Review Letter

RECENT FINDINGS IN THE STRUCTURE–FUNCTIONAL CHARACTERISTICS OF BACTERIORHODOPSIN

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1. Introduction

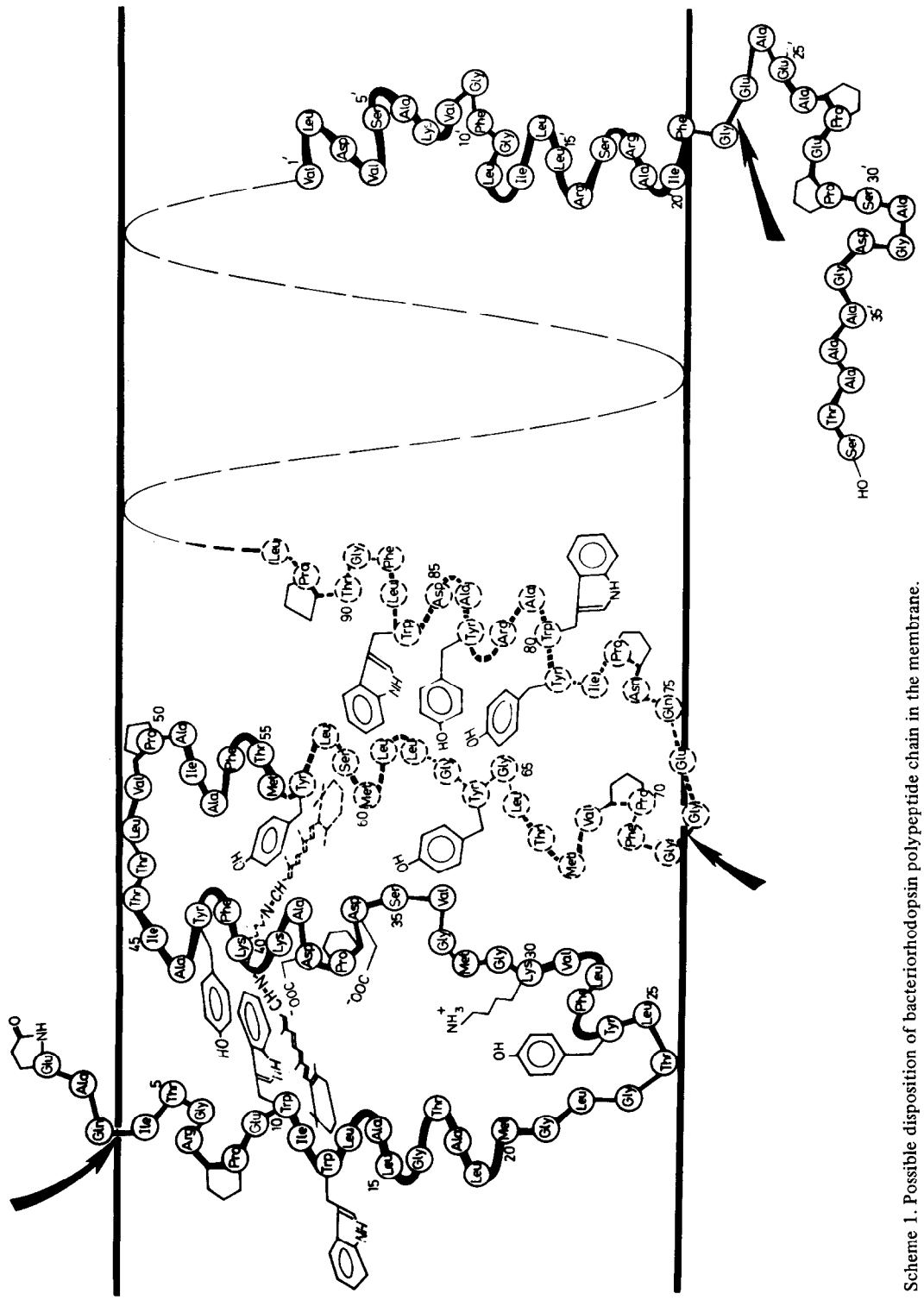
As is well known, bacteriorhodopsin is the key element in the non-chlorophyllic photosynthesizing system of *Halobacterium halobium.* This comparatively small membrane chromoprotein (mol. wt 22 000–26 000) fulfills the function of a primary proton translocase, utilizing the energy of the light quanta absorbed by a retinal prosthetic group for active transport of the protons [1,2]. However the exact details of the mode of action of this unique molecular machine still remain obscure, due largely to the absence of precise and reliable data on its structure in the membrane and the rearrangements it undergoes in functioning. The membrane packing of the bacteriorhodopsin molecule as elucidated in the elegant work of Henderson and Unwin [3] has excited one's fantasy as to the way it acts, leaving unanswered the question of the actual build up of its 'active center' and of the arrangement of the functional groups in the molecule.

