The Histaminase Activity of Ceruloplasmin (36913)

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Whether or not diamine oxidase (DAO) and histaminase are identical enzymes has been vigorously debated (1–6) and we have previously contributed to the controversy with the finding that DAO and histaminase values of plasmas from many primates do not correlate (7). In further studies (8) we showed that acrylamide gel electrophoresis of plasmas from man and all of many primates examined separated an oxidase fraction which readily utilized histamine as a substrate, but not putrescine (as is characteristic of DAO), further supporting the view that two genetically different enzymes may exist.

Since this electrophoretic fraction with histaminase activity was found at a position in the gel comparable to ceruloplasmin (Cp), the copper-carrying plasma protein known to have oxidase activity, it raised the question of whether or not this fraction was in fact ceruloplasmin (8). The present study indicates that Cp and this histaminase fraction are the same but it does not resolve the issue first raised, *i.e.*, whether or not DAO and histaminase are identical.

Materials and Methods. Enzymic oxidative deamination of either putrescine or histamine results in the production of H_2O_2 (1, 4, 5). Thus, the method of Gordon and Peters (9), based on secondary oxidation of *o*-dianisidine, was modified to locate such activity zones in acrylamide gels. Rhesus monkey (*Macaca mulatta*) plasma (heparinized) was used due to its high DAO and histaminase activities. Also, heparinized plasmas from pregnant (3rd trimester) and nonpregnant humans were examined. All plasma had been stored frozen before use. Electrophoresis was carried out as described by Ornstein (10) and Davis (11) for the separation of sera. Following electrophoresis, the gels were incubated overnight in a solution consisting of 0.03 *M* substrate, 0.1% peroxidase (Calbiochem, horseradish, B grade), 0.01% *o*-dianisidine and 0.0126 *M* Tris buffer (pH 7.0).

The method of Owen and Smith (12), for the detection of ceruloplasmin by *o*-dianisidine oxidation at a relatively low pH, was modified to give a final buffer concentration of 0.06 *M* pH 5.7. Commercial anti-human Cp (Hyland, Div. Travenol Laboratories, Inc., Los Angeles, CA) was used after determining that it cross-reacted with rhesus plasma by immunoelectrophoresis on cellulose acetate (13), giving a single arc of precipitation. Ceruloplasmin was prepared according to Deutsch (14) except that the material was fractionated on a DEAE column twice and subsequently fractionated on a Sephadex G-200 column.

Results. Figure 1 illustrates that, under the conditions used to stain for oxidative deaminating activity, absence of an added substrate (gel 1) precluded oxidation of *o*-dianisidine by rhesus monkey plasma. However, when histamine was added (gel 2) to the incubation mixture, two distinct bands appeared. The band which migrated fastest was the predominant zone, the slower one being rather faint. When putrescine was the available substrate (gel 3) the faster band was absent as it was in the control gel (gel 1) in which no substrate was present. The slower band, however, did appear to be able to utilize putrescine as evidenced by its faint persistence. Only the faster band is present in plasmas from pregnant humans or human males and no putrescine oxidation is evident (8).

The faster, most conspicuous histaminase band in rhesus plasma (gel 2, Fig. 1) had a position similar to that observed for Cp (gel 6, Fig. 1). The similarity of the histaminase band and specifically stained Cp band is fur-

