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Studies of Pig Kidney Diamine Oxidase

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Preparations of pig kidney histaminase were analyzed for total compositions, *N*-terminal residues, patterns of tryptic peptides and for subunits under dissociating conditions. The crystalline enzyme is pure by several criteria but not completely homogeneous. It is rich in acidic or amidated residues and has a low content of cysteine/halfcystine. Preparation analyzed consistently contained a few percent of carbohydrate. Subunits are concluded to be identical or highly similar with a molecular weight of about 90 000, which is consistent with other analyses.

Pig kidney histaminase or diamine oxidase (diamine: oxygen oxidoreductase, EC 1.4.3.6) has been crystallized [1] and is known to contain pyridoxal phosphate and copper.[1–5] From the metal content, a minimum molecular weight of about 87 000 was determined [1–3] but the whole enzyme is larger,[3] probably twice this size.[1] Various amine oxidases differ in molecular weights, in subunit arrangements and in cofactor contents (flavin, copper, pyridoxal phosphate) but few other structural characteristics are known. Similarities in monomer sizes (80 000 – 100 000) between different amine oxidases have been suggested [3] but the subunits of some other flavin-linked oxidases are smaller.[6]

In the present work, crystalline enzyme preparations were analyzed for total compositions, *N*-terminal residues and subunit sizes. The result give further characteristics of the enzyme, and conclusions from these chemical analyses are compatible with those obtained from physicochemical and metal analyses.

MATERIALS AND METHODS

Enzyme protein. The enzyme was purified from fresh pig kidney and crystallized as previously described.[1] The crystalline preparation migrated as a single band on agarose gel electrophoresis, pH 8.6, but in some preparations a minor, more acidic component was also present. Histaminase activity was determined by incubation of the sample with [ring-2-^{14}C]labelled histamine for 15 min at 37 °C in 0.04 M sodium phosphate buffer, pH 7.5, and subsequent separation of substrate and reaction products on phosphocellulose columns,[7] from which eluted fractions were measured in a Packard Tri-Carb liquid scintillation spectrometer. Total protein was determined by weights of samples dried at 105 °C. The crystalline enzyme preparations converted 0.33 μmol of histamine per min and mg protein.

Analyses. Samples for amino acid analysis were hydrolyzed in evacuated tubes with 6 M HCl containing 1 % phenol, at 110 °C for 20, 48 and 72 h, and analyzed on a Beckman Unichrom amino acid analyzer. Cysteic acid was determined after oxidation with performic acid [8] and tryptophan after hydrolysis with 4 M methanesulfonic acid containing 0.2 % 3-(2-aminoethyl)indole.[9,10] Neutral monosaccharides were determined with the orcinol-H_2SO_4 method [11] and identified by thin-layer chromatography on silica gel (20 × 20 cm, Merck AG) in 1-propanol:water:2-butanone (2:1:1, v./v.) after hydrolysis in 0.5 M H_2SO_4 at 100 °C for 8 h. Glucosamine was determined with the amino acid analyzer and sialic acid with the thiobarbituric acid method.[12]

Reduction of histaminase was performed in 8 M urea, 0.1 M Tris, 2 mM EDTA, pH 8.1 (10 mg protein/ml) with dithiothreitol (0.08 μg/mg protein) for 2 h at 37 °C, and subsequent carboxymethylation with iodoacetate (1.5 μmol/mg protein) under identical conditions. Reagents were removed by dialysis against distilled water. *N*-Terminal residues were determined by the dansyl method after coupling in 8 M urea.[13] Electrophoresis in 10 % polyacrylamide slab gels containing 0.1 % sodium dodecylsulfate,[14] and chromatography on Sepharose 4B in buffered 5 M guanidine.HCl[15] were performed as previously described. Tryptic peptides were mapped by different steps of

Table 1. Composition of a crystalline histaminase preparation analyzed by different methods as given in the text.

