PURIFICATION OF PIG KIDNEY DIAMINE OXIDASE BY GEL-EXCLUSION CHROMATOGRAPHY

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Received 15 July 1976

1. Introduction

Diamine oxidase or histaminase was identified about fifty years ago by Best [1]. Although various papers have been published concerning this enzyme and related proteins from different sources, no clear statement until now has been made about the molecular properties of these enzymes. This is partly due to the difficult purification procedures which allowed only minute amounts of homogeneous material to be obtained at one time.

Affinity chromatography has introduced a powerful tool to solving similar problems. Thus Toraya et al. [2] proposed the use of AH–Sepharose (an aminohexyl derivative of a polysaccharide) as affinity resin. Our trial with pig kidney diamine oxidase gave completely different results. A fairly peculiar purification procedure was set up which appears to overcome most of the previous limiting steps in the purification of diamine oxidase.

2. Materials and methods

All chemicals were of the best commercially available purity and were used without further purification. AH–Sepharose 4B was from Pharmacia, Uppsala, Sweden. Diamine oxidase activity was tested either by convential Warburg apparatus or by Gilson oxygraph equipped with a rotating platinum electrode.

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The incubation mixture contained the enzyme (50–150 μg) dissolved in 0.1 M phosphate buffer, pH 7.4, in the presence of 30 μg catalase and 0.11 M ethanol. The reaction was started by addition of 17 mM cadaverine. The center well of Warburg apparatus contained 0.1 ml of 10% NaOH.

Spectrophotometric measurements were done with a Beckman DB recording spectrophotometer.

SDS–gel electrophoresis was conducted according to Weber and Osborn [3].

Diamine oxidase was purified from pig kidney cortex up to the step 4 according to Mondovì et al. [4] with minor modifications.

Beef plasma amine oxidase was purified up to the step 2 according to Yasonobu and Smith [5].

AH–Sepharose chromatography was performed as follows. 1 g of AH–Sepharose was suspended in 0.5 M NaCl and stirred for 2 h, washed with water and equilibrated with 0.01 M phosphate buffer pH 7.4 in a glass column (1 × 10 cm). About 200 mg of crude diamine oxidase resulting from step 4 [4] were poured onto the column. The column was washed with the same buffer. The bulk of the protein was eluted with 0.05 M phosphate buffer, pH 7.4, containing 1 M NaCl.

3. Results and discussion

Toraya et al. [2] reported that AH–Sepharose strongly binds *Aspergillus niger* amine oxidase, which can be eluted only by buthylamine containing buffer. This behavior is consistent with affinity chromatography. Buthylamine is a substrate of this enzyme and is chemically related to the resin's side chain,

presumably involved in the binding of the protein. Being a diamine oxidase, also able to oxidize monoamines though at a lower rate than diamines [6], it seemed worthwhile to try the same purification procedure. The elution profile of an AH–Sepharose column is reported in fig.1. It is clearly seen that diamine oxidase activity is completely excluded from the column, while other proteins are more or less retained. The protein coming just after diamine oxidase has been identified as hemoglobin. The yield of this chromatography may be as high as 90%, with a purification factor of about 32-fold. In order to exclude trivial effects, the chromatography was performed at higher pH. Here the dissociation of the free NH_2 group is lower, thus reducing the ability of the resin to act as an anion exchanger. No difference in the overall behavior was observed, though a lower purification was achieved. In view of the different

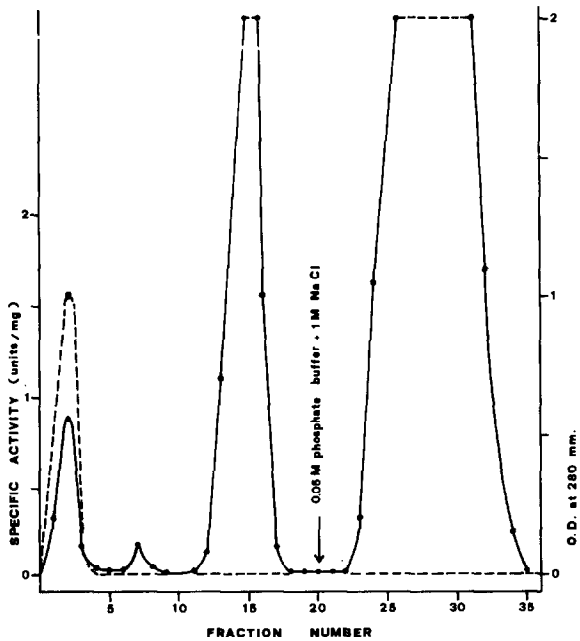

Fig.1. Elution pattern of pig kidney diamine oxidase. A 1 × 10 cm column of AH–Sepharose equilibrated with 0.01 M phosphate buffer, pH 7.4, was poured with about 200 mg of crude diamine oxidase after step 4 (see text) and eluted with the same buffer. Fraction volume was 3 ml. The buffer was changed where indicated. The specific activity is expressed as micromoles of substrate transformed per minute and per mg of protein. Continuous line: optical density. Dashed line: specific activity.

