PREPARATION AND PROPERTIES OF ANTIBODY AGAINST PIG KIDNEY DIAMINE OXIDASE

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1. Introduction

Amine oxidases form a class of enzymes devoted to the oxidation of amines by molecular oxygen with the production of hydrogen peroxide, ammonia and an aldehyde [1]. They constitute a very important class of enzymes since they can remove such active compounds as biogenic amines, diamines and polyamines. However the current literature on this subject is often misleading due to a not yet established terminology. Hence some authors indicate as 'monoamineoxidases' the serum amine oxidases which share most properties with tissue diamine oxidase (histaminase) but are completely different from the mitochondrial FAD-containing monoamine oxidase.

In an attempt to bring new data on this aspect we produced an antibody against pig kidney diamine oxidase. We now report that this antibody does not show cross-reactivity with related enzymes except with bovine kidney diamine oxidase.

2. Materials and methods

All the chemicals used were commercially available. Diamine oxidase was purified from pig kidney by the method of Bardsley et al. [2] with some modifications (Guerrieri et al., to be published). The enzyme sample used for rising antibody had a specific activity of 0.96 units/mg and showed in polyacrylamide gel electrophoresis a faint secondary band ($\simeq$ 10% of the total protein).

Pig and human plasma amine oxidase and human pregnancy plasma amine oxidase were grossly purified by fractional precipitation of plasma by ammonium sulfate (30–60% saturation). Beef plasma amine oxidase was obtained as homogeneous protein by the method of Turini et al. [3]. Beef kidney diamine oxidase was purified up to the step 4 of the method described for the pig enzyme [2].

Antibody against pig kidney diamine oxidase was raised in rabbits. It was purified from plasma by ammonium sulfate precipitation followed by extensive dialysis against NaCl-borate, pH 8.4.

The antibody was conjugated with fluorescein by the method of Clark and Shepard [4]. Radial immunodiffusion was performed according to Outcherlony [5]. Immunoelectrophoresis was done at pH 8.5 according to an established procedure [6]. The plates were selectively stained by incubation in a solution containing 7 mM cadaverine, 0.25 mg/ml *o*-dianisidine and 20 μg/ml horse radish peroxidase in 0.1 M phosphate buffer, pH 7.5. In this way the H_2O_2 produced by diamine oxidase using cadaverine as substrate was responsible for the development of color from the chromogen. Diamine oxidase activity was determined either spectrophotometrically at 25°C [7] or polarographically at 38°C.

Fluorescence polarization was measured using a FICA 55 L spectrofluorimeter equipped with

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Glan-Thomson polarizers. The polarization was $F_{\parallel} - F_{\perp} / F_{\parallel} + F_{\perp}$.

3. Results

The antibody raised in rabbits against pig kidney diamine oxidase was tested by immunodiffusion and immunoelectrophoresis. Unfortunately the small contamination in the enzyme used for sensibilization of the animals exhibited high antigenic power. Thus as shown in figs.1A and 2A, the antibody has two