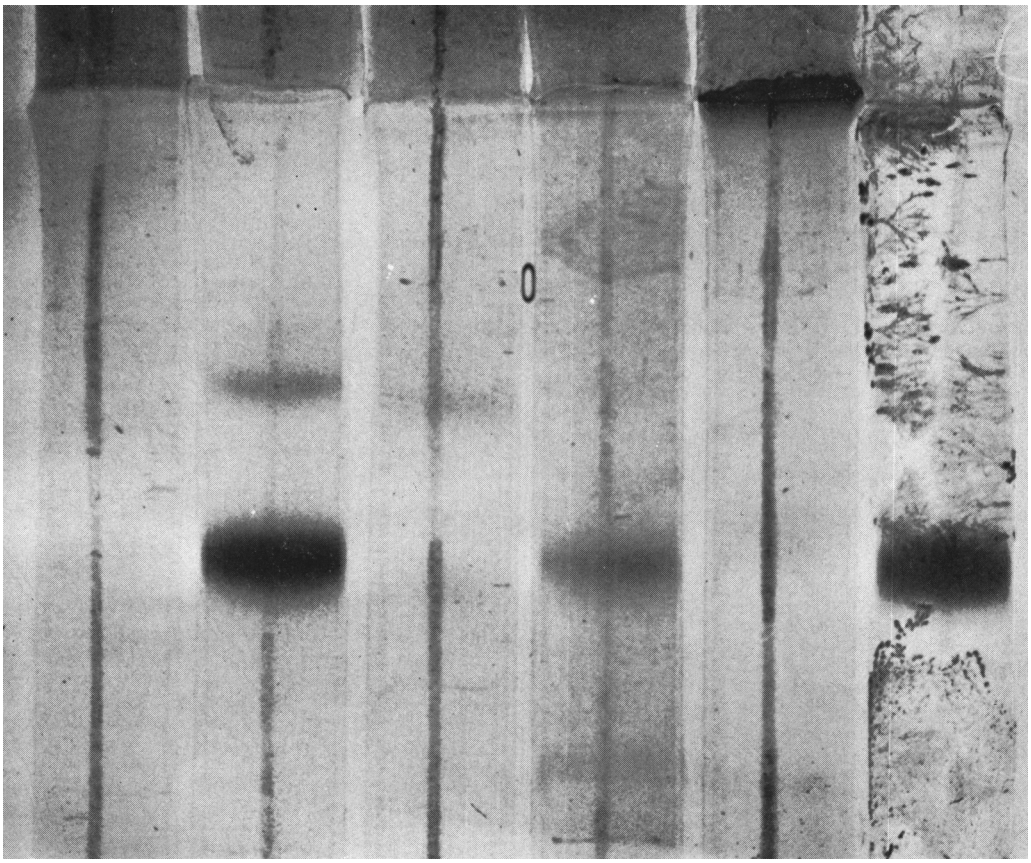

FIG. 1. Disc electrophoresis of rhesus monkey plasma (cathode at top), stained for histaminase (gels 1–5, pH 7.0) or Cp (gel 6, pH 5.7). Amount of plasma and type of substrate as follows (left to right): (gel 1) 20 μl without added substrate; (gel 2) 20 μl with histamine; (gel 3) 20 μl with putrescine; (gel 4) 5 μl with histamine; (gel 5) 5 μl + 15 μl anti-human Cp with histamine; (gel 6) 20 μl, specific stain for Cp.

ther seen in Fig. 2 (gels 1 and 2) in which a human plasma, having a normal copper value (126.9 μg %), is examined by applying both the histaminase and specific Cp staining procedures.

Although the two observed types of oxidative behavior could be compatible with one enzyme, such as Cp, and each activity was found at the same location on acrylamide gels, the fact that Cp was responsible for the histaminase activity was confirmed by the following experiments, illustrated in Fig. 1 (gels 4 and 5) for rhesus plasma and Fig. 2 for human plasma. When anti-human ceruloplasmin was mixed with plasmas before electrophoresis (gel 5, Fig. 1; gels 3 and 4, Fig. 2), not only was the histamine dependent activity absent at the usual position in the running gel, it was detected at the spacer-running gel junction, showing that it could not enter the running gel. This doubtlessly represents antibody bound enzyme in large molecular aggregates. A trace of Cp activity persisted after antibody treatment in the human pregnancy plasma (gel 4, Fig. 2). Note that plasma samples in gels 4 and 5 (Fig. 1) were 5 μl, instead of 20 μl, in order to accommodate 15 μl of antiserum without exceeding an appropriate sample volume. In Fig. 2, only 2 μl samples were used throughout.

Finally, a sample of partially purified Cp was electrophoresed (Fig. 3) and stained for histaminase (gel 1) and Cp (gel 2). This showed that substantial purification of Cp did not remove the histaminase activity which we have attributed to it.

