A computational analysis of Bacteriorhodopsin and four mutants reveals local conformational changes correlating to changing probabilities in unfolding pathways

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Motivation

Integral membrane proteins are widely involved in many essential cellular processes, and misfolding of membrane proteins due to point mutations plays an important role in many human diseases such as retinitis pigmentosa or diabetes insipidus. Single molecule force spectroscopy (SMFS) is a novel technique, which measures the force necessary to pull a protein out of the native membrane. In general SMFS experiments generate hundreds of force-distance curves that contain valuable information on inter- and intra-molecular interactions that stabilise the protein and exhibit patterns of different probabilities of occurrence of different force peaks. These peaks are thought to correspond to different unfolding pathways of the protein during the process of mechanical unfolding.

Fully and objective automated analyses of different force curces datasets are needed, as well as detailed bioinformatics analysis and correlation of different types of intra-molecular interactions with the patterns observed in the SFMS data.

This study is focused on the analysis of SMFS data from Bacteriorhodopsin (BR) and four mutants, as with increasing knowledge of its structural and functional properties, BR has become a paradigm for alpha-helical membrane proteins.

Furthermore, as Proline residues are recognised as playing a significant role in helix kinks formation and in general in protein folding, we analyse four BR proline mutants in order to see if the structural stability of the protein is affected.

Our goal is to understand the origin of the interactions that establish the observed unfolding barriers and correlate preferential routes in the energy landscape with the location of computed intra-molecular interactions.

Methods

Dataset: five datasets relative to pulling experiments of Bacteriorhodopsin Wild Type (BR WT) and four mutants (P50A, P91A, P186A, M56A).

Automated analysis of SMFS data: for classification of different unfolding pathways we apply a pattern recognition algorithm (described in a previus paper from us) that pre-processes the curves and cluster them on the basis of their similarity score derived form all the possible pairwise alignments between the curves. We identify subclusters in the tree that possibly correspond to different unfolding pathways and characterize them in terms of positions, magnitude and frequency of force peaks (unfolding barriers).

Residue-residue contacts analysis: we quantify the total number of residue-residue contacts in BR WT and the four mutants according to a classical analysis that defines a contact between two residues when the distance between their Calpha atoms is less than 8 ï¿½ We distinguish between polar and hydrophobic contacts on the basis of atom type and strict geometrical constraints. We calculate the number of missing and additional contacts between BR WT and each mutant in every detected unfolding barrier and we evaluate the significance of differences in residue-residue contact area.

Results

Since the intermediates that occur during unfolding can be identified from the clustering procedure, we construct the complete unfolding pathways of BR WT and the four mutants.

From our automated analysis we find that BR WT and the four mutants exhibit the same unfolding patterns inside the experimental errors. Furthermore we observe that, while the frequencies of main peaks (peaks that appear in every curve of the dataset) does not change from WT to mutants, the frequences of minor peaks show remarkable changes.

Residue-residue contact area calculations point out that the analysed point mutations do not significantly affect the stability of a protein and that local changes in the contact area, due to local alterations in the arrangement of intra-molecular interactions, compensate each other all over the structure, leaving the stability of the protein unaffected.

Nevertheless, we observe that certain pathways occur with higher frequency in the BR WT than in a given mutant, whereas some others are more frequently chosen from the mutants' unfolding routes. We hypothesise that this observation correlates with the location and type of altered molecular interactions

between WT and mutants, detected from our computational analysis. We prove our hypothesis showing that different probabilities of peaks between BR WT and mutants P50A and M56A can be explained in terms of additional or missing interactions, involving mostly side chain polar contacts and highly conserved residues.
Overall we conclude that the unfolding pathways of BR and the four mutants find an interpretation at structural level. Furhtermore, changes in intra-molecular interactions in P50A and M56A with respect to the WT do not introduce a new or remove an existing intermediate in the force-distance curves, but an analysis of missing and additional contacts clarifies the influence of single point mutations: they bring to changes in local residue-residue interactions that alter the preferred unfolding routes on the energy landscape of the protein.
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