In this study an attempt has been made to give a fuller chemical description of bacteriorhodopsin, including its native structure localized within purple membrane and to obtain independent data confirming and elaborating Henderson and Unwin's model.

3. Results and discussion

According to the model, bacteriorhodopsin is the form of somewhat elongated helices spanning the entire width of the membrane, more or less perpendicularly to the surface, and one could try to identify the rather obscure chain segments near the membrane surface that were not very clearly observed by these authors in their electron diffraction study. To this end we decided to treat the native purple membrane with nonpermeating reagents, primarily proteolytic enzymes [4,5]. Simultaneously we started an investigation into the primary structure of bacteriorhodopsin, particularly its functionally important centers. As a preliminary result of this analysis we have arrived at scheme I, which, although containing much conjecture, should give a generally correct idea of the topography of the active groups in bacteriorhodopsin. In the following a brief description of the results obtained up till now is presented.

Determination of the primary structure of bacteriorhodopsin proved to be a quite difficult task, owing to the extraordinary hydrophobicity of both the protein and its fragments, to cope with which required the development of novel procedures. By which it became possible to determine the sequence of the most important, N-terminal region, and also of a comparatively large part of the C-terminal region. The results are shown in Scheme 1, where the amino acid residues identified with sufficient reliability are given in bold faced type and the regions requiring additional proof by dotted lines. It turned out that the N-terminus of bacteriorhodopsin is pyroglutamic acid, making clear why attempts to identify it by conventional means [6] were unsuccessful. The chromophore-bonded lysine residue is 41, i.e., it is relatively close to the N-terminus, whereas the retinal-carrying segment Asp–Pro–Asp–Ala–Lys–Lys basically coincides with that proposed by Bridgen and Walker [7].



Scheme 1. Possible disposition of bacteriorhodopsin polypeptide chain in the membrane.

If we compare the segments of known primary structure with Henderson and Unwin's model, assuming a given membrane thickness and estimating the helicity of the peptide chain according to Chou and Fasman [8] on the basis of the amino acid sequence, it turns out that the fragments 25–30, 39–45, 51–62, 66–69, 78–88, and also 1′–8′ and 12′–21′, marked by bold-faced connecting lines, have the greatest tendency for α-helix formation, whereas the remaining fragments would be expected to be non-helical.

Short-time papain treatment of purple membranes at low (1:200) enzyme/bacteriorhodopsin ratios cleaves off a 17 amino acid fragment from the C-terminus. At least five of the terminal amino acids can also be cleaved by carboxypeptidase A. Hence the C-terminal region of bacteriorhodopsin must be exposed to the aqueous phase rather than inside the membrane.

The existence of a quite long water-exposed 'tail' in the bacteriorhodopsin molecule is not unexpected if one bears in mind that this 'tail' is rich in glutamic and aspartic acid residues and hence quite hydrophilic.

At higher (1:10) enzyme/substrate ratios and more prolonged digestion, the bacteriorhodopsin molecule is cleaved in two more places, namely, at the Gln–Ile (positions 3 and 4) and the Gly–Gly (72–73) bonds.

We thus see that in addition to the C-terminal region, the N-terminal region and a segment containing the Gly–Gly (72–73) residues are also accessible to the membrane non-permeating enzymes, although with greater difficulty.

Support for the projection of the N-terminus into the aqueous phase can be seen in the presence of the tripeptide < Glu–Ala–Gln in the supernatant liquid.