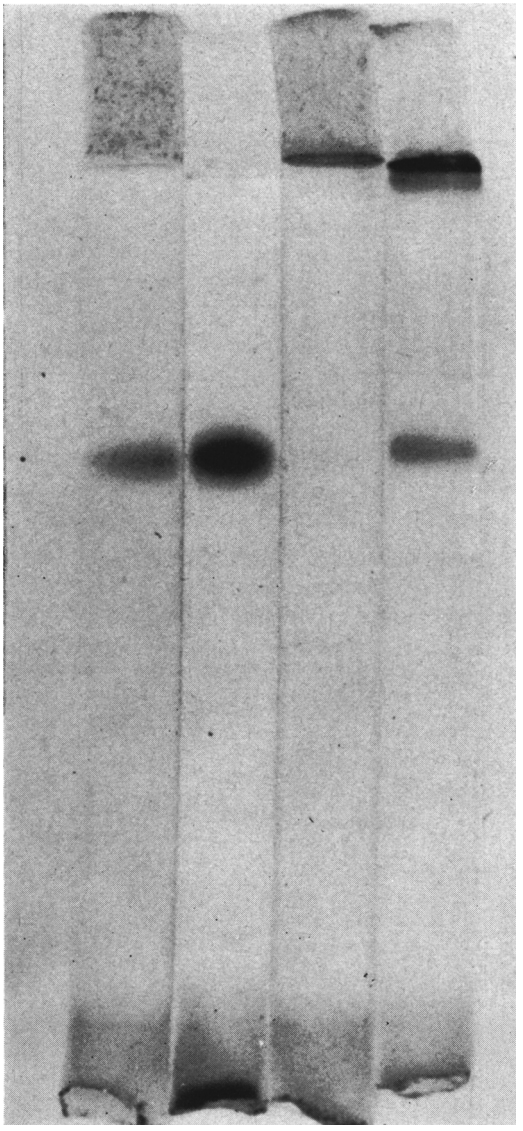

FIG. 2. Disc electrophoresis (cathode at top) of human plasma with copper (126.9 μg %) in the normal range, stained for histaminase (gels 1 and 3) and Cp (gels 2 and 4). Two microliter samples were used and 2 μl of commercial anti-Cp were mixed with plasma samples on gels 3 and 4. Gels numbered from left to right.

Discussion. Ceruloplasmin was initially described as an enzyme with ozidase activity (15). However, it has generally been shown to function as such *in vitro* only at a low pH (such as 5–6) and against such substrates as *p*-phenylenediamine (15, 16), ascorbic acid (15), benzidine (17) and, to some extent, serotonin (18). Cp oxidation of *p*-phenylenediamine at pH 5.5 causes no release of NH_3 (16) or H_2O_2 production (15), and a similarly direct oxidation of *o*-dianisidine at this pH readily occurred (gel 6, Fig. 1; gel 2, Fig. 2; gel 1, Fig. 3). The fact that NH_3 release and H_2O_2 production are characteristic of amine:oxygen oxidoreductase deaminating) enzymes such as histaminase (1, 4, 5), suggests that at a low pH, Cp oxidized the *o*-dianisidine by one mechanism but nearer to physiological pH, another, histamine dependent mechanism functioned. Both of the enzymic activities complex with anti-Cp serum. The finding that Cp, with its 7 or 8 copper atoms (19), could function in two different ways oxidatively was not surprising since Malkin and Malmstrom (20) have pointed out that its copper is in several forms ("blue", "nonblue" and "EPR-nondetectable") and that the forms of copper confer several catalytic functions on various copper containing oxidases.

A primary role as a plasma copper transport protein has not been validated for Cp (21). Recently it has been suggested that Cp has a physiological role as a ferroxidase which catalyzes the oxidation of ferrous iron in a process by which the iron–transferrin complex is formed (22). Objection to this theory comes from the report (23) that no relationship exists between serum ferroxidase activity and changes in iron absorption known to occur after bleeding, iron deficiency or iron loading. Furthermore, there is relatively normal iron transport capacity in persons with Wilson's disease, a condition characterized by low plasma Cp (24). However, a nonceruloplasmin ferroxidase (ferroxidase II) has been described in human plasma and identified in patients with Wilson's disease (24). This study, by showing that Cp can oxidatively deaminate histamine at a pH of 7 or above, provides a new avenue for pursuit of its physiological role. It is especially important to determine whether this histaminase activity is the only histaminase activity of plasma. Certainly it is the only one seen following acrylamide gel electrophoresis of rhesus monkey and human plasmas.

Summary. The plasmas of man and rhesus monkey contain a protein which oxidatively deaminates histamine, but not putrescine, at near physiological pH. Purified ceruloplasmin

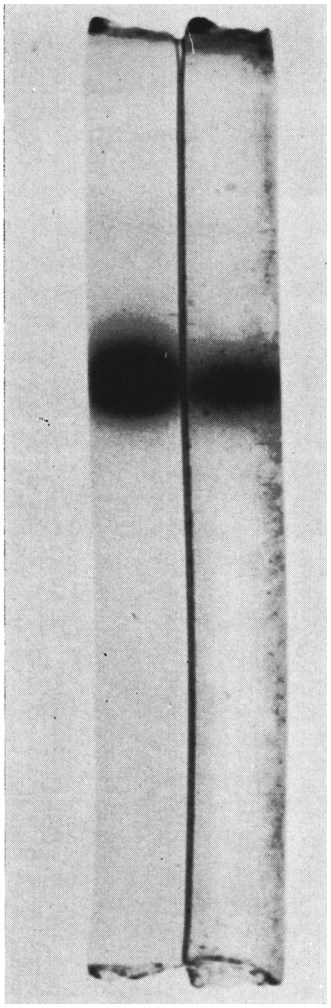

FIG. 3. Disc electrophoresis (cathode at top) of purified Cp stained for histaminase (gel 1) and Cp (gel 2). Twenty microliters of a 1:10 dilution of Cp prepared from pooled sera was used.

also gives this histaminase reaction. It migrates as a distinct zone in acrylamide disc electrophoresis except when complexed with anti-ceruloplasmin, indicating its identity with that copper-bearing protein. It is not yet established whether or not the histaminase activity of ceruloplasmin represents all or only part of the histaminase activity of plasma.

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