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Partial Purification of Histaminase from Guinea-Pig Liver by Gel-Filtration at High Temperature

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ABSTRACT

Histaminase was partially purified by Sephadex G-200 gel filtration at high temperature. Guinea pig liver histaminase was extracted with heparin. The extract was fractionated by Sephadex G-200 gel filtration at 4°C. In the fractions containing histaminase, 7 dense and 2 faint protein bands were detected on SDS-gel electrophoresis. Further fractionation of this sample by Sephadex G-200 gel filtration at 55°C markedly decreased the number of bands, i.e. only 1 dense and 2 faint bands were observed in the fractions in which histaminase activity was detected, and the enzyme activity was increased by approximately ten times. The results suggest that gel filtration at high temperature may be useful for partial purification of histaminase.

Histamine is one of the important mediators in inflammation, especially in anaphylactic reaction in skin. It has been suggested that histaminase might be an important factor modulating the magnitude of histamine-mediated anaphylactic reaction in guinea pig skin[5].

In the present study, in order to investigate the physicochemical and biological properties of histaminase, purification of the enzyme was attempted from guinea pig liver which is one of the major sources of histaminase[2]. The present results indicate that gel filtration of guinea pig liver extract at 55°C may be useful for purification of histaminase.

MATERIALS AND METHODS

Guinea pig liver extract: The livers obtained from normal male Hartley guinea pigs weighing 300—400 g were stored at −20°C until use. The frozen livers were cut into small pieces and incubated in two volumes (v/w) of 0.01 M phosphate buffer (pH 7.0) containing heparin (20 units/ml) at 37°C for 20 min with shaking. After incubation, the pieces were removed by filtering by cotton gauze. The filtrate was centrifuged at 21,000 g for 20 min at 4°C. The supernatant was dialyzed against 0.01 M phosphate buffer (pH 7.0) which was changed three times daily for 2 days at 4°C, and concentrated by ultrafiltration to one fifth of original volume.

Gel filtration: A 2.6 × 94 cm column of Sephadex G-200 was equilibrated with 0.01 M phosphate buffer (pH 7.0). A 8 ml sample of the concentrated liver extract was applied to the column. This was run at flow rate of 10 ml/hr at 4°C. Fractions of 5 ml were collected and stored at −20°C until use. For gel filtration at 55°C, a 2.6 × 66 cm column of Sephadex G-200 was used.

DEAE-cellulose chromatography: A 2.6 × 38 cm column of DEAE-cellulose was equilibrated with 0.01 M phosphate buffer (pH 7.6). A 21 ml sample of the enzyme solution (50 mg protein/ml) which was dialyzed against the starting buffer (0.01 M phosphate buffer, pH 7.6) was applied to the column at 4°C. Elution of the pro teins was started with the same buffer, and then

salt concentration gradient of elution was applied. The final buffer was 0.01 M phosphate buffer containing 0.6 M NaCl. The column was run at flow rate of 30 ml/hr. Fraction of 10 ml were collected and stored at −20°C until use.

Sodium dodecyl sulfate (SDS) gel electrophoresis: SDS-gel electrophoresis was performed by the method of Weber and Osborn[3].

Determination of protein concentration: The determination of protein concentration was carried out by the method of Lowry et al[1].

Assay of histaminase activity: Aliquots of the enzyme preparations from guinea pig liver were incubated with histamine (histamine dihydrochloride, Wako Pure Chemical Ltd., Osaka) at 37°C. The final volume of the reaction mixtures was 1.0 ml. After incubation, the reaction was terminated by adding 0.1 ml of 10^{-3} M aminoguanidine, histaminase inhibitor, to the reaction mixtures, following by diluting the reaction mixtures to 4 ml with ice cold Tyrode solution. The histamine contents in the reaction mixtures were determined by bioassay, using an isolated atropinized guinea pig ileum preparation or high-performance liquid chromatography (Simazu, LC-4A).

