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Studies on the heat and urea induced rearrangement of subunits measured by small-angle X-ray scattering; the case of hyperthermophilic glutamate dehydrogenase produced in *Escherichia coli*

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Introduction

A number of proteins from hyperthermophiles have been produced as the recombinant proteins by introducing cloned genes in *Escherichia coli*. Some of these recombinant proteins are known to be produced as forms different from those of the natural ones. Recently, we cloned and expressed the gene encoding GDH of the hyperthermophilic archaeon *Pyrobaculum islandicum* (pis-GDH) in *E. coli*[1]. The recombinant enzyme was produced as a hexameric form with an extremely low specific activity, and the specific activity of the enzyme purified by a heat treatment was similar to that of the natural one[1]. We have also observed that urea has a significant effect on the proper rearrangement of the enzyme at 37˚C. To investigate the structural changes between the low activity form and heat or urea treated form, small-angle X-ray scattering analysis (SAXS) was carried out. As a result, a large difference in the radius and quaternary structure of the molecules was measured by SAXS.

Materials and Methods

Production and purification of the low-activity form of recombinant GDH

The expression of the protein in *E. coli* was performed as follows. *E. coli* BL21 (DE3)-codon plus-RIL cells carrying the recombinant plasmid were grown at 37°C in Luria-Bertani medium containing ampicillin (50 µg/ml) and the enzyme expression was induced by addition of 1 mM IPTG. Cells were collected by centrifugation and disrupted by sonication. For the purification of the recombinant enzyme, three successive column chromatographies of Red Sepharose CL-4B, DEAE-Toyopearl and GIGAPITE K-100S were carried out at 4˚C to avoid the activation of the enzyme by heat. Enzyme assay was carried out as previously described[2].

SAXS measurements and data analysis

SAXS experiments were performed with the optics and detector system of SAXES[3,4] installed at Beamline BL-10C. All measurements were done at 25°C. Measured time for SAXS measurements of GDH solutions at a concentration of 2 to 5 mg/ml was 300 seconds for each measurement and multiple scattering data were accumulated 4800 seconds to improve signal to noise ratio. The experiment and simultaneous analysis of the data using the SAXSANA program in MS Visual Basic[5] enabled an immediate on-site check of the SAXS results. Detailed description of the SAXS measurements has been described elsewhere[6].

Results

SAXS measurements were carried out to measure the structural changes of the GDH molecules with the non-treated, heat-treated and urea-treated. The *Rg* values of non-treated, heat-treated and urea-treated GDH were 60.5, 46.5 and 53.7 Å, respectively. The large changes in the *Rg* of the molecules were seen between the heat-treated GDH and the non-treated GDH. *D*max also decreased with the heat-treated (105.5 Å) and urea-treated (114.4 Å) compared to that of the none-treat GDH (120.7 Å). These results showed the shape of the molecules became compact by the heat or urea treatment. $J(0)/C$ (molecular weight) was independent on the states of heat-treated, urea-treated and non-treated, indicating the molecular mass of these three protein states did not change. A comparison of the Kratky plot profiles offers a clue to find the change of the quaternary structure[6]. The profile of non-treated, urea-treated and heat-treated GDHs had the first peaks at about Q = 0.033, 0.034 and 0.037Å^{-1} and the second ones at 0.11, 0.12 and 0.13Å^{-1}, respectively. The ratio of the height of the second peak to the first one unchanged to be around 0.26 for all samples. Klatky plots showed that the quaternary structures of GDHs changed by rearrangement of the subunit, whereas the molecular masses of these molecules were same. It was remarkable case that the large differences in the molecular radiuses were seen in the protein species with the same molecular mass but different quaternary structures.

References

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