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Studying the spatial organization of membrane proteins by means of tritium stratigraphy: bacteriorhodopsin in purple membrane

A.V. Shishkov [a], A.L. Ksenofontov [b,*], E.N. Bogacheva [a], L.V. Kordyukova [b], G.A. Badun [c], A.V. Alekseevsky [b], V.I. Tsetlin [d], L.A. Baratova [b]

[a] *N.N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, 117977 Moscow, Russia*
[b] *A.N. Belozersky Institute of Physico-Chemical Biology and Moscow State University, 119899, Moscow, Russia*
[c] *Department of Chemistry, Moscow State University, 119899, Moscow, Russia*
[d] *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117871 Moscow, Russia*

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Abstract

The topography of bacteriorhodopsin (bR) in situ was earlier studied by using the tritium bombardment approach [Eur. J. Biochem. 178 (1988) 123]. Now, having the X-ray crystallography data of bR at atom resolution [Proc. Natl. Acad. Sci. 95 (1998) 11673], we estimated the influence of membrane environment (lipid and protein) on tritium incorporation into amino acid residues forming transmembrane helices. We have determined the tritium flux attenuation coefficients for residues 10–29 of helix A. They turned out to be low (0.04 ± 0.02 Å^{-1}) for residues adjacent to the lipid matrix, and almost fourfold higher (0.15 ± 0.05 Å^{-1}) for those oriented to the neighboring transmembrane helices. We believe that tritium incorporation data could help modeling transmembrane segment arrangement in the membrane.

Keywords: Bacteriorhodopsin; Transmembrane helix; Tritium stratigraphy; Attenuation coefficient

1. Introduction

The elucidation of the spatial and functional organization of biological membranes is one of the most important tasks in cellular biology. Specifically, membrane proteins represent extraordinary technical difficulties to the mostly common used in structural biology methods such as crystallography, solution and solid-state NMR spectroscopy.

In 1988, the topography of bacteriorhodopsin (bR) in situ was studied by using the tritium bombardment approach [1]. In essence, in this approach a specially prepared target maintained at the temperature of liquid nitrogen is exposed to a beam of thermally activated (2000 K) tritium atoms to substitute hydrogen for tritium [2]. The action of "hot" tritium atoms on the rapidly frozen aqueous suspension of native purple membranes revealed bR segments localized to the membrane interior and those lain outside or close to membrane surface. The bombarding resulted in 10-fold lower incorporation of tritium in transmembrane helices in comparison with loops. Now, having the X-ray crystallography data of bR at atom resolution [3], we address that work again to analyze the values of tritium incorporation in the residues of membrane-spanning segments as a function of their position in the membrane. In this respect, the tritium bombardment approach as was offered earlier [4] may be regarded as "tritium stratigraphy."

2. Experimental

The coordinate system of the bR trimer (Ref. [3], PDB accession number 1 BRR) was transformed so that z-axis was arranged along the membrane normal, and z coordinates of NZ atoms of Lys 40, 41 and 159, and OD2 atoms of Asp 36, 38, 102 and 104 being in the vicinity of lipid headgroups became close to zero. Then z coordinates of CA atoms of each amino acid residue indicated its distance from the cytoplasmic side of the membrane.

The accessible surface areas of the amino acid residues in bR trimer were calculated using WhatIf package of programs for tritium atom effective radius of ~ 0.9 Å [5].

* Corresponding author. Tel.: +7-95-939-54-08; fax: +7-95-939-31-81.
E-mail address: ksenofon@belozersky.msu.ru (A.L. Ksenofontov).

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3. Results and discussion

Bacteriorhodopsin is a membrane protein comprising seven transmembrane α-helices (named A–G) and extramembrane loops. It is organized into trimers in situ (Fig. 1, inset). The experimental data of the work [1] allowed us to analyze the values of specific radioactivities of the amino acid residues forming transmembrane helices A, B, C, F and G. Here are the results obtained with the most fully characterized helix A.

Helix A is located at the periphery of the bR protein complex and is surrounded by lipid matrix as well as protein helices B and G (Fig. 1). It crosses the membrane at an angle of 23° to the membrane normal.

We have studied the dependences of ratios of the amino acid residue-specific radioactivities normalized by the tritium incorporation probability coefficients and the residue accessible surface areas upon the positioning of the residues in the membrane using the general formula

$$(A_i/k_iS_i)/(A_j/k_jS_j) = \exp(-\mu_i(x_i - x_j)),$$

where A_i, A_j are the specific activities of amino acid residues i and j (taken from the work [1]); k_i, k_j — the corresponding tritium incorporation probability coefficients [6]; S_i, S_j — the residue accessible surface areas; x_i, x_j — the distances of the residue CA atoms from the cytoplasmic side of the membrane; μ_i — the tritium flux attenuation coefficient of ith residue.

