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*Hypothesis*

# The leader peptides from bacteriorhodopsin and halorhodopsin are potential membrane-spanning amphipathic helices

Gunnar von Heijne and Jere P. Segrest*

*Research Group for Theoretical Biophysics, Royal Institute of Technology, S-100 44 Stockholm, Sweden* and **Departments of Pathology, Biochemistry and Medicine and Atherosclerosis Reserach Unit, University of Alabama at Birmingham Medical Center, Birmingham, AL 35294, USA*

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We show that the N-terminal leader peptides from the bacterial membrane proteins bacteriorhodopsin and halorhodopsin can be expected to form amphipathic α-helices with a highly hydrophobic nonpolar face and a narrow, negatively charged polar face. This finding is discussed in terms of a model for the integration of these proteins into the bacterial membrane.

Bacteriorhodopsin; Halorhodopsin; Membrane protein; Leader peptide; Amphipathic helix

## 1. INTRODUCTION

Bacteriorhodopsin (bR) from the purple membrane of *Halobacterium halobium* is one of the best characterized of all complex transmembrane proteins. It has seven $\alpha$-helical membrane-spanning segments connected by short loops protruding into the aqueous phase [1]. The N- and C-termini of bR face the extracellular medium and the cytosol, respectively [2]. In vivo, bR is made with a 13-residue long leader peptide [3] of unknown function that does not have the usual features of a typical secretory signal peptide [4]. Here we show that this leader, and the recently sequenced 21-residue leader from the related *H. halobium* halorhodopsin (hR) protein [6], have the information in their amino acid sequences to form amphipathic helices [1,2,5] containing a highly hydrophobic, nonpolar face and a relatively narrow, negatively charged polar face. Based on this observation, we suggest that both bR and hR are inserted into the membrane as four 'helical hairpins' [7,8], thus initially forming eight-helix bundles across the membrane.

Correspondence address: G. von Heijne, Research Group for Theoretical Biophysics, Royal Institute of Technology, S-100 44 Stockholm, Sweden

## 2. RESULTS AND DISCUSSION

Helical wheel plots of the bR and hR leader sequences (fig.1) indicate that, in both cases, the more polar residues cluster in a region representing approximately one-quarter of the circumference of the helix. The remaining three-quarters of the helix is composed of nonpolar residues. This structural motif is similar in topography to known amphipathic membrane-spanning $\alpha$-helices [10].

Since helical wheel plots do not always provide statistically valid information [9], we carried out more detailed analyses. First, the hydrophobic moment [10,11] (a measure of the amphipathic character or 'sidedness' of a stretch of regular secondary structure) was calculated as a function of the angle $\delta$ between successive residues in the sequence. The resulting plot for bR (fig.2) shows a



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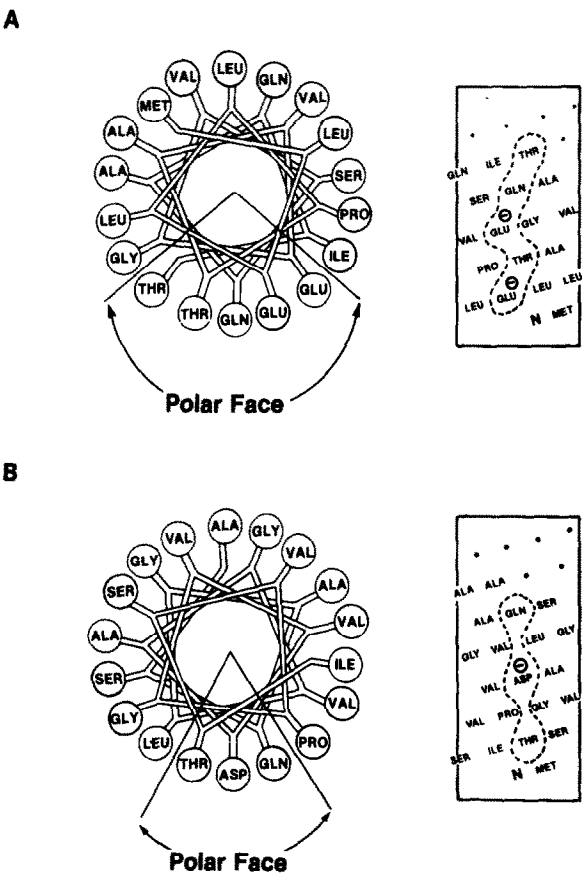


Fig.1. Helical projections of the bacteriorhodopsin (A) (bR 1–18) and halorhodopsin (B) (hR 3–20) leader peptides. The polar patches are shaded. Bacteriorhodopsin is cleaved preferentially after residue 13, halorhodopsin after residue 21.

