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PURIFICATION OF HISTAMINASE (DIAMINE OXIDASE) FROM HUMAN PREGNANCY PLASMA BY AFFINITY CHROMATOGRAPHY

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Summary

The present study describes the use of affinity chromatography to achieve a high degree of purification of histaminase (diamine oxidase, EC 1.4.3.6) from plasma of women in the third trimester of pregnancy. The procedure is based upon the binding of histaminase to cadaverine, a diamine substrate for the enzyme, which is coupled to Sepharose. Contaminant proteins were removed by high concentrations of NaCl (up to 1.0 M), and the histaminase was then eluted from the column with a buffer containing 300—400 units/ml of sodium heparin. The purification technique has the following characteristics: (1) in optimal experiments, 3000-fold purification of enzyme was obtained; (2) the yield of enzyme was as great as 25%; (3) the binding of histaminase to the amine groups of the cadaverine appears to represent a true "affinity" phenomenon since enzyme bound to DEAE-cellulose under neutral pH conditions was eluted at much lower concentrations of NaCl (less than 0.4 M).

The enzyme purified by the present procedure has the following properties: (1) disc gel polyacrylamide electrophoresis showed two protein bands for 1000—3000-fold pure histaminase; the major band may represent a contaminant protein, while the minor band corresponded to the position of histaminase activity; (2) a 90 000 molecular weight subunit for the plasma histaminase was identified on calibrated sodium dodecyl sulfate gels; this value agrees well with previous estimations for the subunit size of human placental histaminase; (3) the purified enzyme behaved as classical histaminase (diamine oxidase) in that it was totally inhibited by low concentrations of aminoguanidine, but was less inhibited by semicarbazide and by inhibitors of monoamine oxidase, and the enzyme was active against histamine and putrescine, but not against the monoamines benzylamine and tryptamine. Also, the enzyme was strongly inhibited by NaCl.

Introduction

Histaminase (diamine oxidase, EC 1.4.3.6) oxidatively deaminates histamine and aliphatic amines such as putrescine and cadaverine [1]. Although the physiological role of this enzyme is poorly understood, high levels of histaminase activity have been described in three diverse biologic situations: (1) activity is high in decidual portions of the placenta in most mammalian species, and plasma histaminase activity increases progressively during the course of pregnancy [2—5]; (2) histaminase is one of several enzymes which increases in activity in plasma following parenteral administration of heparin [6]; a reduced response to heparin is found in the genetic disorder, Type I hyperlipoproteinemia [7]; and (3) histaminase activity is also high in the endocrine malignancy, medullary carcinoma of the thyroid gland [8,9].

To date, little has been done to compare the histaminases found in various tissues of human and other animals. The enzyme has been partially purified from kidney and placenta [10—14] by lengthy procedures that require large amounts of starting material and achieve rather low yields of enzyme. The present report describes a high degree of purification of histaminase from pregnancy plasma by an affinity chromatography technique which may aid the study of the enzyme in many tissues. The procedure is rapid, simple, and requires comparatively small amounts of crude starting material. Also reported are the substrate specificity, inhibitor sensitivity, and molecular weight of the purified enzyme from pregnancy plasma.

Experimental procedure

Materials

The following radioisotopic substrates were used to assay amine oxidase activities: [β-^{3}H]histamine (1400 Ci/mol), a gift from Dr Michael Beaven (National Heart and Lung Institute, National Institutes of Health, Bethesda, Md.), was prepared as previously described [15]; [2,3-^{3}H]putrescine (diaminobutane, 182.8 Ci/mol) and [^{14}C]tryptamine (47 Ci/mol) were purchased from New England Nuclear Corp.; and [^{14}C]benzylamine (8.6 Ci/mol) was obtained from I.C.N. Pharmaceuticals, Inc.

Other materials used in the studies include Dowex AG-50 W-X2, 200—400 mesh, electrophoresis grade acrylamide, bisacrylamide, $(NH_4)_2S_2O_8$, (*N,N,N'-N'*-tetramethylenediamine), Coomassie Blue, and bromophenol blue, all from BioRad; $(NH_4)_2SO_4$, scintillation grade toluene, glacial acetic acid, glycine and semicarbazide from Fisher Scientific Co.; cadaverine, as the free base, Trizma base, and iproniazid phosphate from Sigma Chemicals; Sephadex G-200 and Sepharose 4B from Pharmacia Fine Chemicals; CNBr from J.T. Baker Chemicals; histamine · 2HCl from Schwartz-Mann; DEAE-cellulose from Whatman; sodium heparin from Upjohn Co.; tranylcypromine from Regis Chemicals; Instrabray scintillation fluid from Yorktown Research; and aminoguanidine from Eastman. The Sepharose-bound cadaverine used in our initial studies was a generous gift from Dr Howard Katzen, Merck Research Institute, Rahway, N.J., and heparin-Sepharose was a gift from Dr Virgil Brown, University of California at San Diego.

Plasma used for purification procedures was obtained by withdrawal of 50 ml of blood by venipuncture from women in the third trimester of pregnancy. The blood was immediately placed in chilled glass centrifuge tubes coated with 0.5 ml of heparin (1000 units/ml) and centrifuged for 20 min at 6000 × *g* in a refrigerated centrifuge (Model B-20, International Equipment Co.). Plasma was stored frozen (—20°C) until time of use.

