11]. Furthermore, it has been shown that all of its photochemical reactions are also photochromic and that there is no photocycle similar to those observed in all other microbial rhodopsins. The relative amount of ASR with cis and Trans forms depends on wavelength of illumination, reflecting a photochromic feature quite similar to phytochrome pigments [11,14].

Subsequent study using full length [1-261] versus 7TM truncated [1-226] ASR shown as repressor of photo-induced regulation of the chromatic adaptive gene expression [15]. The repression is clearly influenced by carboxyl terminus sequence or by mutation in charged residues on carboxyl terminus [15]. It further outlined the significance of charged residues in carboxyl terminus.

Structural Outlook of Carboxy-Terminus

Most available resources for secondary structure prediction indicated that the missing carboxyl terminus sequence is likely to fold primarily into helix. Solution in protein secondary structure prediction, PSSP is important as tertiary structure is based on structural folds according to how secondary elements are packed and permutated [16,17]. The accuracy of protein secondary structure prediction directly impacts the accuracy of protein structure prediction, prediction of solvent exposure of amino acid residues and discrimination of structured from unstructured, intrinsically disordered protein regions [17].

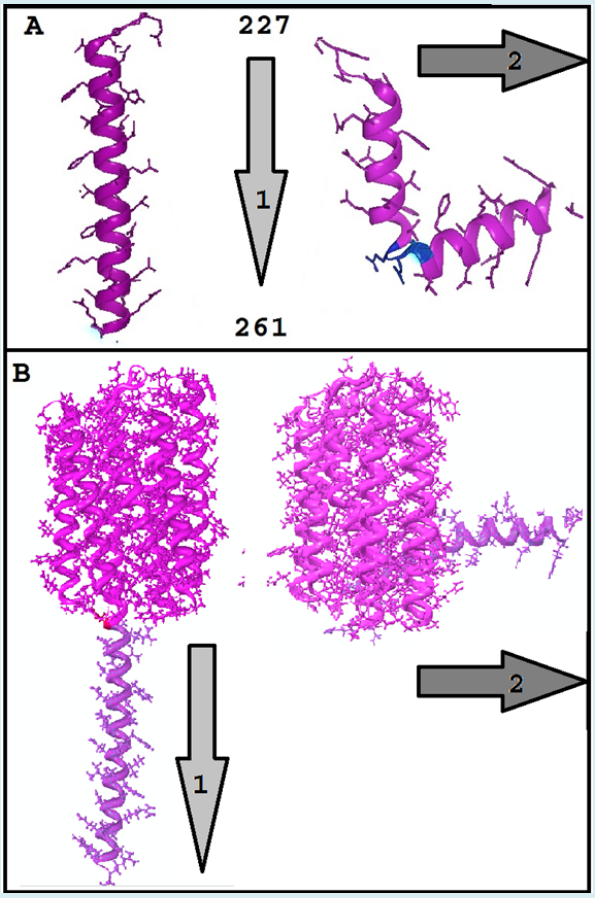

Figure 2: NovaFold AI predicted structure of carboxyl terminus domain of ASR [227-261] in upper panel A. Two [model 1 and 2] of the most distinct predicted structures out of 5 predicted models are included. Remaining structures are broadly similar to either group of predicted included [on left model A1: typical helical extension towards cytoplasmic side as indicated by downwards arrow and model 2 (on right) is two helices with twist (shown in blue) at position 244-246 [amino acids E-N-I] with turn at with tilt towards inner membrane]. In lower panel [B] complete sequence of ASR [1-261] predicted with two distinct models [1 of left with LDDT value of 94.12 and on right model 2 with LDDT value of 93.86]. The prediction was made using NovaFold AI [Powered by AlphaFold2 [18,19] under licensed DNAStar Inc]. Since, sequences of 227-230 is not ordered in either model, it is evident that first model [extending as helical motif downwards, away from membrane], while second model [B2] on right positioning the helical domain towards membrane side is remarkably distinct. Arrows indicate the direction of carboxyl motif in each panel A and B. The model on right side in panel B is altered with minor color change and rotation to provide better view without much influence on TM comparative position while visualization.

Highly successful NovaFold and NovaFold AI, an algorithm from Deep Mind uses two unique strategies to create highly accurate models that are unattainable though standard modeling methodologies [18,19]. It utilizes the Iterative Threading Assembly Refinement, I-TASSER protein structure prediction algorithm. It was used to reconstruct the missing carboxyl terminus motif, 227-261. Two of the models, out of 5 constructed structures are shown in Figure 2. Models A1 has a lower RMSD value compared to A2. It is interesting to note that the sequence of 227-230 [amino acids S-R-Q-T] sequence is not ordered in either model. Therefore it is likely that it could play a vital role towards membrane mimic environment, even though it is a clear extended helix. Another model shown in A2 reflects an unordered region between the sequences of 244-246 by splitting a continuous helix into two helical segments. Such conformation would provide more flexibility to interact with transducer protein.

Conclusion and Future Perspective

The AlphaFold2, AF2 [AI Deep Mind algorithm] generated model [Figure 2B (lower panel)] outlined two distinct models. Both are consistent with prediction of carboxyl terminus domain. Though truncated, 7TM only ASR excluding the carboxyl terminus and its mutant focused without sensory emphasis [20,21], but the role of excluded domain at carboxyl terminus can't be ignored. The ASR provided a unique pair involving membrane photoreceptor and cytoplasmic transducer protein that is distinct from other microbial sensory rhodopsin. It is analogous to the visual rhodopsin model. The proposed model highlights the role of charged residues in interaction with transducer. Downstream protein-protein crosstalk and phosphoryl transfer via transducer could impact the receptor binding as proposed earlier [22]. Authors plan to complete molecular dynamics study and anticipate to complements the outcome of modular docking study to probe ASR-transducer interaction via carboxyl terminus.

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