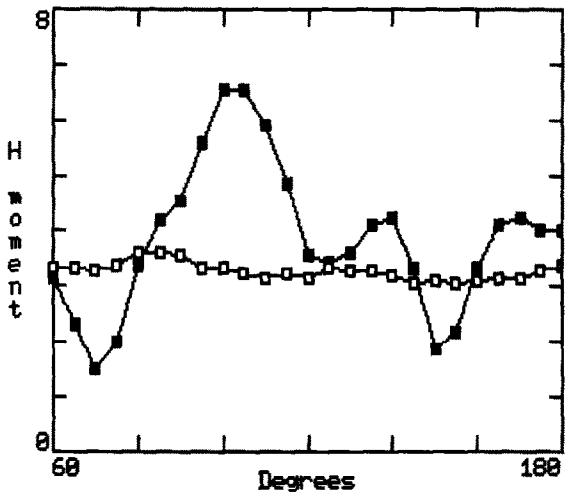


Fig.2. Total hydrophobic moment as a function of the angle $\delta$ between successive residues. The hydrophobic moment $\mu$ is calculated as [10]:

$$\mu = [(\Sigma H_n \sin(\delta n))^2 + (\Sigma H_n \cos(\delta n))^2]^{1/2}$$

where $H_n$ is the hydrophobicity of the $n$-th residue and $\delta = 100°$ for an ideal $\alpha$-helix and $\delta = 160–180°$ for a $\beta$-structure. For each value of $\delta$, the summations in the expression were carried out over 11–18 residues, and the maximum value of $\mu$ in this range was plotted. As a control, 100 scrambled copies of the bR leader peptide were generated by random shuffling of the residues in segment 1–18 and subjected to the same analysis. (■) bR leader, (□) mean values for randomized sample.

pronounced peak at $\delta = 100°$, corresponding to an ideal $\alpha$-helix encompassing residues 1–15 (the secondary peaks at $\delta = 140$ and $\delta = 170°$ are typical for a short amphipathic helix [11]). A similar plot of the mean hydrophobic moment for a sample of 100 randomly scrambled versions of the bR leader shows no similar peak. The hR leader, with only a single charged residue, has a corresponding, though less pronounced, peak at $\delta \approx 100°$; its maximum hydrophobic moment ($\mu = 5.2$, not shown) is obtained for residues 3–20.

Second, we compared the maximal hydrophobic moments at $\delta = 100°$ of the wild-type leaders (residues 1–15 in bR and 3–20 in hR) with the distributions found for 1000 randomly scrambled versions of these two peptides (including residues 1–18 for bR and 1–21 for hR). Only 2% of the scrambled bR copies had a hydrophobic moment larger than that of the wild type; the corresponding value for hR was 18%. We conclude that the bR leader, and apparently the hR leader as well, are evolutionarily selected to be able to form amphipathic helices; these are of the narrow polar face variety typical for transmembrane helical bundles and are of a length sufficient to span a lipid bilayer.

According to the helical hairpin hypothesis [7,8], integral membrane proteins with multiple membrane-spanning segments insert into the membrane by pairing neighboring hydrophobic or amphipathic $\alpha$-helices to form nonpolar 'hairpins' that penetrate the membrane. The bR and hR leaders can easily be envisaged to form such hairpins together with the first membrane-spanning segment of the respective mature proteins, which would result in an initial eight-helix bundle. We

note that a centrally placed negatively charged residue is found in both leaders ($Glu_{10}$ in bR and $Asp_{11}$ in hR); these might interact with positively charged residues in one of the other membrane-spanning amphipathic helices. After membrane insertion, proteolytic removal of the leader peptide from the extracytosolic side would generate the mature form of the protein with the N-terminus outside.

This picture is supported by the observation that *H. halobium* spheroplasts synthesize and accumulate the bR precursor in a form that is integrated into the membrane in the correctly folded conformation, as judged by sensitivity to various proteases and retinal attachment [12]. Interestingly, *Staphylococcus aureus* protease cleaves the bR leader after $Glu_3$ but not after $Glu_{10}$, suggesting that the latter is buried in the membrane.

There are no published data to indicate whether bR can be reconstituted into a functional form without its leader peptide and thus whether the bR (and by implication the hR) leader is absolutely required for correct assembly. If not found to be absolutely required, the leaders may serve to speed up or otherwise facilitate membrane insertion.

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