Methods

Enzyme assays. Histaminase activity was measured by the method of Beaven and Jacobsen [15], using as substrate 100 μl of [β-^{3}H]histamine (15 pmol or 50 000 cpm), in 0.1 M phosphate buffer, pH 6.8. Samples for determination of enzyme activity were diluted to a volume of 100 μl with the same buffer and added to the substrate in 1.5 ml Eppendorf vials; incubations were carried out for various times at 37°C. A modification of the original procedure was used to separate ^3H_2O, formed during deamination of the substrate, from undeaminated [β-^{3}H]histamine*; rather than using Thunberg tubes to capture the ^3H_2O by sublimation, unreacted substrate was removed by adsorption onto Dowex AG-50 ion-exchange resin. Specifically, the enzyme reaction was terminated by adding 200 μl of 0.1 M histamine · 2HCl containing 0.2 g of Dowex AG-50. Following vigorous mixing, the incubation vials wer e centrifuged at top speed in a table-top centrifuge, and a 100-μl aliquot of the supernatant was removed for determination of radioactivity. Samples were counted in a Beckman LS-1006 scintillation counter with 40% efficiency for ^{3}H. Results were expressed as cpm ^3H_2O/100 μl, or, percent [β-^{3}H]histamine deaminated, or as units/ml solution where one unit = 1 pmol [β-^{3}H]histamine deaminated/h. The method yields values of enzyme activity identical with the original Thunberg tube method [15] when tested against pregnancy plasma.

Diamine oxidase activity was determined by measuring the deamination of [β-^{3}H]putrescine using Tryding and Willert's modification [4] of Okuyama and Kobayashi's method [16]. The assay was further modified so that incubation volumes were the same as those used for the histaminase assay. Labeled putrescine was diluted in 0.1 M phosphate buffer, pH 7.6, and 100 μl (300 pmol or 150 000 cpm), was added to 1.5 ml Eppendorf vials. Enzyme samples were similarly diluted to a volume of 100 μl, and added to the substrate. Incubations were performed for 60 min at 37°C. Blanks were run either with buffer alone or with enzyme in the presence of $2 \cdot 10^{-5}$ M aminoguanidine, a specific inhibitor of histaminase [17]. The deaminated product was extracted by two successive additions of 1.0 ml of toluene, rather than the 10 ml of PPO/POPOP/toluene solution originally used [4,16], and 200-μl aliquots of the pooled extracts were counted in 10 ml of Instrabray. Under these conditions, the reaction reached completion in 60 min using 100 μl of pregnancy plasma, and up to 80% of the total added counts was recovered as deaminated product in the toluene extracts. Blanks of only 40—50 cpm were obtained for both buffer and aminoguanidine incubations.

Monoamine oxidase activity was assayed by the method of Robinson et al.

* Based on a personal communication from Dr Beaven.

[18], modified to assess deamination of [^{14}C]benzylamine, and [^{14}C]-tryptamine. The labeled substrates were prepared in Sorensen's buffer, pH 7.2, and 100-μl aliquots were added to 1.5 ml Eppendorf vials. Total activity in each 100 μl aliquot was 40 000 cpm for 300 pmol of tryptamine, and for 3000 pmol of benzylamine. Samples for assay of enzyme activity were diluted with the same buffer to a total volume of 600 or 800 μl, added to the substrate, and incubated at 37°C for 60 min. Aldehyde products were extracted exactly as described for the measurement of [^{3}H]putrescine deamination, and 200-μl aliquots were counted for ^{14}C with an efficiency of 70%. Under the conditions, 5—10 μl of a 1 : 80 (w/v) homogenate of rat liver or a 1 : 1 (w/v) dilution of a pellet of rat liver mitochondria gave 70—80% deamination of the labeled tryptamine in 1 h, and 300 μl of crude human plasma produced 15—20% deamination of the labeled benzylamine. Buffer blanks gave 40—50 cpm for [^{14}C] tryptamine and 200—250 cpm for [^{14}C] benzylamine.

Preparation of Sepharose-bound cadaverine (diaminopentane). The coupling was accomplished using the method of Cuatrecasas [19]. In brief, 12.5 g of CNBr was added to 50 ml of settled Sepharose suspended in water to a total volume of 100 ml. NaOH (8.0 M) was added until the pH stabilized at 11.0. Following washes with several volumes of water, the Sepharose was added immediately to 100 mmol of cadaverine which had been previously titrated to pH 10.0 with 6.0 M HCl, and brought to a volume of 50 ml with water. After this mixture was stirred in the cold for 20—24 h, the Sepharose was washed successively with water, 6 M guanidine · HCl, and 0.005 M phosphate buffer, pH 7.2, prior to use.

