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# Structural study on the heat activation process of a recombinant glutamate dehydrogenase from a hyperthermophilic archaeon *Pyrobaculum islandicum*

Yoshimi Nishikawa[1], Shuichiro Goda[1], Haruhiko Sakuraba[1], Toshihisa Ohshima[1], Yuzuru Hiragi*[2,]
[1]The Univ. of Tokushima, 2-1 Minamijosanjima, Tokushima 770-8506, Japan
[2]Kansai Medical Univ., 18-89 Uyama-Higashi, Hirakata 573-1136, Japan

## 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 as being produced in forms different from those of the natural ones. Recently, we cloned and expressed the gene encoding glutamate dehydrogenase (GDH) of the hyperthermophilic archaeon *Pyrobaculum islandicum* (pis-GDH) in *E. coli* [1]. Initial recombinant enzyme prepared as a hexameric form with an extremely low specific activity, gains natural enzymatic activity by heat treatment at 90°C for 15 min [1,2].

To investigate the process of changes in the size and shape of GDH in relevant with the heart activation, small-angle X-ray scattering analysis (SAXS) was carried out.

## Materials and Methods

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

The expression of the protein in *E. coli* [2] and enzyme assay [3] were carried out as previously described.

*SAXS measurements and data analysis*

SAXS measurements were performed with a camera of SAXES [4,5] installed at Beamline BL-10C at the Photon Factory. All measurements were done at 25°C. Measured time for SAXS of GDH solutions at a concentration of 5 to 6 mg/ml was 900 seconds for each measurement and multiple scattering data were accumulated up to 1800 seconds to improve signal to noise ratio. For the experiment and simultaneous analysis of the data, the SAXSANA program in MS Visual Basic [6] was used. Detailed description of the SAXS measurements was described elsewhere [7].

## Results

SAXS measurements were carried out to measure the structural change in the GDH molecules during heat activation. Large change in the *Rg* value was seen (Fig.1, filled circle). The *Rg* value of inactive recombinant GDH was 56.8 Å. By heat-treatment at 70°C, the *Rg* value decreased sharply below 100 min. After 550 min incubation at 70°C, the *Rg* value reached 46.2 Å, similar to heat-activated GDH (45.5 Å) at 90°C for 15 min. The change in the *Rg* value fitted well to exponential curve (Fig.1), suggesting inactive GDH transformed to active form without passing through an intermediate structure. Increase in the enzyme activity accompanied to the change in the *Rg* value as seen in Fig. 2. Additionally, even after 550 min, specific activity of GDH was not reached the value that of heat-activated one at 90°C for 15 min. These results suggest that the change in the size of the GDH molecule follows the activation of the GDH.

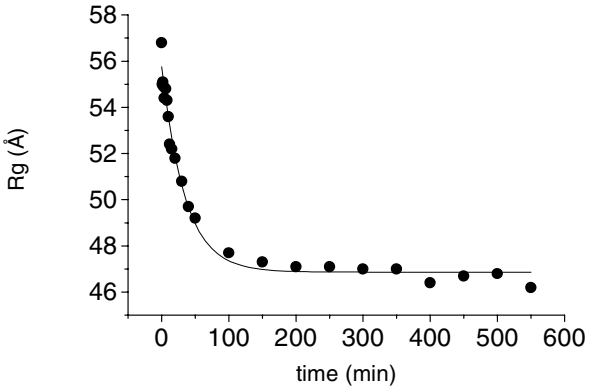


Fig. 1. The time course of the Rg value of pis-GDH at 70ºC

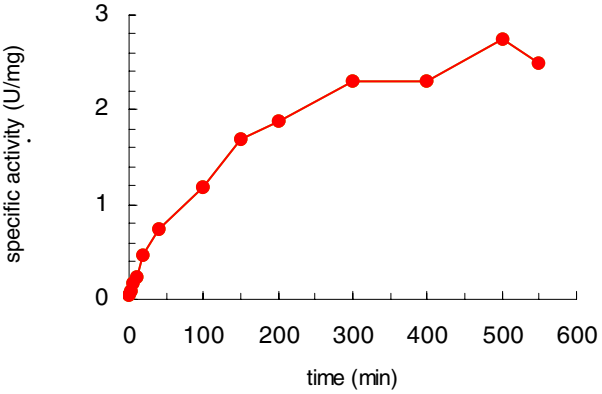


Fig. 2. The time course of specific activity of pis-GDH at 70ºC

## References

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* yhiragi@ybb.ne.jp &_hiragiy@makino.kmu.ac.jp