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Prediction of the mutation-induced change in thermodynamic stabilities of membrane proteins from free energy simulations

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Abstract

Comparative protein structure modeling and free energy perturbation simulation have been applied in a consecutive manner to investigate the mutation-induced stabilization of membrane proteins (MPs) in aqueous solution without knowledge of their three-dimensional structures. The calculated difference in protein solvation free energy between the wild type and a mutant compares well with their relative thermodynamic stabilities in solution. For monomeric MPs, a mutant reveals a higher stability than the wild type if the calculated solvation free energy indicates a favorable change. On the contrary, for oligomeric MPs the stability of a mutant increases as the solvation free energy of a mutated monomer becomes less favorable, indicating that the oligomeric MP mutant would be stabilized in solution due to the reduced desolvation cost for oligomerization. The present computational strategy is expected to find its way as a useful tool for assessing the relative stability of a mutant MP with respect to its wild type in solution.

Keywords: Membrane proteins; Protein engineering; Solvation free energy; Molecular dynamics; Free energy simulation

1. Introduction

Although 25% to 30% of the proteins coded by the human genome are embedded in membranes, only about 2% of the known protein structures belong to the category of membrane proteins (MPs) [1]. This disparity in structural information stems from the instability of MPs in aqueous solution due to the possession of large hydrophobic patches on their surfaces. Nevertheless, considering the fundamental biological functions of MPs and their importance as drug targets, the development of methods for overcoming such a technical barrier in the structural determination of MPs represents the next frontier of structural genomics [2].

One way to maintain the MPs stable in aqueous solutions is to use detergent molecules that equilibrate between a monolayer covering the transmembrane surface of the protein and protein-free micelles [3]. However, the detergents often inhibit the crystallization of an MP in its active form, and the size of MP-detergent mixed micelles may be too large for NMR experiments [4]. An alternative way is to identify mutations that can enhance the stability of MPs in solution without involving loss of activity and significant structural change [5]. Although such mutations have been found in several MPs [6–10], most of them have been identified from randomly generated mutant libraries in which more than 90% of mutant proteins exhibited decreased stability. Therefore, researchers in structural biology are in urgent need of a rational engineering method for finding mutant MPs with the desired physical characteristics.

In this study, we propose to use comparative protein structure modeling and free energy perturbation (FEP) calculation in a consecutive way for predicting the stability-enhancing point mutations of MPs. Based on this method, we calculate the solvation free energy (SFE) differences between the wild type (WT) and mutants of

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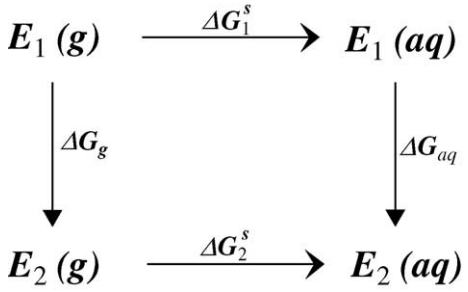

Fig. 1. Thermodynamic cycle used in calculating the difference in solvation free energies ($\Delta\Delta G_{sol}$) between the WT (E_1) and a mutant protein (E_2).

bacteriorhodopsin [11], OmpF porin [12], M13 coat protein [13], and lactose permease [14] in the monomeric states. Although the X-ray structures of these proteins have already been reported, we use the homology-modeled structures because we aim to propose a computational method for predicting the stability-enhancing point mutations in the membrane proteins for which 3D structures are unknown due to the instability in solution. The results will then be compared with the experimental thermodynamic stabilities to assess the predictive power of our computational strategy.

2. Computational methods

The peptide sequences of the targets (bacteriorhodopsin, OmpF porin, and M13 coat protein with accession numbers P02945, P02931, and P03617, respectively) and their respective templates (archaerhodopsin 1, phosphoporin, and IF1 coat protein with accession numbers P19585, P02932, and P03619, respectively) were retrieved from the SWISS-PROT protein sequence data bank (http://us.expasy.org/sprot). Sequence alignments between the targets and the templates were then derived with the CLUSTAL W package [15]. Based on these sequence alignments and X-ray structures of archaerhodopsin 1 [16], phosphoporin [17], and IF1 coat protein [13] with PDB ID's 1UAZ, 1PHO, and 1IFK, respectively, the 3D structures of bacteriorhodopsin, OmpF porin, and M13 coat protein were constructed using the MODELLER program of version 6v2 [18].

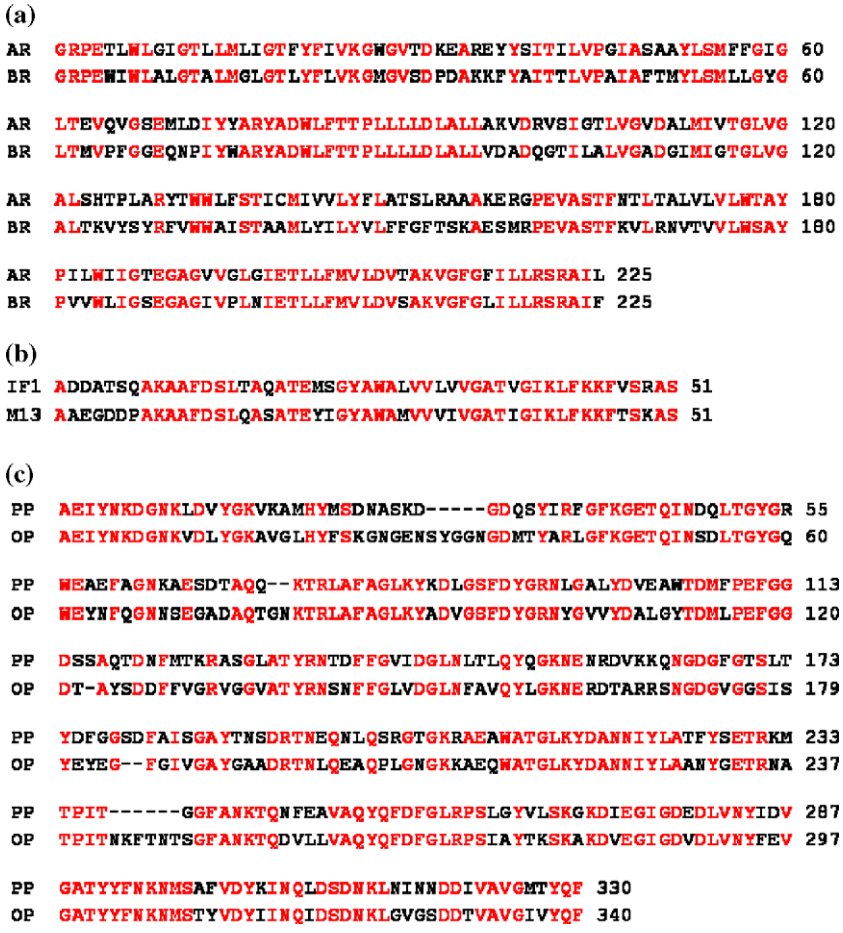

Fig. 2. Sequence alignments (a) between archaerhodopsin 1 (AR) and bacteriorhodopsin (BR), (b) between IF1 and M13 coat proteins, and (c) between phosphoporin (PP) and OmpF porin (OP).