Component	%[a]	Component	%[a]
Aspartic acid/Asparagine	8.4	Phenylalanine	5.1
Threonine	5.5	Lysine	4.2
Serine	5.8	Histidine	2.9
Glutamic acid/Glutamine	12.2	Arginine	5.1
Proline	6.7	Cysteine/Half-cystine	1.5
Glycine	7.8	Tryptophan	1.2
Alanine	7.2	Galactose	0.4
Valine	7.0	Mannose	1.0
Methionine	1.8	Fucose	0.2
Isoleucine	4.0	*N*-Acetylglucosamine	1.4
Leucine	9.5	*N*-Acetylneuraminic acid	0.1
Tyrosine	4.1		

[a] Content per 100 amino acid residues.

electrophoresis and chromatography on paper [16] or by two-dimensional thin-layer separation on cellulose.[14]

RESULTS AND DISCUSSION

Purity and dissociation experiments. The enzyme is crystalline and pink in colour. The best preparations moved as a single band on agarose gel electrophoresis at pH 8.6, revealed only one *N*-terminus (below) and are previously known to be homogeneous in the ultracentrifuge.[1] These results suggest that the histaminase preparations are reasonably pure. The subunit composition was analyzed on the carboxymethylated protein under dissociating conditions by sodium dodecylsulfate polyacrylamide gel electrophoresis and by Sepharose chromatography in guanidine.HCl. In both cases, one major component was obtained, with a molecular weight against marker proteins (β-galactosidase, adenovirus hexon, bovine serum albumin, and horse liver alcohol dehydrogenase) of about 85 000-90 000. The protein was not completely homogeneous and there was some variation between different preparations. Apart from trace amounts of different components, a minor component with an apparent molecular weight of 40 000 was present. The origin of this fragment is unclear. It may be unrelated to the enzyme or, due to the variability, proteolytically obtained.

Analytical results. The total compositions of the enzyme preparation calculated from different hydrolysates is shown in Table 1. Values for serine and threonine were extrapolated to zero time (addition of 4 and 3 %, respectively, to the 20 h values), those for valine and isoleucine are the maximum yields, whereas those for remaining residues are average values. Different preparations showed highly similar compositions, including carbohydrate and the less abundant residues. The results show that the histaminase preparation is rich in acidic or amidated residues and contains small amounts of carbohydrate.

End-group analysis of the carboxymethylated protein in 8 M urea with the dansyl method [13] revealed the presence of *N*-terminal threonine apart from trace amounts in variable yield of a few other residues (Gly, Ser, Leu). If the possibility of blocked polypeptide chains is disregarded, this result is compatible with an essentially pure protein preparation containing *N*-terminal threonine.

Mapping of tryptic peptides was performed on cellulose thin-layer and on paper. All spots, especially the neutral ones, were not resolved on thin-layer but on paper at least 55 spots (26 basic, 9 acidic, 20 neutral) were revealed. With the ^{14}C-carboxymethylated protein, 11 of these were radioactively labelled. Due to partial cleavages, overlapping of peptide positions, and presence of weakly staining peptides,

correlations between peptide mapping and total compositions are extremely difficult with large proteins [15,17] and all fragments are usually not detected. Nevertheless, the number of total and labelled tryptic peptides detected, compared with the content of lysine, arginine and halfcystine/cysteine in the enzyme (Table 1) is compatible with the presence of one unique monomer with a size similar to that of the 90 000 dalton component shown in the dissociation experiments.

Structural characteristics. The present results show that crystalline histaminase is pure by several criteria but not completely homogeneous. The enzyme is rich in dicarboxylic residues and has a low content of cysteine/half cystine. Carbohydrate was consistently found in the preparations, and if histaminase-bound would correspond to a glycoprotein with a few percent carbohydrate. A protein subunit with a molecular weight of 90 000 is demonstrated by electrophoresis and chromatography under dissociating conditions. This is compatible with the results of peptide mapping and of analysis for total composition, which combined further suggest that all subunits are identical or highly similar. These data support conclusions from previous determinations of only copper and pyridoxal phosphate in the protein [1-5] and are consistent with analyses of another diamine oxidase.[18]

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