

Microbiosensor immobilized histamine oxidase

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Histamine is known as a putrefactive amine, which cause an allergy-like food poisoning. Though histamine is not present in fresh fish, it is formed when the fish has been contaminated with microorganisms which have a strong histidine decarboxylase (EC 4.1.1.22) activity; the histamine is formed by decarboxylation from L-histidine. Therefore, histamine in fish meat extracts is a marker of fish freshness, and the determination of histamine in fish meat is important for evaluation of the degree of freshness and the prevention of food poisoning incidents.

Fluorometry and HPLC are the main methods that have been used for histamine determination, but they are slow and complicated. The HPLC methods are reliable, but they produce an excessive amount of information that is not required for the purpose and result in high cost per analysis. Therefore, enzymatic methods with histamine-oxidizing enzyme have been proposed for a more selective determination of histamine.

A thermostable histamine oxidase (EC 1.4.3.6) was found in cells of *Arthrobacter crystallopoietes* KAIT-B-007 isolated from soil. The enzyme was purified about 715-fold over the cell free extracts by ammonium sulfate fractionation and various column chromatographies. The purified enzyme was thermostable. When the enzyme was kept at 70-degreeC for 10 min, the activity was decreased to 9% of the initial level at 70-degreeC (Fig.). The enzyme showed potent activity toward histamine, whereas it was inactive toward putrescine, cadaverine, spermine, and spermidine (table).

In this study, the thermostable histamine oxidase was immobilized on microelectrode, and then the histamine concentration in fish meat extracts were measured by the enzyme-immobilized microelectrode.

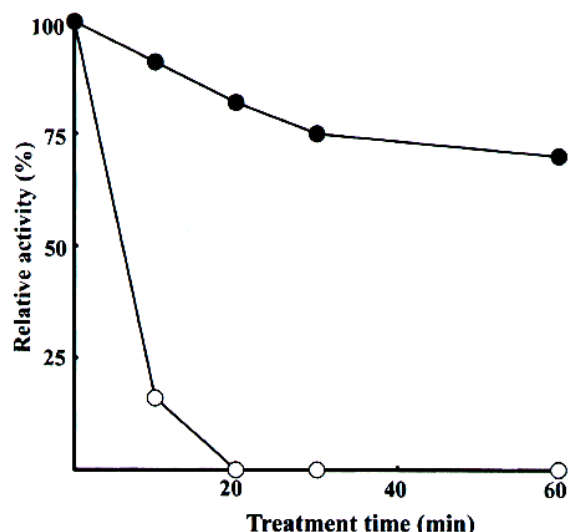


Fig. Effect of temperature on stability of histamine oxidase. Histamine oxidase was incubated at 70-degreeC for different lengths of time and the histamine oxidase activity was measured at 37-degreeC. Symbols: closed circles, histamine oxidase from *A. crystallopoietes* KAIT-B-007; open circles, histamine oxidase from *A. globiformis* IFO12137.

Table. Substrate specificity of histamine oxidase from *A. crystallopoietes* KAIT-B-007

Substrate	Relative activity (%)
Histamine	100
Methylamine	0
Ethylamine	0
Propylamine	7
Butylamine	22
Pentylamine	32
Hexylamine	41
Tyramine	36
Dopamine	51
Tryptamine	15
1-Phenylethylamine	44
Putrescine	0
Cadaverine	0
Spermidine	0
Spermine	0