SUMMARY OF Ph.D. DISSERTATION

School	Student Identification Number	SURNAME, First name BAKKE Mikio

Title

The development and improvement of useful oxidoreductases for enzymatic determinations of biological substances.

Abstract

In the field of clinical and food chemistry, it is very important to measure only the target substance in biological samples including various materials. Compared with many analytical methods, enzymatic determination is especially attractive from the point of simplicity, quickness, accuracy, safety and automatization. Many oxidoreductases are practically employed for the enzymatic determinations because their reaction products, such as hydrogen peroxide and NAD(P)H, can be detected as optical or electrical signals.

The development of novel enzymatic assays and amelioration of existing methods are still required. To meet these demands, many efforts have been devoted to the development of enzymes with excellent performances, in terms of purity, stability, substrate specificity, and affinity toward their substrates. In this study, five oxidoreductases were developed and improved. The contents of the research are as follows.

1. A novel diacetylspermine oxidase (DASpmOX), which seems suitable for enzymatic determination of N^1,N^{12}-diacetylspermine as a tumor marker, was isolated from *Debaryomyces hanseii*. Its stability and substrate specificity have been improved by additives. The gene encoding DASpmOX was cloned, and recombinant enzyme was prepared and characterized.

2. L-Fucose dehydrogenase originated from *Pseudomonas* was stabilized by the addition of chelating reagents for the use of NADPH recycling.

3. Porcine kidney D-amino acid oxidase, which is useful for the determination of D-amino acids, was stabilized by chemical modification and single amino acid substitution.

4. The gene encoding histamine dehydrogenase from *Rhizobium* sp. was cloned and overexpressed in *Escherichia coli*. The recombinant enzyme exhibited the most excellent substrate specificity toward histamine among all amine oxidases or dehydrogenases found up to this time. The enzyme could be used for the determination of histamine in seafood samples.

5. A characteristic acyl-coenzyme A oxidase with only a few *N*-ethylmaleimide (NEM)-reactive amino acid was found from *Arthrobacter ureafaciens* by the gene cloning. The recombinant enzyme showed NEM-resistance, which is the advantage for the determination of free fatty acids.