Biochemical Characterization and Molecular Cloning of Thermostable *Aeropyrum pernix* Glutamate Dehydrogenase

The discovery of microorganisms that can live at high temperature near or above boiling point that called hyperthermophiles has been opening the understanding about the thermostability mechanism of thermostable enzymes, since these hyperthermophiles are sources of enzymes with uncommon thermostability. However, the general determinant of the thermostability could not be concluded so far from empirical methods. The available data are still too few to deduce a general determinant factor for thermoadaptation mechanism. Further research results show that different enzyme has undergone different mechanism to maintain their adaptation in high temperature. Hence, more data are still required in order to understand thermoadaptation mechanism. In other respect, enzymes are biocatalyst. Enzymes are proteins; thus they are typically labile molecule and affected adversely when exposed to any type of extreme condition. However, this characteristic is not appropriate when enzymes will be applied as biocatalyst for technological purpose or as labeling material or recognition molecule in bioassay or bioanalytical field, because in this kind of application enzymes have to be stable and functional even in condition far from their physiological environment. Thus, thermostable enzymes from hyperthermophiles are potential materials in such application.

This research focuses on biochemical characteristics and molecular cloning of thermostable glutamate dehydrogenase (GDH), one of oxidoreductases which is very applicable for bioanalytical use, from a high temperature microorganism, *Aeropyrum pernix* (*A. pernix*). This work is aimed as the first step in more understanding about the thermostability adaptation and the difference between natural enzyme and its recombinant. I hope it could be a significant contribution in adding more data about thermostability factor. I also expect that characterized enzyme would be a potential material in bioassay or bioanalytical application.

Chapter 2 describes the approach of purification and the biochemical characterization of GDH from the native organism. The approach of purification of native enzyme directly from its original source is conventional method. However, it is still valuable and challenging in understanding the difference between natural enzyme and its recombinant. Furthermore, because of the interesting properties of the *A. pernix* itself, that it is the first strictly aerobic archaeon that live at temperature near boiling point (90°C) and at neutral pH, the direct biochemical characterization of the native GDH could be expected to give some interesting features. The native A. pernix GDH is homohexamer and NADP-dependent enzyme. The NADP-dependent characteristic is unusual property of archaeal GDH which most of them recognize both NAD and NADP. The enzyme is very stable at 100°C and it still retains it activity above 70% although after 5-h incubation at 100 °C. The N-terminal amino acid sequence analysis and comparison to the nucleotide sequence from genome information reveals that this enzyme has TTG as an initial codon, and the N-terminal is 5 residues shorter than that of the predicted ORF. This is a novel characteristic of an archaeal GDH.

Chapter 3 describes the molecular cloning and expression of the enzyme's gene in E. coli. This approach is performed based on the N-terminal amino acid sequence finding in the Chapter 2, in order to know the effect of the N-termini difference between native and predicted ORF on enzymatic prperties. The enzyme gene with the same amino acid to that of the native enzyme (sGDH), and to that of the predicted ORF (lGDH) are expressed. One mutant which Arg 420 is replaced by Trp (R420W) is also introduced. The all-recombinant GDHs are homogeneously purified and ready for further characterization.

Chapter 4 discusses the biochemical characterization of the recombinants and the comparison among their properties. The native GDH and the recombinant of native (sGDH) reveals differences in thermostability and specific activity, although they have the same amino acid sequence. From Circular Dichroism (CD) spectra observation and comparison of HPLC elution profile and peptide mapping, it is proposed that the differences is not due to the modification of native GDH. It is likely due to the improper folding of the recombinant sGDH because it is produced in lower temperature environment (*E.coli*). The most stable GDH is the recombinant with longer N-terminal amino acid (lGDH). It shows that longer N-termini has a stabilization effect to the overall enzyme structure. This observation is a novel finding for hyperthermophilic GDH. Using structure model as an interpretation tool, I consider that hydrophobic interaction in the region of this N-termini may have important role in maintaining structure in high temperature. R420W mutant is the most unstable recombinant. By comparing to other hyperthermophilic GDHs, it reveals that this Arg is conserved, and it suggests the importance of Arg residue in this position. The previous reported *Pyrococcus furiosus* GDH shows that the ion pairs network is the significant factor in high temperature adaptation, and this network is involving Arg residue in this postion. Based on this previous finding, probably ion pairs network may be also significant contributor to the *A. pernix* GDH stability.

Chapter 5 introduces the preliminary examination of this enzyme in possible application field like bioassay. The preliminary examination shows this thermostable GDH is feasible to be used as a recognition molecule in amperometric biosensor or as a stable labeling enzyme in DNA hybridization application.

By this way, this thesis contains findings that important from a view of basic field and I hope this thesis may become a significant contributor to the science in general.