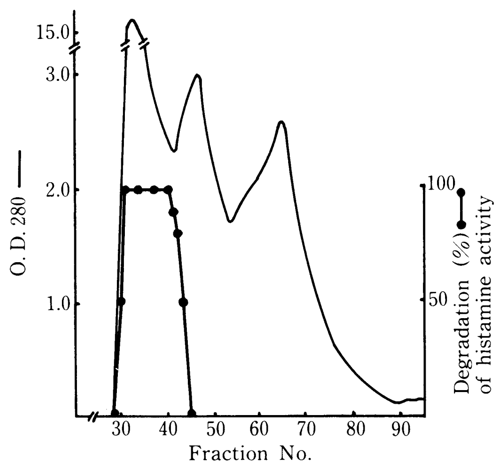

Fig. 1. Sephadex G-200 gel filtration at 4°C of the extract obtained from guinea pig liver

RESULTS

Sephadex G-200 gel filtration of guinea pig liver extract at 4°C: Fig. 1 shows a typical chromatogram of guinea pig liver extract on Sephadex G-200 gel filtration at 4°C. Histaminase activity corresponded to the first peak of the protein which eluted just after void volume. The fractions in which histaminase activity was detected were pooled and concentrated by ultrafiltration. The concentrated sample was centrifuged at 21,000 g for 20 min at 4°C to remove the insoluble substances formed during concentration. The protein concentration in the supernatant (sample-1) was 6.2 mg/ml.

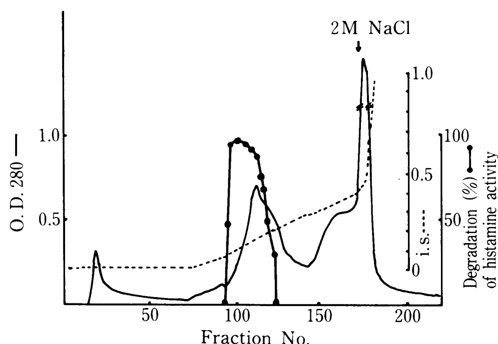

Fig. 2. DEAE-cellulose chromatogram of the sample-1 (fractions containing histaminase activity in Fig. 1)

Sephadex G-200 gel filtration of the sample-1 at 55°C: Ten ml of the sample-1 was applied to the column of Sephadex G-200 at 55°C. Results of an experiment are shown in Fig. 2. Two main peaks of proteins were obtained. Histaminase activity nearly corresponded to the first peak of the protein. The fractions of the first peak were pooled. The conspicuous precipitate was observed in the pooled sample of the first peak of the protein after storing the sample at −20°C for overnight. The precipitate was removed by centrifugation at 21,000 g for 20 min at 4°C. The supernatant was concentrated by ultrafiltration. The protein content in the concentrated sample (sample-2) was 0.65 mg/ml.

DEAE-cellulose chromatography of the sample-1: Twenty one ml of the sample-1 (50 mg protein) was applied on DEAE-cellulose column. As shown in Fig. 3, histaminase activity was detected in the fractions eluted at ionic strength between 0.08 and 0.208. The fractions containing histaminase were pooled and concentrated by ultrafiltration. The precipitate which was formed during concentration was removed by

centrifugation at 21,000 g for 20 min at 4°C. The concentration of the protein in the supernatant was 80 mg/ml (sample-3).

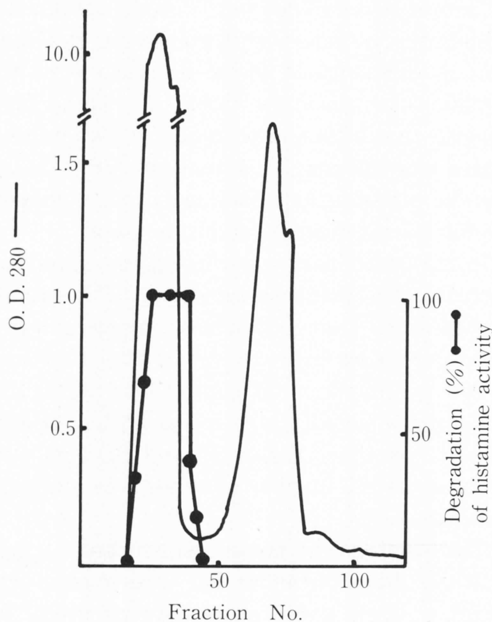

Fig. 3. Sephadex G-200 gel filtration at 55°C of the sample-1 (fractions containing histaminase activity in Fig. 1)

