HISTAMINASE AND ITS INHIBITORY FACTOR IN GUINEA PIG SKIN

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ABSTRACT

The crude histaminase preparation obtained from guinea pig skin was fractionated by Sephadex G-200 at 4°C and 55°C. The histaminase activity corresponded to the descending limb of the first peak of protein eluted just after void volume at 4°C and to the second peak at 55°C. The histaminase activity in the fractions eluted at 4°C was higher at 55°C than at 37°C. On the contrary, no significant difference between the histaminase activity assayed at 55°C and 37°C was observed in the fractions eluted at 55°C. On Sephadex G-200 gel filtration, histaminase was eluted in the later fractions at 55°C, compared with the fractions eluted at 4°C, indicating that the molecular weight of histaminase may become smaller at 55°C. These facts suggest that histaminase obtained from guinea pig skin may bind with an inhibitor-like substance at low temperature and that the enzyme may separate from the inhibitor at high temperature.

INTRODUCTION

Our previous study indicated that histaminase may play an important role in the regulation of histamine-mediated inflammatory reactions in guinea pig skin1). In the physicochemical analysis of histaminase in guinea pig skin, histaminase in the crude enzyme preparation was more active at 55°C than at 37°C, when synthesized histamine was used as a substrate2. The histaminase activity enhanced at 55°C in the crude enzyme preparation gradually reduced at 37°C3. These facts suggest that in the crude enzyme preparation from guinea pig skin there may be a histaminase inhibitor-like substance whose effect may be reduced at 55°C and reverted at 37°C. It was posited that histaminase may in part form a complex with the inhibitor-like substance in the enzyme preparation at 37°C and that the enzyme may become more active by dissociation from the enzyme-inhibitor complex at 55°C.

In the present experiments, therefore, to confirm these possibilities, the crude histaminase preparation from guinea pig skin was fractionated by gel filtration at 55°C.

MATERIALS AND METHODS

The homogenate of guinea pig skin was obtained by the method described in the previous report4. The supernatant of the homogenate was precipitated with powered ammonium sulphate to 60% saturation by slowly adding

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*山本昇: モルモット皮膚のヒスタミナーゼとその阻害因子
ammonium sulphate (42.4 g/100 ml of the supernatant). The precipitate was centrifuged at 15,000 × g for 30 min at 4°C and the supernatant was decanted. The precipitate was dissolved in 0.01 M phosphate-buffered saline at pH 7.3 and was dialyzed against the same buffer which was changed three times during 24 hr at 4°C. The dialyzed sample was centrifuged at 15,000 × g for 30 min at 4°C to remove the insoluble substance and the precipitate formed during dialysis in the dialysis sacs. The supernatant was stored at −20°C until use. This was used as a crude histaminase preparation of guinea pig skin. No histamine activity was detected in this preparation.

Histaminase activity was determined by the method described in our report[3]. The concentration of histamine as a substrate was 100 ng/reaction mixture. The determination of histamine content in each reaction mixture was performed by the method of the previous report[3].

Gel filtration was carried out as follows: a 1.9 × 40 cm column of Sephadex G-200 was equilibrated with 0.01 M phosphate-buffered saline (pH 7.3). A 2 ml sample of the enzyme solution from guinea pig skin was applied to the column. This was run at flow rate of 4.3 ml/hr. Fractions of 3.0 ml were collected and stored at −20°C until use.

DEAE-cellulose chromatography was carried out by standard methods[4]. The 1.5 × 6.6 cm column of DEAE-cellulose was equilibrated with 0.005 M phosphate buffer (pH 8.0). A 7 ml sample of the enzyme solution (2.7 mg protein/ml) which was dialyzed against 0.005 M phosphate buffer (pH 8.0) for 48 hr was applied to the column at 18°C. Elution of the proteins was started with the same buffer, and then a salt concentration gradient of elution was applied; 0.005 M phosphate buffer was the starting buffer and 0.005 M phosphate buffer containing 0.5 M NaCl the final one. The column was run at a flow rate of 30 ml/hr. Fractions of 3.0 ml were collected and stored at −20°C until use.

RESULTS

Gel filtration of the crude histaminase preparation at 4°C: Figure 1 represents a typical chromatogram of the crude enzyme preparation at 4°C. Histaminase activity corresponded to the descending limb of the first peak of the protein which eluted just after void volume. The histaminase activity of each fraction at 55°C (○) was higher than that at 37°C (●). Histamine degradation by each fraction was completely reversed by the histaminase inhibitor, aminoguanidine (10−4 M); this indicated that the histamine degradation by each fraction was due to histaminase.