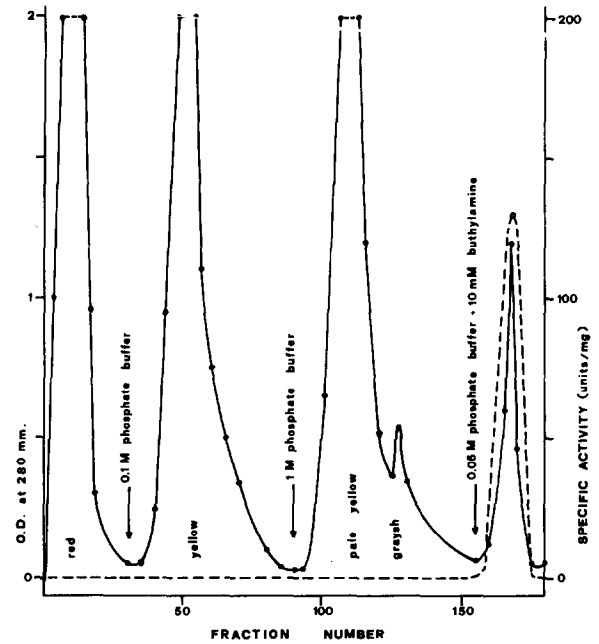

Fig.2. Elution pattern of beef plasma amine oxidase. An AH–Sepharose column as above was poured with about 1600 mg of crude beef plasma amine oxidase and eluted with the indicated buffers. A number of peaks of different color, as indicated, were eluted before amine oxidase. The enzymic activity was determined according to [5]. For other details see fig.1.

result obtained with respect to those reported previously [2], control experiments were performed using the bovine plasma as a source of amine oxidase. The relative elution pattern from AH–Sepharose is presented in fig.2. In this case most protein is eluted before amine oxidase, which does not come out from the column unless buthylamine is added to the elution buffer. It is interesting to note that ceruloplasmin is also bound to the column as a clearly visible blue band, which disappears on washing the column with concentrated buffer. The purification factor of plasma amine oxidase is about 50.

The protein obtained from the column is only partially purified, at variance with diamine oxidase, which gives only one band in SDS–gel electrophoresis (fig.3).

The specific activity and optical spectra of the purified enzyme are quite similar to those reported for homogeneous diamine oxidase preparations

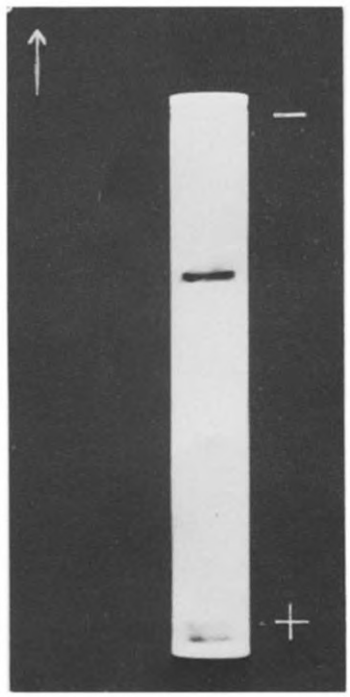

Fig. 3. SDS–gel electrophoresis of purified diamine oxidase.

obtained by much more tedious and time consuming methods.

A further observation concerns the different interaction between AH–Sepharose and the two related amine oxidases from beef plasma and kidney cortex.

It seems that one should take into account the role of the matrix besides that of the side chains considering beef plasma enzyme is rather strongly retained, as ceruloplasmin, while diamine oxidase is completely excluded. It is very difficult at the moment to decide which interaction is predominant in the two cases. It could well be that diamine oxidase is not adsorbed to the polysaccharide matrix as are contaminant proteins, because it interacts with the protruding side chains.

References

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Table 1
Purification of pig kidney diamine oxidase

Step	Total protein (mg)	Specific activity (units/mg)	Purification (-fold)	Total activity (units)	Yield (%)
1. Crude homogenate	19 160	0.0024	1	46.0	100
2. Controlled heat denaturation	3600	0.008	3	29.0	63
3. Ammonium sulfate fractionation	2600	0.01	4	26.0	56.5
4. Precipitation at pH 5.3	143	0.05	21	7.1	15.4
5. Column chromatography on AH–Sepharose	4	1.6	670	6.4	13.9

The starting material was 300 g of kidney cortex