

Immobilization of plant histaminase for medical applications

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Introduction

Histamine in food has been shown to induce intolerance reactions. The concept of pseudoallergic reactions to food or false allergies (FFA) was suggested first in 1975 [1]. In this context an abnormal intake of biogenic amines such as histamine and tyramine represents one of the major mechanisms involved [2]. Diamine oxidase is the main histamine degrading enzyme acting predominantly in the gastrointestinal tract. Food histaminosis was described by Sattler et al. [3] and demonstrated the clinical evidence for histamine intolerance. The mechanisms of pseudoallergic reactions caused by food seem to involve intestinal hyperpermeability, or changes in enzymatic activities suggesting that the use of purified histaminase, in order to eliminate the excess of histamine, could be of use in the treatment of histaminosis. In this study, we immobilized pea seedling histaminase on CNBr-Sepharose 6MB (Pharmacia). The plant enzyme was employed because of its higher efficacy in comparison to that found in mammals.

Materials and methods

Histamine, putrescine, spermidine, agmatine, pepsin (4,500 EU/mg) and trypsin (9,820 EU/mg) were obtained from Sigma (St. Louis, MO, USA) and CNBr-Sepharose was obtained from Pharmacia.

Pea seedling histaminase was purified according to Mc Guirl et al. [4], with a specific activity of 38 EU/mg. The enzyme was immobilized on CNBr-Sepharose 6 MB (Pharmacia). Briefly, 250 mg of the activated support was swollen in 0.001 M HCl, washed rapidly with distilled water and equilibrated with 0.01 M phosphate buffer pH 7.2. Plant histaminase (2 mg) was added to 0.7 mL of activated gel and the mixture was incubated for 2 h at room temperature and then 15 h at 4°C. Proteolysis studies were performed with free and immobilized enzyme

measuring the enzymatic activity in either the absence or presence of 0.1 % trypsin at pH 7.2 and 0.01 % pepsin at pH 2, using putrescine 10^{-3} M as substrate. The assays were incubated for various intervals at 37°C. The enzyme concentration for the assays was 0.5 mg/ml for the free plant histaminase and 0.29 mg/ml suspension for the immobilized form. Enzymatic activity was determined by a polarographic method (YSI 53 device) measuring the rate of oxygen consumption [5] in the presence of the substrates. The protein concentration was determined by Biuret method as previously described [6].

Results and discussion

The plant enzyme was immobilized (approx. 100% coupling yield) with about 64% of enzymic activity retained on the gel. Table 1 shows the K_m values of plant histaminase measured for various substrates. These values are of the same order of magnitude for both forms (free and immobilized) of the enzyme. Table 2 shows that the immobilized histaminase maintains about 11 % of its specific activity after 60 min. of incubation in the presence of pepsin and the free form is completely inactivated. In the presence of trypsin, the immobilized enzyme was completely stable after 60 min incubation, while the free form retained 67% of its specific activity. Moreover, the immobilized enzyme retained 100% of its initial specific activity after 6 h of incubation (data not shown). The good immobilized yield (64%), the similar K_m values for free and immobilized enzyme towards histamine and the high stability to proteolytic attack by trypsin suggest that the chosen immobilization system on CNBr-Sepharose is probably adequate for the continuation of the studies on the use of the plant histaminase as antihistaminergic agent. Further experiments are in progress to improve the gastro-resistance of the enzyme and to study the histaminase activity in biological models.

Substrate	Free histaminase		Immobilized histaminase	
	Km (M)	V _{max} EU/mg prot.	Km (M)	V _{max} EU/mg prot.
Histamine	1.6×10^{-4}	27	3.57×10^{-4}	16
Putrescine	5.0×10^{-5}	61	8.50×10^{-5}	23
Spermidine	4.3×10^{-4}	17	8.33×10^{-3}	16
Agmatine	6.6×10^{-4}	20	2.70×10^{-4}	15

Table 1. Km and V_{max} values measured for various substrates with free and immobilized plant histaminase.

Time (min)	Free histaminase % activity		Immobilized histaminase % activity	
	Pepsin (0.01 %) pH 2	Trypsin (0.1 %) pH 7.2	Pepsin (0.01 %) pH 2	Trypsin (0.1 %) pH 7.2
0	100	100	100	100
15	21	100	50	100
30	14	100	17	100
45	4	100	14	100
60	0	67	11	100

Table 2. Stability at proteolysis of free and immobilized plant histaminase.

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