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Conformational change in *Aeropyrum pernix* 5'-methylthioadenosine phosphorylase under extremely high temperature conditions

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Introduction

To find out extremely thermostable proteins in hyperthermophilic archaea, we screened soluble proteins even after autoclave (121°C, 20 min) treatment. As the result, some autoclave resistant proteins were found in the cell extract of the hyperthermophilic archaeon *A. pernix*. Among them, we found a protein whose molecular mass was estimated to be 135 kDa by SDS-PAGE and the N-terminal amino acid sequence was identical to that of the APE1885 gene product. This protein has been annotated as a putative 5'-methylthioadenosine phosphorylase (MTAP) via genome analysis. The subunit molecular mass was calculated to be 30.7 kDa from the predicted amino acid sequence. This indicates that the MTAP forms SDS-resistant oligomeric structure (aMTAP) by autoclave treatment. To elucidate the difference in the structure between MTAP and aMTAP, small-angle X-ray scattering (SAXS) measurements were carried out.

Materials and Methods

Recombinant MTAP was expressed in *Escherichia coli*. The *E. coli* strain BL21(DE3) codon plus RIL was transformed with pET11a/MTAP. The transformants were cultivated at 25°C in 200 ml of a medium containing 2.4 g of trypton, 4.8 g of yeast extract, 1 ml of glycerol, 2.5 g of K_2HPO_4, 0.76 g of KH_2PO_4, and 10 mg of ampicillin, until the optical density at 600 nm reached 0.6. Induction was carried out by the addition of 1.0 mM isopropyl-β-D-thiogalactopyranoside to the medium, and then the cultivation was continued for 3 h. MTAP was purified by a Butyl-Toyopearl chromatography. SAXS measurements and analysis were described elsewhere [1, 2]. aMTAP was purified by autoclave treatment at 121°C, 20min followed by a Butyl-Toyopearl chromatography.

Results and Discussion

MTAP was expressed in *E. coli* and the subunit molecular mass of recombinant MTAP was determined to be 31 kDa by SDS-PAGE, while that of the autoclaved recombinant enzyme (aMTAP) was 135 kDa. On the other hand, both the proteins exhibited similar mobility in non-reducing SDS-PAGE, which corresponds to 135 kDa. SAXS analyses showed that both the enzymes, MTAP and aMTAP form quaternary structure and the R_g values were 36.5 Å (Fig. 1 (a) and (b)). No difference in shape of

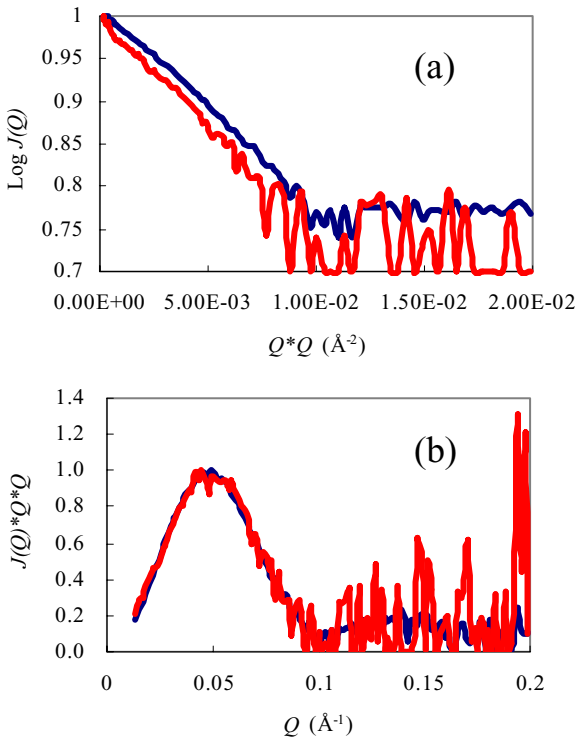

Figure1. Guinier (a) and Kratky (b) plot of the MTAP (black line) and aMTAP (red line).

overall molecule and in circular dichroism spectra was observed.

We changed the strain of *E. coli* from BL21(DE3) codon plus RIL to Rosetta-gami that promotes disulfide bond formation in the cell. In the results, product from the Rosetta-gami formed SDS-resistant oligomeric structure. These results suggest that the conformational change induced by autoclave may be related to the formation of disulfide bond in the protein.

References

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