Regarding the two large fragments formed as the result of the splitting of the Gly–Gly (72–73) bond, a more detailed analysis showed that these fragments, containing Ile and Gly at the N-termini, remain attached to the membrane. In harmony with this are the data obtained in electrophoresis of the papain-treated purple membrane in polyacrylamide gel in the presence of sodium dodecylsulfate.

From the fact that the Gly–Gly (72–73) segment must be in the region of the membrane surface and, bearing in mind that the part of the bacteriorhodopsin molecule embedded in the membrane should consist of some 200–220 amino acid residues, i.e., on an average of about 30 amino acid residues for each of the seven rods and their connections, it follows that the region of the polypeptide chain near residue 70 should constitute the connection between rods 3 and 4 counting from the N-terminus and that the first three rods should have somewhat less and the last 4 somewhat more than 30 amino acid residues.

A number of tentative conclusions concerning the structure–function relation of bacteriorhodopsin can be drawn from the schematic representation in the figure. According to Skulachev et al. [9] the papain treatment has practically no effect on the ability of bacteriorhodopsin to act as a proton pump, either directly in the purple membranes or in proteoliposomes derived from them. Hence the bacteriorhodopsin molecule most probably retains almost all of its activity when split into the two fragments and the eliminated C-terminal and N-terminal segments are apparently not essential for the transmembrane proton transport function. Whether this is the result of retention of the native, membrane conformation of the protein due to non-covalent interaction between the rods, or the retinal-containing N-terminal segment is itself responsible for the whole activity of the protein will be the subject of a further study. The aldimino group connecting Lys-41 with the retinal residue should be about 15–17 Å from the membrane surface through which is projecting the N-terminus of the chain. Since the first, rapid phase of proton efflux from the membrane at the surface facing the periphery of the bacterial cell and the second, slower phase absorption of the protons from the surface facing the cell interior [10], we have reason to assume that the aldimino group should lie close to the outer-facing surface of the membrane. Such an assumption is confirmed in studies of the location of retinal residues in the membrane by both optical and electrical means (Bogomolni, R. A., personal communication).

It is to be noted that the degree of helicity of the elucidated bacteriorhodopsin segments is about 50%, which is substantially less than that (70%) ascribed to the entire molecule on the basis of CD [11] and X-ray data [12,13]. From this it follows that the as yet structurally unelucidated rods should have a particularly high degree of helicity. In fact, these rods are impoverished of amino acids that are conspicuously rare in helices.

The chain segments which, according to the

proposed scheme, are near the membrane surface do not contain basic amino acids capable of interacting with the negatively charged phosphate and sulfonyl groups of the lipids at the membrane/water interface (cf. Konishi et al. [14]). Hence, such interactions apparently play no part in the packing of at least some of the rods in the membrane.

The Lys-41 residue carrying the retinal aldimino group is close to the end of a helical segment. This will permit the local conformational changes, that probably accompany both the *cis–trans* isomerization of the retinal C_{13} double bond and the photochemical transformation cycle in bacteriorhodopsin. Already Bridgen and Walker [7] have shown that two aspartic acid residues must be at a rather short distance along the chain from the aldimino group. According to the model proposed these two residues should form part of the direct environment of this group. They should not only interact with the latter, thus affecting the spectral characteristics of bacteriorhodopsin (cf. [15,16]) and the pK_a of the aldimine, but very likely constitute part of the active center of the protein participating directly in the proton transport.

We still do not know just how the retinal residue is positioned within the system of its surrounding protein rods (the scheme shows two such possible orientations) nor the relative locations of these rods. Therefore, it would be premature to discuss the possible participation of other groups in the active center. However, it could hardly be accidental that several tryptophan residues are located in the region of possible contact with retinal. Also noteworthy is the location of at least four tyrosine residues and one lysine residue in the region between the aldimino group and the inner facing surface of the membrane. These residues may possibly take part at one of the slow stages of the photochemical cycle in the proton transport chain to the aldimine group.

Clearly this analysis has only brought us to the threshold of solution of the problem. But the chemical findings presented here, opening a fold of the curtain before the structure–functional relation, are in harmony with the majority of the known facts concerning this interesting protein and hopefully will serve as a stimulant for further research.

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