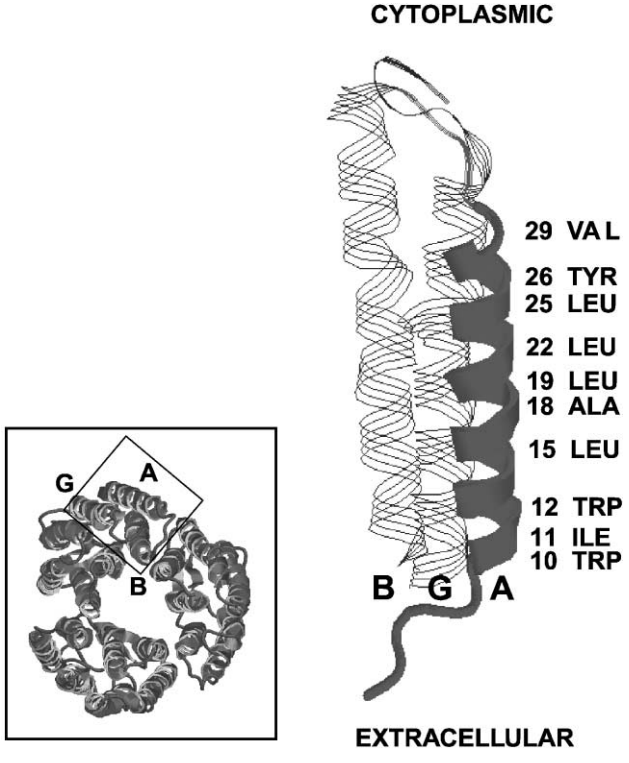

Fig. 1. Cartoon and strands representation of the bR fragment spatial structure (Ref. [3], PDB accession number 1BRR; visualization with the program Ras Win Molecular Graphics). Helix A sterical surroundings are lipid molecules and protein helices B and G. The residues adjacent to the lipid matrix are indicated. Inset: bR trimer viewed along the bilayer normal. The fragment of the bR monomer including A, B and G helices is in frame.

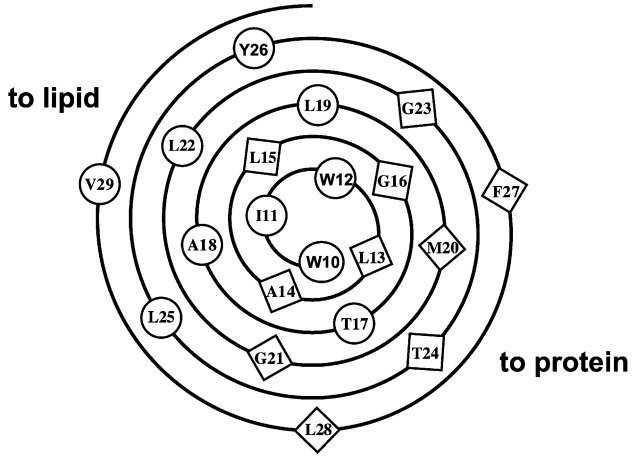

Fig. 2. Schematic representation of helix A (the butt reamer) indicating amino acid residues numbers. The residues having the attenuation coefficients equal to 0.04 ± 0.02 Å^{-1} are denoted by circles; 0.15 ± 0.05 Å^{-1}, by diamonds.

To analyze in detail the influence of environment (lipid and protein) on tritium incorporation, we have determined the tritium flux attenuation coefficient μ_i for amino acid residues 10–29 of helix A. The residues could be combined in two groups in accordance with their attenuation coefficients, and each group turned out to be located on a certain side of the helical surface (Fig. 2). The attenuation coefficients are low (0.04 ± 0.02 Å^{-1}) for residues positioned in the vicinity of the lipid matrix. This value is close to that we have determined earlier for the acylglicerol residue region of the liposome bilayer [7]. The attenuation coefficients for another group of residues oriented to the adjacent protein helices are almost fourfold higher (0.15 ± 0.05 Å^{-1}). Apparently, protein helices shield the neighboring helix surface from the tritium flux substantially, while the lipid matrix is almost transparent for hot tritium atoms. It is in accordance with the earlier results: the penetration of hot tritium atoms into globular proteins does not exceed 5 Å [2], whereas lipid membrane attenuates the tritium flux only partly [8].

Thus, the values of attenuation coefficients for transmembrane helix residues indicate the nature of their sterical surrounding: if it is a protein or a lipid. Therefore, basing on the tritium incorporation data about a segment of a transmembrane protein, we could suppose about its arrangement in the membrane.

4. Conclusion

Tritium bombardment data could be used in protein structure modeling to distinguish the transmembrane helix

areas oriented to the lipid membrane from those located near the protein secondary structure elements. Knowledge of the tritium intramolecular distribution combined with the structural predictions allows to conclude about the number, lengths, and also the arrangement of membrane-spanning α-helices.

Acknowledgements

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