Disc gel electrophoresis. Methods described by Davis [20] were used for disc gel electrophoresis. Polyacrylamide gels of varying concentrations were poured in 6 mm × 9 cm glass tubes to a depth of 7.5 cm. All gels were pre-electrophoresed prior to use since this appeared to enhance detection of enzyme activity*. All protein samples were mixed in 0.2 M Tris · HCl buffer, pH 6.8, with three drops of glycerol and 2.5 μl of 0.1% bromophenol blue to a total volume of 400—500 μl prior to application to the gels. Electrophoresis was carried out at room temperature for 4—6 h, using 2—3 mA/gel, in Tris/glycine electrode buffer, pH 8.4. Gels were stained with Coomassie blue and destained and stored in 10% acetic acid. Gels were scanned in a Guilford 2400 recording spectrophotometer, using a wavelength of 600 nm. For analysis of enzyme activity, unstained gels were sectioned by hand with a scalpel in 2—2.5-mm slices which were then incubated overnight at 4°C in 200 μl of 0.1 M phosphate buffer, pH 6.8. Aliquots (100 μl) were subsequently assayed for histaminase activity as previously described. Sodium dodecyl sulfate gel electrophoresis was performed by the method of Weber and Osborn [21].

For all studies, the method of Lowry et al. [22] was used for direct determinations of protein concentrations. Protein estimations of column fractions by $A_{280\,nm}$ readings were performed on a Perkin-Elmer, Coleman 55 spectrophotometer.

* Unpolymerized acrylamide and $(NH_4)_2S_2O_8$-inhibited histaminase activity.

Results

Affinity chromatography of crude plasma

Batch methods were used to test whether cadaverine coupled to Sepharose (cadaverine-Sepharose) would bind histaminase from pregnancy plasma. Neither untreated Sepharose nor heparin-Sepharose, a preparation which binds lipoprotein lipase, another enzyme which increases in plasma following parenteral administration of heparin [23], removed histaminase from plasma. The cadaverine-Sepharose removed 100% of the enzymatic activity from plasma during 5 min of incubation (100 μl of plasma, and 300 μl of a 70% suspension of cadaverine-Sepharose).

Initial efforts to purify histaminase from pregnancy plasma utilized 0.6 × 4.0 cm columns of cadaverine-Sepharose. As with the batch procedure, the cadaverine-Sepharose columns bound histaminase effectively, but attempts to remove the enzyme with salt solutions up to 1.0 M in concentration were unsuccessful. Addition of heparin (300 units/ml) to the elution buffer successfully removed the enzyme. When heparin was added immediately following application of plasma, only a 10-fold purification of histaminase was achieved. However, elution of contaminating proteins with salt solutions of increasing molarity, followed by addition of heparin, provided a successful purification procedure. The pattern of histaminase elution in an experiment using 2.0 ml of pregnancy plasma is shown in Fig. 1. During application of the sample, the column turned progressively blue, probably due to the retention of ceruplas-

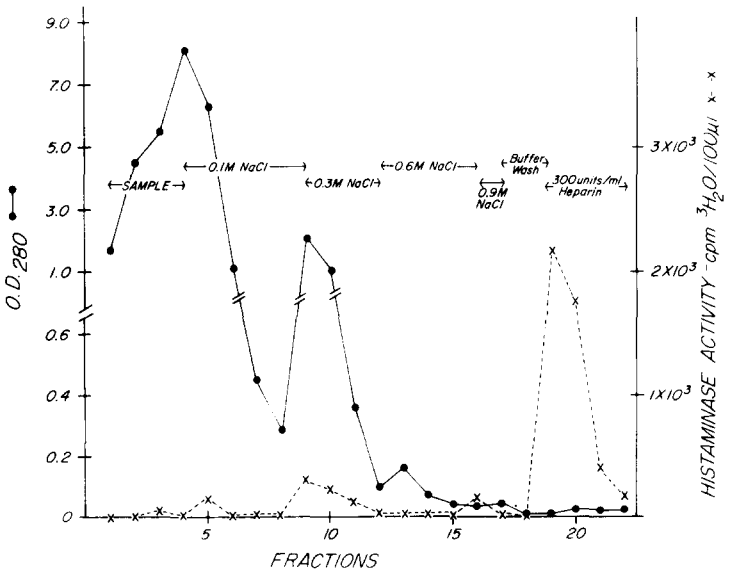

Fig. 1. Cadaverine-Sepharose chromatography of pregnancy plasma. Plasma (2.0 ml) was diluted to a total volume of 25 ml with 0.005 M phosphate buffer, pH 7.2, containing 0.05 M NaCl, and then placed on a 0.6 × 5.0 cm cadaverine-Sepharose column. The same buffer was used to prepare NaCl solutions of increasing concentration which were applied to the column in step-wise additions following application of the sample, and to perform the buffer wash prior to heparin elution, and to elute the enzyme with the final heparin wash. The volume of each fraction was 5 ml, and the flow rate was 20—30 ml/h. The entire procedure was performed in a cold room at 4° C.