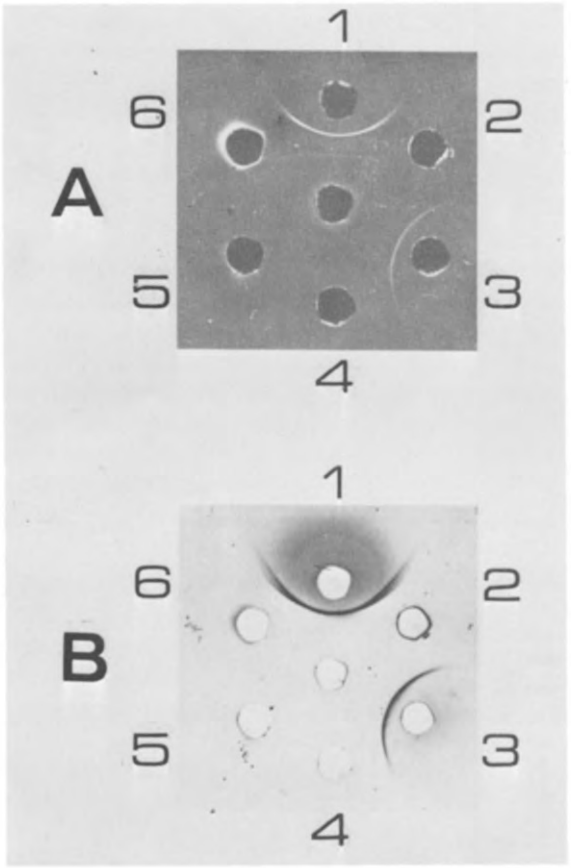

Fig.1. Radial immunodiffusion in agar of antibody against pig kidney diamine oxidase (central well) with various amine oxidases: (1) purified diamine oxidase from pig kidney; (2) purified amine oxidase from beef plasma; (3) diamine oxidase from beef kidney; (4) amine oxidase from normal human plasma; (5) amine oxidase from pig plasma; (6) amine oxidase from human pregnancy plasma. After the dark background photograph (A) was taken, the plate was stained as described in the text, and the brown spots appeared in the white background photograph (B).

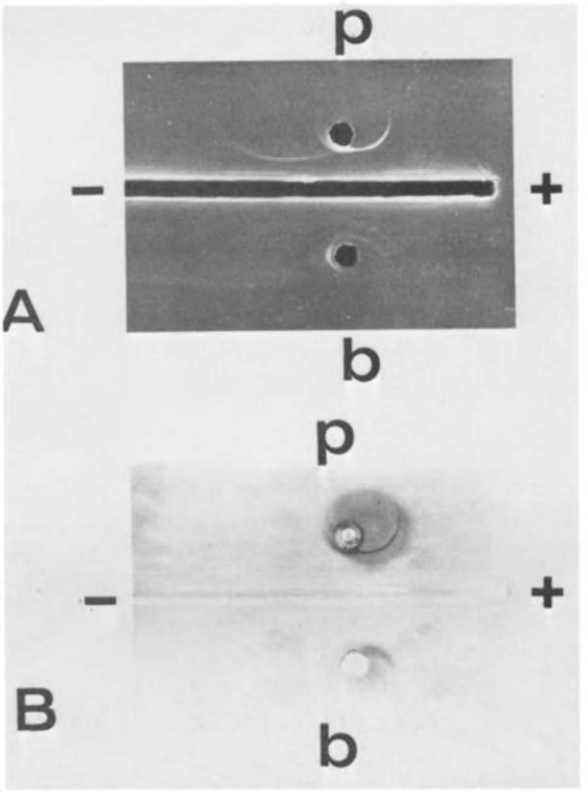

Fig.2. Immunoelectrophoresis of pig (P) and beef (B) kidney diamineoxidase, in the presence of antibody against the pig kidney enzyme. A: dark background before staining; B: white background after staining.

precipitation lines against the antigen. The slower diffusing antigen, dissociating in the anionic form at the pH of the immunoelectrophoresis, has been shown to be diamine oxidase (see below). The antigen–antibody reaction was also tested in solution by determining the increase in fluorescence polarization of the antibody-bound fluorescein. Here the polarization of the chromophore increased from 0 to 0.07 without any change in shape on mixing the labeled antibody with the antigen.

The antibody binding does not affect the catalytic power of the enzyme which is fully active in the presence of excess antibody, provided that a saturating amount of substrate is present. The Lineweaver-Burk plot reported in fig.3 shows that while the V_{max} of the enzyme acting upon cadaverine is not affected by the presence of antibody, the