Gel filtration of the crude histaminase preparation at 55°C: If one speculates that enhancement of histaminase activity at 55°C in each fraction is due to dissociation of the enzyme from the enzyme–inhibitor complex, the histaminase activity may be eluted in the fraction with a smaller size of molecule from the Sephadex G-200 column at 55°C. To confirm this possibility, the crude histaminase preparation was fractionated by Sephadex G-200 at 55°C. The enzyme solution was preincubated at 55°C for 10 min before application to the column. The conditions of elution were the same as those of elution at 4°C, apart from temperature.
Histaminase Activity in Guinea Pig Skin

Figure 2. Sephadex G-200 gel filtration at 55°C of the crude histaminase obtained from guinea pig skin. Degradation of histamine activity at 37°C (●) and 55°C (○).

Figure 2 represents a typical chromatogram of the crude enzyme preparation at 55°C. Four main peaks of proteins were observed. Histaminase activity corresponded exactly to the second peak of the protein. This indicated that on gel filtration histaminase was eluted in the later fractions at 55°C, compared with the fractions eluted at 4°C in which the histaminase activity was detected. In the fractions eluted at 55°C, the magnitude of enhancement of histaminase activity at 55°C was markedly smaller than that in the fractions eluted at 4°C.

DEAE-cellulose chromatography of the crude histaminase preparation: Figure 3 shows a DEAE-cellulose chromatogram of the crude histaminase preparation. The histaminase activity was detected in the fractions eluted at ionic strength between 0.125 to 0.150. The fractions containing histaminase activity were pooled and the activity was determined at 37°C and at 55°C. The activity at 55°C was markedly higher than at 37°C, as shown in Figure 4.

DISCUSSION

In the previous study, we proposed that histaminase in the skin may modulate histamine-mediated cutaneous anaphylactic reactions in guinea pigs. In addition, it was indicated that the histaminase activity in the crude enzyme preparation obtained from guinea pig skin was maximum at 55°C. The activity attained by preincubating the crude enzyme preparation at 55°C was decreased by allowing it to stand at low temperature. The facts suggested that histaminase binds with inhibitor-like substance at low temperature in the crude enzyme pre-
paration obtained from guinea pig skin and that the enzyme may become dissociated from the enzyme-inhibitor complex at high temperature.

In the present study, in the Sephadex G-200 gel filtration at 4°C, histaminase activity was detected in the fractions which corresponded to the descending limb of the first peak of the protein which eluted just after void volume. The activity was significantly higher at 55°C, compared with that at 37°C. On the other hand, the Sephadex G-200 gel filtration at 55°C revealed a shift of histaminase activity to the region of the second peak (Fig. 2). The first, second and third peaks of the proteins were eluted exactly in the same position both at 4°C and at 55°C. These facts suggest that the size of the enzyme molecule with histaminase activity may become smaller at 55°C than at 4°C. In the fractions eluted at 55°C, the magnitude of increase of histaminase activity at 55°C was markedly smaller than in the fractions eluted at 4°C. This suggests that histaminase in the crude enzyme preparation may separate from histaminase inhibitory factor during the fractionation at 55°C.

The histaminase activity in the fractions eluted from DEAE-cellulose column at 18°C was significantly higher at 55°C, compared with that at 37°C (Fig. 3, 4). This suggests that histaminase bound with the inhibitor-like substance may be eluted from DEAE-cellulose column under the present experimental conditions.

In the present study, the histaminase activity at 55°C and at 37°C was completely abolished aminoguanidine when histamine was used as a substrate, and no loss of histamine activity was observed during the incubation at 55°C and at 37°C in the absence of the enzyme. These facts indicate that the degration of histamine by each fraction was due to histaminase. Hansson reported that preincubation of human blood plasma for 30 min at 45°C and 55°C caused an increase of histaminase activity of the plasma by a few percent, but no explanation about the mechanism of the finding was mentioned.

In the present study, although the histaminase inhibitor-like substance could not be detected in the crude enzyme preparation from guinea pig skin, the increase of the histaminase activity at 55°C is perhaps most easily explained by the enzyme dissociation from enzyme-inhibitor complexes at 55°C. If this speculation is correct, the histaminase inhibitory substance may also have a role in the regulation of histamine-mediated inflammatory reactions in skin. The detection of this inhibitor-like substance is under investigation in this laboratory.

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REFERENCES