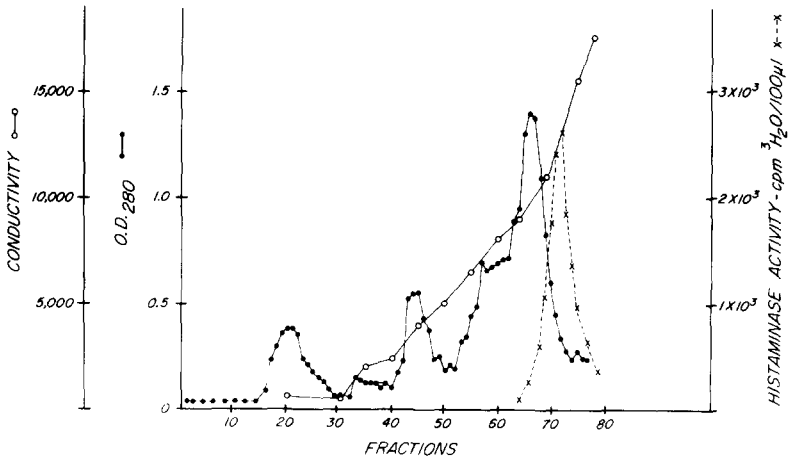

Fig. 2. DEAE-cellulose chromatography of pregnancy plasma. Plasma (2.0 ml) was diluted to a total volume of 30 ml with 0.005 M phosphate buffer, pH 7.6, prior to application to a 2.6 × 30 cm column. The DEAE-cellulose had been equilibrated previously with the same buffer. Following application of the sample, the column was washed with an equal volume of the buffer, and then eluted with solutions of the same buffer containing increasing concentrations of NaCl. Three chambers of a Varigrad mixer were charged in order with 0.05, 0.2, and 0.4 M NaCl and salt elution was started at Fraction 15 with a flow rate of 60 ml/h. The volume of each fraction was 5.0 ml. Conductivity was measured with a Beckman Conductivity Bridge (MHO-OHM Model RC-16B2).

min. This blue protein eluted in a single fraction (No. 6) during the application of 0.1 M NaCl. Histaminase activity was retained on the column as the salt concentration of the buffer solution was increased to 0.9 M, but was eluted in a sharp peak with salt-free buffer containing 300 units of heparin/ml. In this particular experiment, 25% of the added enzyme was recovered from the column in the heparin eluate. Contaminant proteins were eluted from the column by the time the NaCl concentration reached 0.6 M in the salt washes and less than 5% of the histaminase eluted during this interval. In similar studies, when 20 ml of plasma was applied to the same size columns, 15% of the added activity was recovered in the heparin wash and the histaminase was purified 1500-fold in the peak fractions.

To test whether histaminase bound to the amine groups of cadaverine-Sepharose by an "affinity" mechanism, the cadaverine-Sepharose procedure was compared to ion-exchange chromatography using DEAE-cellulose under similar pH conditions (Fig. 2). Histaminase activity was eluted from DEAE-cellulose near a major peak of contaminant protein before the NaCl concentration reached 0.4 M. The resultant purification of the enzyme was only 10-fold.

After the elution pattern of histaminase from cadaverine-Sepharose was established, larger scale purifications of the enzyme were attempted with a 30—65% $(NH_4)_2SO_4$ fraction of pregnancy plasma. Initial trials, with columns 0.6—1.0 cm in diameter and up to 30 cm in length, resulted in poor yields of enzyme (less than 2%), and relatively low degrees of purification (200-fold). Also, under these conditions 10—30% of the histaminase activity was eluted during washes with low concentrations (0.3—0.6 M) of NaCl. By increasing the ratio of the column diameter to its length, and using flow rates of 60—100 ml/h, strikingly better purification and yields were achieved. Results from an

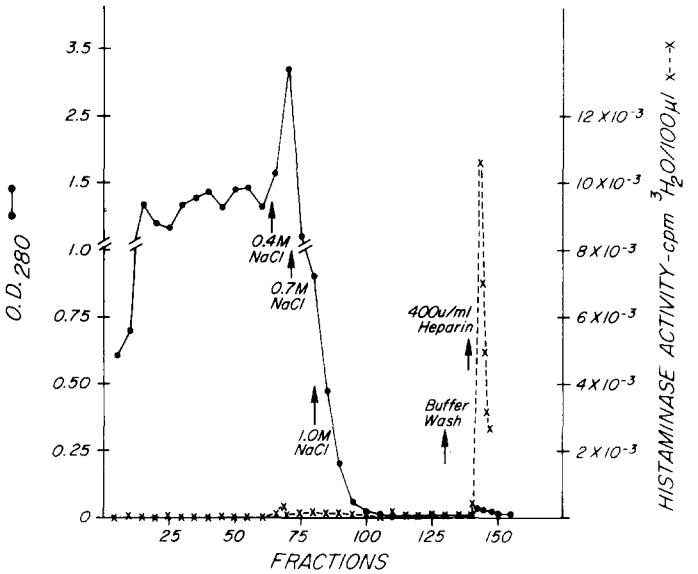

Fig. 3. Cadaverine-Sepharose chromatography of an $(NH_4)_2SO_4$ fraction from pregnancy plasma. A 30—65% precipitate was prepared from 500 ml of plasma and the pellet was diluted in 400 ml of 0.01 M phosphate buffer, pH 7.2. The solution was dialyzed for 18 h against 2 l of the same buffer; the final volume was 440 ml. An aliquot (140 ml) was then diluted to a total of 700 ml (protein concentration, 9 mg/ml) with 0.01 M phosphate buffer, pH 7.2, which contained 0.1 M NaCl. This sample was applied to a 2.6 × 7.0 cm column of cadaverine-Sepharose. The column was then washed with 0.01 M phosphate buffer, pH 7.2, containing the salt concentrations and heparin as indicated. The flow rate was 60—100 ml/h and 10-ml fractions were collected.