Histaminase activity in each partially purified sample: Histaminase activity in each sample was determined, using histamine as a substrate, in 0.01 M phosphate buffer, pH 7.0. One tenth ml of the enzyme sample was added to 0.9 ml of the same buffer containing 200 ng of histamine, and incubation was carried out at 37°C for 5 min. The histaminase activity was expressed as amount (ng) of the substrate, histamine, degraded for 5 min by 1 mg of protein of the enzyme sample.

As shown in Table 1, the histaminase activity in the sample-1 was 259.3, and in the sample-2 which was obtained from sample-1 by DEAE-cellulose chromatography was 580.0. On the other hand, the activity was about ten times higher (2866.0) in the sample-3 which was obtained by fractionating the sample-1 on Sephadex G-200 at 55°C, compared with that of the sample-1.

Table 1. Histaminase activities in the samples obtained by different procedures

Procedure	Histaminase activity***
Column chromatography on	
Sephadex G-200 (4°C) (sample-1*)	259.3
DEAE-cellulose** (sample-2)	580.0
Sephadex G-200 (55°C)** (sample-3)	2866.0

*Sample-1 was obtained by Sephadex G-200 chromatography of the guinea pig liver extract at 4°C.
**The samples applied on the columns were sample-1.
***Histaminase activity was expressed as amount (ng) of histamine degraded for 5 min by 1 mg of protein of the enzyme preparations.

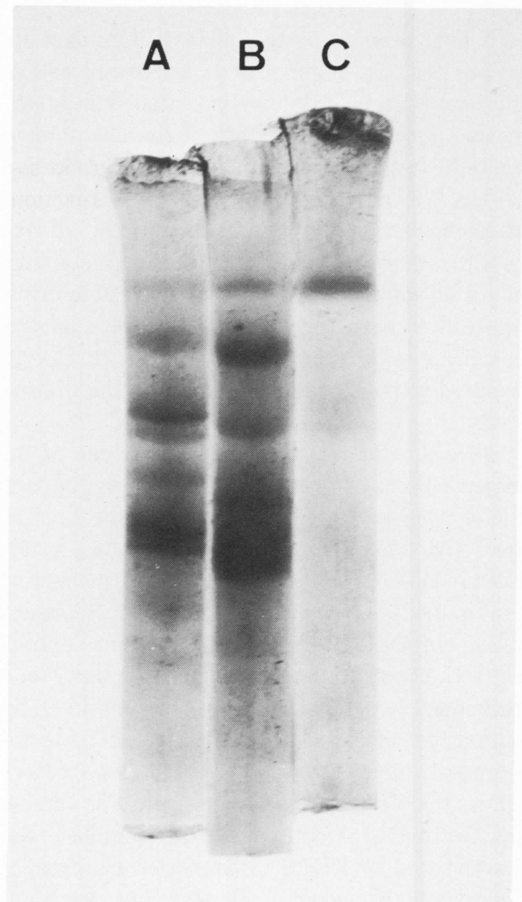

Fig. 4. SDS-gel electrophoresis on 5% polyacrylamide gels of the preparations of the guinea pig liver histaminase. A: sample-1 (fractions containing histaminase activity in Fig. 1), B: sample-2 (fractions containing histaminase activity in Fig. 2), C: sample-3 (fractions containing histaminase activity in Fig. 3). Approximately 75 μg for A and B, and 7.5 μg for C.

