Structural Characterization of Transmembrane Protein Rhodanese YgaP by EPR

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Introduction

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. YgaP is a membrane protein that contains a rhodanese domain and a C-terminal transmembrane segment found in Escherichia coli. It consists of 174 amino acids and is located on the membrane as a dimer. Recently, the structures of YgaP rhodanese domain and transmembrane (TM) domain have been determined separately by solution NMR.[1] However, until now no atomic structure of full length YgaP protein has been reported. EPR is becoming a powerful and effective method used to study structure, dynamics and even function of membrane proteins. In the present research, we combine several different EPR techniques to characterize the structure of full length YgaP.

Experimental

Single cysteine mutations were introduced into YgaP from amino acid site L111 to G160 and double cysteine substitutions were also constructed. Proteins were overexpressed in *E. coli*, and purified in DPC micelles using nickel affinity chromatography.
Purified proteins were labeled with MTSL for EPR studies. 40% glycerol was supplemented as cryo-protectant prior to snap freezing in liquid nitrogen for EPR distance measurements. CW EPR distance measurements were performed at 150 K. DEER data was collected at 65 K on a Bruker E680 spectrometer at the NHMFL.

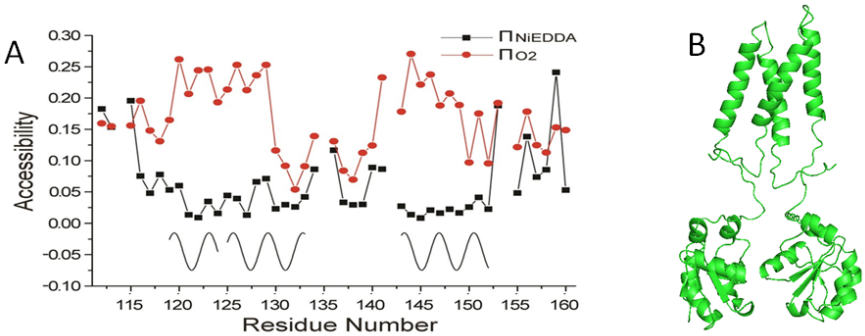

Fig. 1: (A) The accessibility data of the transmembrane region of YgaP. (B) The full length structure of YgaP protein calculated using EPR and NMR data in DPC micelles.

Results and Discussion

All the single cysteine mutants of YgaP protein were expressed, purified, and subsequently labeled by MTSL spin labels. CW EPR spectra and accessibility data (in air,N_2 and 50 Mm Ni-EDDA) were collected on consecutive amino acid sequence from L111C to G160C in DPC micelles at room temperature. The accessibility data show two helical segments in the TM region of Ygap (Fig. 1A). One segment starts at residue R114 and ends at L135, and the other one spans from F142 to L160. In order to determine the structures of the TM segments, 30 double cysteine-substitution mutants were expressed and labeled for distance measurements. DEER experiments were performed on 22 double mutants to obtain distances between the spin labels placed on different sites of Yagp (data not shown). Additionally, distances of 8 double mutants were collected by cw-EPR method to acquire short spin-spin distances. Two steps were taken for full length YagP structure calculations. First, all the distance constrains of the transmembrane domains were introduced into the secondary structures (two TM α helixes) derived from the accessibility data, and a following Xplor calculation was used to obtain topology structures of YgaP TM domains. Second, the structure of full length YgaP was calculated by combining the solution NMR structures of rhodanese domains (not shown), the EPR topology structures of the TM domains, and the distance constrains between these domains obtained from DEER data, shown in Figure. 1B. In conclusion, combining distance constraints obtained from EPR techniques with conventional NMR, we successfully determined the structure of full length YgaP membrane protein in DPC micelles.

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References

[1] Wang, W., *et al*., Biochemical and Biophysical Research Communications, **452**, 817-821 (2014).