experiment using a 2.6 × 7.0 cm column are shown in Fig. 3. With this method histaminase activity was purified 3000-fold from pregnancy plasma (specific activity of the crude plasma = 12.4, of the $(NH_4)_2SO_4$ preparation = 20.0, and of the enzyme in the two most active fractions after heparin elution, 35 358 and 41 133, respectively). A total of 0.5 mg of protein was obtained, and the yield of enzyme was 15.1% of the original crude plasma, and 21.7% of the materials placed on the column. Enzyme purified in this manner was used for all subsequent studies detailed below.

Disc gel electrophoresis of purified histaminase

Fig. 4 compares the electrophoretic patterns on 5% polyacrylamide gels of pregnancy plasma, a 1000-fold purified enzyme preparation from a 0.6 × 7.0 cm cadaverine-Sepharose column, and a 3000-fold purified preparation from a 2.6 × 7.0 cm column. The 1000-fold purified preparation (Fig. 4A) contained one dense protein band and a faint band which migrated faster. Electrophoresis of the 3000-fold purified enzyme was complicated by pronounced aggregation of protein at the gel origin (Fig. 4B), but the major and minor bands could again be identified. When the enzyme preparation was incubated with 0.1% mercaptoethanol prior to electrophoresis, aggregation was essentially abolished; enzymatic activity was completely lost under these conditions.

When the position of histaminase activity on the gels was compared to the location of the protein peaks (Figs 4A and 4B), the enzyme activity was de-

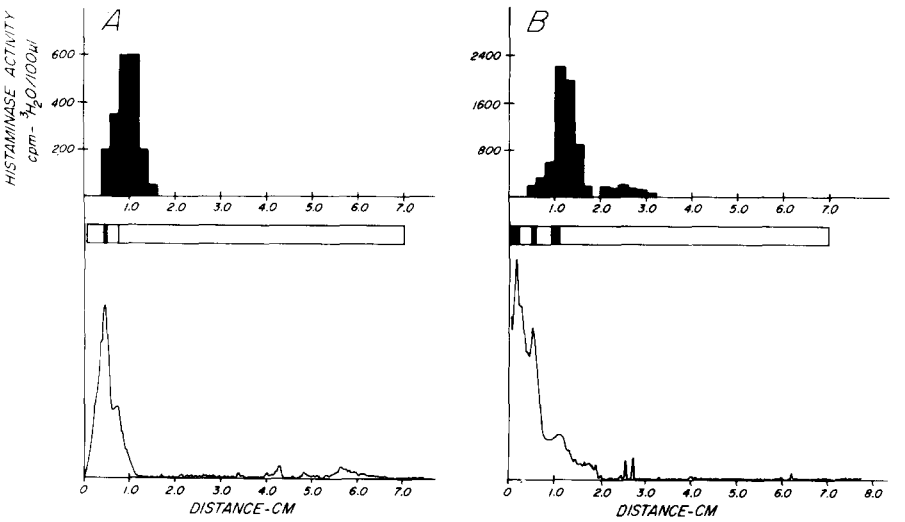

Fig. 4. Disc gel electrophoresis on 5% polyacrylamide gels on 1000-fold (A), and 3000-fold (B) purified plasma histaminase. Approx. 15 µg of the purified protein was applied to the gels. Enzyme activity was determined from sectioned gels (see Methods) run simultaneously. A schematic of the stained gel is shown above the scan pattern.

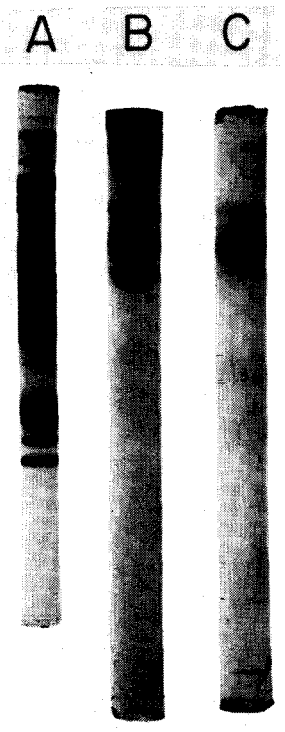

Fig. 5. Sodium dodecyl sulfate gel electrophoresis on 7.5% polyacrylamide gels of pregnancy plasma (A), 3000-fold purified histaminase incubated with 1.0% sodium dodecyl sulfate alone (B), and 3000-fold purified histaminase incubated with 1.0% sodium dodecyl sulfate and 1.0% β-mercaptoethanol (C). Approx. 60 µg of plasma protein was applied for A and approx. 10 µg of purified protein was applied for B and C.

tected only in the areas where protein was stained. For both the 1000- (Fig. 4A) and 3000- (Fig. 4B) fold purified preparations, the enzyme activity seemed to correspond to the faster moving minor protein band. This relationship was somewhat difficult to see in the 1000-fold purified preparation where the minor band presented as a shoulder on the main protein peak (Fig. 4A), but was easier to appreciate in Fig. 4B where the time of the electrophoretic run was longer and the proteins moved further into the gel, allowing the minor protein to be seen as a more distinct peak.