SDS-gel electrophoresis of partially purified histaminase: Fig. 4 compares the electrophoretic patterns on SDS-gel electrophoresis of sample-1, sample-2 and sample-3. The sample-1, which was obtained by Sephadex G-200 gel filtration of the liver extract at 4°C, contained 7 dense and 2 faint protein bands. The sample-2, which was obtained by DEAE-cellulose chromatography of the sample-1, contained 5 dense bands. On the other hand, only 1 dense and 2 faint bands were observed in the sample-3, which was obtained by fractionating the sample-1 on Sephadex G-200 chromatography at 55°C.

DISCUSSION

It has been reported that histaminase in guinea pig skin might play an important role in the regulation of histamine-mediated skin inflammation, since administration of the histaminase inhibitor, aminoguanidine, caused amplification of the 72 hr homologous passive cutaneous anaphylaxis in this species[5]. In view of the possible role of this enzyme in histamine-mediated inflammation, it is of interest to investigate the characteristics or localization of this enzyme in skin. For these purposes, it was attempted to purify the histaminase in the present study.

Since it is known that the liver is one of the major sources of histaminase in guinea pigs and the enzyme is liberated chiefly from the liver after i.v. heparin injection[2], the crude histaminase extract was prepared by incubating the guinea pig liver with heparin. Sephadex G-200 chromatography of the crude liver extract at 4°C showed that histaminase activity corresponded to the first peak of the protein which eluted just after void volume (sample-1). In the sample-1, at least 9 protein bands were detected on the SDS-gel electrophoresis. For further purification of histaminase, the sample-1 was fractionated by DEAE-cellulose chromatography (sample-2). However, 5 dense bands were still detected in the sample-2, although histaminase activity was approximately double, compared with that in the sample-1.

The results of the previous studies indicated that the histaminase activity in the crude histaminase preparation obtained from guinea pig skin was strikingly increased by incubating the reaction mixtures at 55°C, compared with that at 37°C. In addition, it was indicated that histaminase might form the complex with inhibitor-like substance in the crude histaminase preparation obtained from guinea pig skin, and that the histaminase might dissociate from the inhibitor-like substance at 55°C[4,6]. These facts suggest that histaminase in guinea pigs in heat-stable and the possibility that gel filtration under the experimental conditions at 55°C may be useful for purification of histaminase.

In the preliminary experiments, no significant increase of histaminase activity at 55°C was observed in the crude histaminase sample prepared from guinea pig liver. However, after Sephadex G-200 gel filtration of sample-1 at 55°C, the histaminase activity was increased by approximately ten times (Table 1), and SDS-gel electrophoresis the number of bands was markedly decreased (Fig. 4)

The results in the present experiments suggest that gel filtration under the experimental condition at high temperature may be useful for purification of histaminase.

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REFERENCES

1. **Lowry, O.H., Rosebrough, N.J., Farr, A.C. and Randall, R.J.** 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**: 265-275.
2. **Schmutzler, W., Goldschmidt, O, Bithge, K. and Knop, J.** 1969. The release of guinea pig liver histaminase and some of its properties. Int. Arch. Allergy **36**: 45-55.
3. **Weber, K. and Osborn, M.** 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacryl amide gel electrophoresis. J. Biol. Chem. **244**: 4406-4412.
4. **Yamamoto, S.** 1981. Histaminase and its inhibitory factor in guinea pig skin. Hiroshima J. Med. Sci. **30**: 251-254.
5. **Yamamoto, S., Francis, D. and Greaves, M.W.** 1976. Enzymic histamine metabolism in guinea pig skin and its role in immediate hypersensitivity reactions. Clin. exp. Immunol. **26**: 583-589.
6. **Yoshikuni, K. Yamamoto, S., Yamura, T., Francis, D. and Greaves, M.W.** 1978. Charactarization of diamine oxidase in guinea pig skin: Increase of diamine oxidase activity at high temperature. Hiroshima J. Med. Sci. **27**: 61-65.