Structure Characterization of Transmembrane Protein Rhodanese YgaP by EPR

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Introduction

YgaP is a membrane protein that contains a rhodanese domain and a C-terminal transmembrane segement found in Escherichia coli. It consists of 174 amino acids and is located on the membrane as a dimer. Rhodanese domains are broadly distributed in bacterial, eukaryotic and archaeal cells. They act as the carrier of reactive sulfur atoms by forming persulfide intermediates in distinct biologic processes, such as sulfur and selenium metabolism, cyanide detoxification and synthesis of ion-sulfur proteins [1,2]. Although it's important in distinct metabolic pathways, up until now, no atomic protein structures were determined by NMR or crystallographic methods. EPR is becoming a powerful and effective method used to study protein structure, dynamic and even function. In the present research, we combine several different EPR techniques to characterize the structure of YgaP.

Experimental

Single cysteine mutants of YgaP from amino acid L111 to G160 were overexpressed in *E. coli*, and purified in detergent DPC using nickel affinity chromatography. Purified proteins were labeled with (1-Oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl) Methanethiosulfonate (MTSL) for EPR studies. For DEER distance measurement between dimer of mutants Q116C, T134C, F139C and F145C, 40% Glycerol was supplemented as cryo-protectant prior to snap freezing in liquid nitrogen. DEER data was collected on a Bruker E680 spectrometer at the NHMFL.

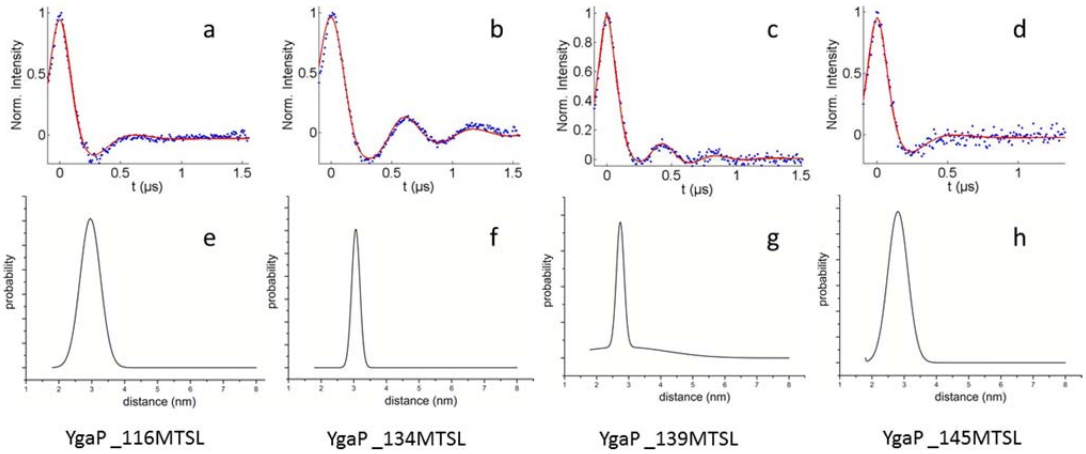

Figure 1: DEER measurement of distance between MTSL labeled monomers of YgaP. Upper panel, DEER spectrum (points) with corresponding best fit (solid red line). Lower panel, Gaussian distance distributions corresponding to the best fit.

Results and Discussion

All the YgaP mutants were expressed and purified, and subsequently labeled by MTSL spin labels. CW EPR spectra and accessibility (in air,N_2 and 50Mm Ni-EDDA) were measured on consecutive amino acid sequence from L111C to G160C in DPC micelles at room temperature. From the accessibility data we find that there are two TM helixes in the C-terminus (not shown). One TM helix starts at residue R115 and end at L131, the other one from F140 to L151. DEER distance measurements were performed on the mutants Q116C, T134C, F139C and F145C described above. Analysis suggests that distances between nitroxides on the dimer at position 116,134,139,145 are 2.96 nm, 3.06 nm, 2.74 nm and 2.78 nm repectively (Fig. 1). Also, DEER spectra of all four positions have narrow distance distributions, especially YgaP_134MTSL and YgaP_139MTSL, which means R1 side chains are very rigid. In sum, our accessibility results confirm that there are two TM helixes in the C-terminus of YgaP dimer. A following study will focus on distance measurements inside the monomers. A combination of distance and accessibility data will be used to determine Ygap dimer structure on the membrane.

Acknowledgements

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References

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