When the 3000-fold purified preparation was incubated for 2 h at 37°C with 1.0% sodium dodecyl sulfate, and then electrophoresed on 7.5% sodium dodecyl sulfate gels (Fig. 5B), aggregation of protein occurred at the gel origin, and four protein bands were seen. When 1.0% mercaptoethanol was added to the incubation, aggregation was abolished, and two bands of unequal intensity were found on the gels (Fig. 5C). Estimated molecular weights obtained from calibration of the sodium dodecyl sulfate gels were 90 000 and 70 000 for the minor and major bands, respectively.

From the data in Figs. 4A and 4B, it appears that the major band identified on the 5% polyacrylamide gels is a contaminant protein, while the minor band is the plasma histaminase. If this assumption is correct, the minor protein band on the sodium dodecyl sulfate gels is a subunit peptide of histaminase, and the major band is a subunit of the contaminant protein. The molecular weight of 90 000 for the plasma histaminase subunit is identical with that found by Bardsley et al. [14] for human placental histaminase; the placental enzyme is thought to be composed of two peptides of this size with a total molecular weight of 180 000 to 190 000 [14]. This molecular weight estimate correlated well with the elution position for pregnancy plasma histaminase from Sephadex G-200; the histaminase activity eluted immediately after the void volume of the column which excluded globular proteins of approx. 200 000 molecular weight.

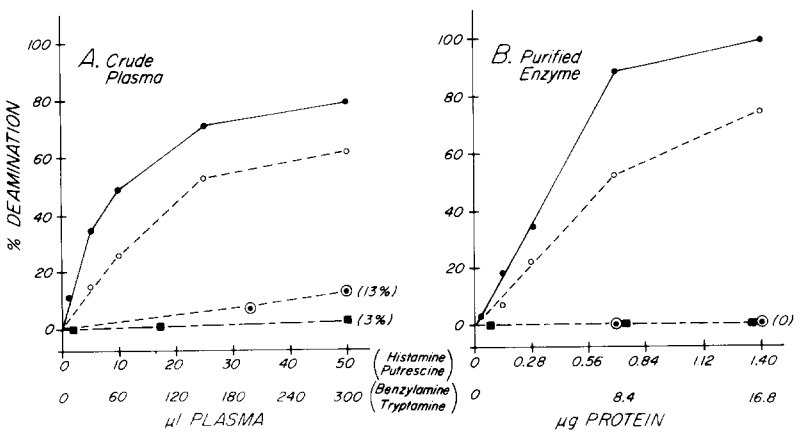

Fig. 6. Activity of pregnancy plasma and 3000-fold purified histaminase against various labeled amines. ●——●, [β-^{3}H]histamine; ○- - - - -○, [β-^{3}H]putrescine; ⊙- - - - -⊙, [^{14}C]benzylamine; ■- - - - -■, [^{14}C]tryptamine. When purified enzyme was used, the amount of protein in each assay was adjusted to give similar activity against [β-^{3}H]histamine and [β-^{3}H]putrescine as obtained with the indicated volumes of pregnancy plasma. Six times more plasma and 12 times more purified enzyme were utilized to measure activity against tryptamine and benzylamine than against histamine and putrescine.

TABLE I

EFFECT OF INHIBITORS ON PURIFIED HISTAMINASE

Protein (0.56 μg) from a 3000-fold purified preparation was assayed against [β-^{3}H]histamine (see Methods) in the presence of the various inhibitors. The data is compared to that of Bardsley et al. [14] for purified placental diamine oxidase.

Inhibitor	Inhibitor concentration (M)	Enzyme activity (cpm ^3H_2O/100 μl)	Inhibition (%)	Inhibition of purified placental diamine oxidase (Bardsley et al. [14]) (%)
None	—	6723	0	—
Tranylcypromine	10^{-4}	5139	24	14
Iproniazid	10^{-4}	2169	68	66
Semicarbazide	10^{-5}	6218	8	50 (5×10^{-5} M)
Semicarbazide	10^{-4}	17	99	
Aminoguanidine	2×10^{-5}	0	100	100 (10^{-3} M)

Substrate specificity

Fig. 6 demonstrates that pregnancy plasma (Fig. 6A) gave high amine oxidase activity against histamine and putrescine. When the volume of plasma assayed was increased 6-fold, considerable activity was also seen against benzylamine, and there was slight deamination of tryptamine. By comparison (Fig. 6B), 3000-fold purified histaminase was inactive against benzylamine and tryptamine even when 12 times more protein was used than the amount assayed to assess deamination of labeled histamine and putrescine. A 1000-fold purified preparation similarly lacked activity against benzylamine and tryptamine.