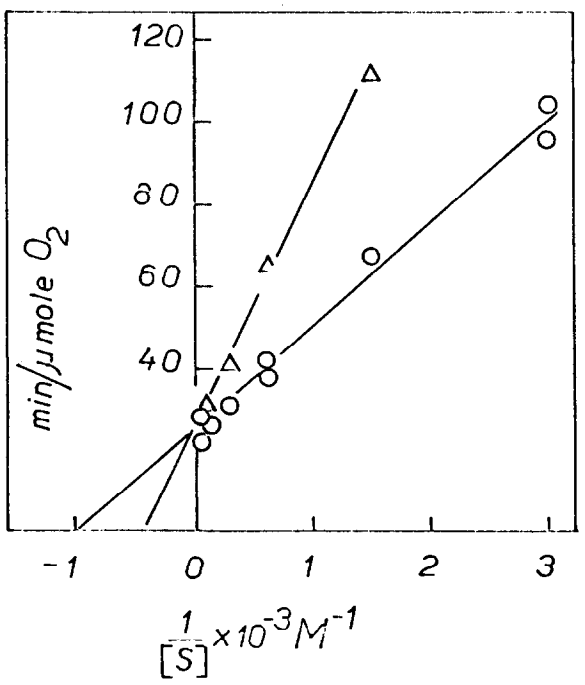

Fig.3. Lineweaver-Burk plot of cadaverine oxidation in the presence (△) and absence (○) of excess antibody. 0.03 units (0.2 mg) of an enzyme preparation (step 4) with a specific activity of 0.15 units/mg were incubated at 38°C in a 1.5 ml polarographic cell containing 0.1 M potassium phosphate buffer at pH 7. The enzyme–antibody complex was obtained by incubating at room temperature for 30 min 0.3 enzyme units with 43.2 mg of purified antibody protein before starting the kinetic experiment.

apparent K_m is 2.3-fold higher. This observation allowed a selective staining of the immunodiffusion and immunoelectrophoresis plates which only evidentiates the diamine oxidase-containing precipitation zones (figs.1B and 2B). From these data it appears that only bovine kidney diamine oxidase cross-reacts with this antibody, while closely related enzymes from plasmas do not react. Worthnoting is the absence of cross reactivity of the autologous plasma. The beef kidney extract which reacts showed both components isolated from pig kidney.

4. Discussion

The present investigation reports on the preparation of an antibody against pig kidney diamine oxidase. This enzyme whose importance is as yet more guessed than proved is related to many similar enzymes present in different tissues (liver, connective, plasma and possible brain). All of them contain copper and are inhibited by carbonyl reagents. However very little is known about their respective protein moieties.

A feasible way of exploring common features in proteins is to study the cross-reactivity by immunological methods. Along this path we found that in our conditions no other amine oxidase besides bovine kidney diamine oxidase and perhaps swine lysyl oxidase (M. P. Cerù et al., to be published) share immunological determinants with the pig kidney enzyme. In particular no immunological similarity was observed between plasma and kidney enzymes. This result appears to be at variance with previous findings obtained using as antigen the purified pig [8] or human [9] plasma amine oxidases. This discrepancy might be due to the presence of plasma in tissues, which could give false positivities. The lack of cross-reactivity and of inhibition of enzymic activity seem to indicate that the active site of the enzyme is not an antigenic determinant.

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References

[1] Buffoni, F. (1966) Pharmacol. Rev. 18, 1163–1199.
[2] Bardsley, W. G., Ashford, J. S. and Hill, G. M. (1971) Biochem. J. 122, 557–567.
[3] Turini, P., Costa, M. T., Sabatini, S. and Mondovì, B. (1978) Ital. J. Biochem. 27, 365–366.
[4] Clark, H. F. and Shepard, C. C. (1963) Virology 20, 642–644.
[5] Outcherlony, Ö. (1956) Acta Pathol. Microbiol. Scand. 26, 507.
[6] Campbell, D. H., Garvey, J. S., Cremer, N. E. and Sussdorf, D. H. (1970) in: Methods in Immunology, W. A. Benjamin Inc. Reading, pp. 260–267.
[7] Bardsley, W. G., Crabbe, M. J. C., Shindler, J. S. and Ashford, J. S. (1972) Biochem. J. 127, 875–879.
[8] Buffoni, F., Della Corte, L. and Hope, D. B. (1977) Proc. Roy. Soc. B London 195, 417–423.
[9] Baylin, S. B. (1977) Proc. Natl. Acad. Sci. USA 74, 883–887.