Sensitivity to amine oxidase inhibitors

Table I illustrates the response of the purified enzyme to several known inhibitors of amine oxidase activity [14]. Complete inhibition was seen with the histaminase inhibitor aminoguanidine, at a concentration of $2 \cdot 10^{-5}$ M while the other inhibitors tested were much less inhibitory at a concentration of $1 \cdot 10^{-4}$ M. As shown, the response of the purified plasma enzyme to

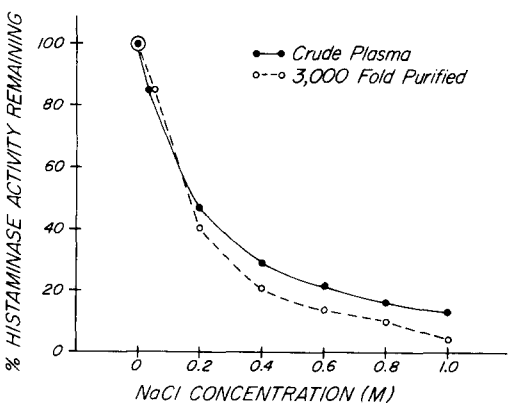

Fig. 7. Salt inhibition of pregnancy plasma and 3000-fold purified histaminase. The increasing salt concentrations were added to the 0.1 M phosphate buffer, pH 6.8, used in the histaminase assay (see Methods).

inhibitors was similar to that found by Bardsley et al. [14] for purified human placental histaminase.

Inhibition by salt

Inhibition of activity by increasing concentrations of NaCl is a property of the histaminase released into blood after heparin injection [7] and of pig kidney diamine oxidase [25]. As seen in Fig. 7, both pregnancy plasma histaminase and purified histaminase from this source showed identical patterns of inhibition by NaCl.

Discussion

The procedure described for the purification of histaminase offers a promising new tool for studies of this enzyme. The technique is relatively simple, and can achieve a high degree of purification of the enzyme from crude preparations within 24—48 h. In this initial study, relatively small amounts of plasma (200—300 ml) were used to obtain enough histaminase to perform kinetic studies, to evaluate substrate specificity, and to study some of the structural characteristics of the protein. The affinity chromatography procedure seems readily adaptable to larger scale purifications to obtain sufficient purified enzyme for more detailed studies, such as a comparison of histaminases from various tissues and preparation of antibodies to these enzymes.

The new affinity technique is based upon binding of histaminase to coupled cadaverine, the best diamine substrate for the enzyme [1]. Under neutral to alkaline pH conditions, however, charged amine groups on the coupled cadaverine may bind other proteins, and thus obscure the specificity of histaminase binding to the coupled diamine. Contaminant proteins do bind to cadaverine-Sepharose at low salt concentrations since the enzyme is purified only 10—40-fold when eluted by heparin solutions prior to washing the columns in buffers with high salt concentrations. On the other hand, the specificity of the interaction between histaminase and the coupled cadaverine is evidenced by binding of the enzyme to the column even after exposure to high concentrations of salt. By contrast, as might be expected for an anion-exchange mechanism, the contaminant proteins are readily eluted before the salt concentration of the buffer reaches 0.6 M. Further evidence for the specific binding of histaminase to cadaverine-Sepharose is provided by the absence of other plasma amine oxidases in the purified preparations. As discussed further below, histaminase is the only amine oxidase in pregnancy plasma that should interact with cadaverine as a substrate [14].

Despite the high degree of purification achieved for pregnancy plasma histaminase by cadaverine-Sepharose chromatography, a contaminant protein probably remains in the most purified preparations. Although the major protein could represent an inactive aggregate of histaminase, comparison of the results obtained by sodium dodecyl sulfate and conventional polyacrylamide gel electrophoresis suggests the presence of a single contaminating protein. There is evidence, however, that further purification of pregnancy plasma histaminase may be possible with the affinity chromatography procedure. Improved purification resulted when the diameter of the cadaverine-Sepharose column was increased relative to its height. The improved results with such columns

may indicate that the contaminant protein is bound to the cadaverine less tightly than histaminase, and is subsequently retarded on the more compact columns after its removal from the binding site by NaCl. Such non-specific binding and retardation on affinity columns has been described previously [26]. Further studies are required to see whether $(NH_4)_2SO_4$ precipitation followed by a affinity chromatography on wide, short cadaverine-Sepharose columns can produce homogeneous preparation, or whether further purification steps will be required.

The use of heparin to elute histaminase from cadaverine-Sepharose was suggested by the fact that parenterally administered heparin liberates histaminase into the bloodstream in those animal species which have been studied [6]. It has been proposed that the negatively charged heparin molecule releases another enzyme, lipoprotein lipase, in vivo, by displacing it from negatively charged binding sites, possibly on capillary membranes [27]. Presumably, such an interaction between charged groups plays a role in the elution of histaminase from cadaverine-Sepharose by heparin. However, certain differences are evident between the effects of heparin on histaminase and on other enzymes, such as lipoprotein lipase. Heparin activates lipoprotein lipase in vitro but has no effect on histaminase activity [7]. Also, whereas heparin-Sepharose binding of lipoprotein lipase provides a useful method for the purification of that enzyme [23], we observed no binding of histaminase to heparin-Sepharose in our studies. An important similarity does exist, however, between histaminase and lipoprotein lipase; inhibition by high concentrations of NaCl, which has long been used to characterize lipoprotein lipase [28], has more recently been described for pig kidney diamine oxidase [25] and for human plasma histaminase released by parenteral heparin [7]. The present study indicates that histaminase from pregnancy plasma is also inhibited by increasing concentrations of NaCl.

The enzymatic activity purified in the present study behaves as a classic histaminase (diamine oxidase) in both its substrate specificity and its response to inhibitors. The purified enzyme differs from the soluble monoamine oxidase from human plasma [18], and the monoamine oxidase in human placenta [14] by its failure to deaminate benzylamine or tryptamine. As is the case for placental diamine oxidase, the purified plasma enzyme is completely inhibited by low concentrations of aminoguanidine and is much less affected by semicarbazide, iproniazid, and tranylcypromine [14].

Although the histaminase activity in human pregnancy plasma is thought to arise from maternal decidual tissue in the placenta, purified histaminase from plasma has not been compared with that found in the placenta. Our data on the properties of the plasma enzyme are in good agreement with those described for purified placental histaminase by other investigators [13,14]. Both the substrate specificity and the potency of inhibition by various compounds tested are similar to that found by Bardsley et al. [14] for purified placental histaminase. Little is known about the structural characteristics of the placental enzyme, but Bardsley et al. [14] have calculated a molecular weight of 180 000 for placental histaminase, and postulated that the protein is a dimer of 90 000 dalton subunits. This data agrees well with our finding of a 90 000 dalton subunit for the purified enzyme from pregnancy plasma.

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Since the completion of this manuscript, the modification of the assay procedure employing [β-^{3}H]histamine as substrate (listed as a personal communication in the first footnote) has been accepted for publication. The reference is Beaven, M.A. and Shaff, R.E. (1975) Biochem. Pharmacol., in the press.

References

1 Zeller, E.A. (1965) Fed. Proc. 24, 766—768
2 Swanberg, H. (1950) Acta Physiol. Scand. 23, Suppl. 79, 1—69
3 Ahlmark, A. (1944) Acta Physiol. Scand. 9, Suppl. 28, 1—107
4 Tryding, N. and Willert, B. (1968) Scand. J. Clin. Lab. Invest. 22, 29—32
5 Hansson, R., Tryding, N. and Tornquist, A. (1969) Acta Obstet. Gynecol. Scand. 48, 8—18
6 Tryding, N. (1965) Scand. J. Clin. Lab. Invest. 17, Suppl. 86, 196
7 Baylin, S.B., Beaven, M.A., Krauss, R.M. and Keiser, H.R. (1973) J. Clin. Invest. 52, 1985—1993
8 Baylin, S.B., Beaven, M.A., Engelman, K. and Sjoerdsma, A. (1970) New Engl. J. Med. 283, 1239—1244
9 Baylin, S.B., Beaven, M.A., Buja, L.M. and Keiser, H.R. (1972) Am. J. Med. 53, 723—733
10 Kapeller-Adler, R. and MacFarlane, H. (1963) Biochim. Biophys. Acta 67, 542—565
11 Mondovi, B., Rotilio, G., Finazzi-Agro, A. and Scioscia-Santoro, A. (1964) Biochem. J. 91, 408—415
12 Mondovi, B., Rotilio, G., Costa, M.T., Finazzi-Agro, A., Chiancone, E., Hansen, R.E. and Beinert, H. (1967) J. Biol. Chem. 242, 1160—1167
13 Smith, J.K. (1967) Biochem. J. 103, 110—119
14 Bardsley, W.G., Crabbe, M.J.C. and Scott, I.V. (1974) Biochem. J. 139, 169—181
15 Beaven, M.A. and Jacobsen, S. (1971) J. Pharmacol. Exp. Ther. 176, 52—64
16 Okuyama, T. and Kobayashi, Y. (1961) Arch. Biochem. Biophys. 95, 242—250
17 Shore, P.A. and Cohn, V.H., Jr. (1960) Biochem. Pharmacol. 5, 91—95
18 Robinson, D.S., Lovenberg, W., Keiser, H. and Sjoerdsma, A. (1968) Biochem. Pharmacol. 17, 109—119
19 Cuatrecasas, P. (1970) J. Biol. Chem. 245, 3059—3065
20 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404—427
21 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
22 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
23 Egelrud, T. and Olivecrona, T. (1972) J. Biol. Chem. 247, 6212—6217
24 Flodin, P. and Killander, J. (1962) Biochim. Biophys. Acta 63, 403—410
25 Bardsley, W.G., Crabbe, M.J.C. and Shindler, J.S. (1973) Biochem. J. 131, 459—469
26 Cuatrecasas, P. and Anfinsen, C.B. (1971) Annu. Rev. Biochem. 40, 259—278
27 Robinson, D.S. (1970) Comprehensive Biochemistry (Florkin, M. and Stotz, E.H., eds), Vol. 18, pp. 51—116, Elsevier, New York
28 Korn, E.D. and Quigley, T.W. (1957) J. Biol. Chem. 226, 833—839