Vol. 98, No. 4, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 27, 1981 Pages 1002-1007

REACTION BETWEEN MAMMALIAN AMINE OXIDASES AND THEIR ANTIBODIES.

Arduino ORATORE°, Grazia BANCHELLI °°, Franca BUFFONI°°, Stefania SABATINI°°°, Bruno MONDOVI' °°° and Alessandro FINAZZI-AGRO' ‡ +

° L.I.U.M.C., L'Aquila; °° Istituto di Farmacologia, Università di Firenze; °°° Istituto di Biochimica Applicata, Università di Roma; ‡ Istituto di Chimica Biologica, Università di Roma, Italia.

Received January 9, 1981

SUMMARY. Antibodies have been raised against purified beef plasma, pig plasma and pig kidney amine oxidases. Despite the overall similarity, no immunological cross-reactivity was observed among these enzymes, even using a very sensitive light-scattering technique. The presence of substrate affects the rate of the reaction between kidney diamine oxidase and its antibody, but not that of other amine oxidases.

INTRODUCTION

Amine oxidases (diamine oxidase type) are copper enzymes which oxidize primary amino groups to the corresponding aldehydes with the production of H_2O_2 (1). They are present in different animal tissues (placenta, intestine, plasma, lung, etc.) and receive their trivial names (e.g. benzylamine oxidase, histaminase, lysyl oxidase) from an "in vitro" specificity often unrelated to their physiological role. This role is mostly inferred taking into account the biological occurrence and significance of the known substrates and the thorough distribution of amine oxidases in living organisms. In fact, besides mammals, similar enzymes have been found in, and isolated from, plants fungi and bacteria.

In previous papers we have reported the production in rabbit and some properties of antibodies against pig kidney diamine oxidase (2) and against pig plasma benzylamine oxidase (3). Pig

+ Author to whom address all the correspondence at: Istituto di Chimica Biologica, Università di Roma, P.le A. Moro, 5, Roma, Italy.

0006-291X/81/041002-06$01.00/0

kidney diamine oxidase-elicited antibodies did not recognize plasma amine oxidases on double immunodiffusion plates (2). The pig kidney enzyme was strongly bound and competitively inhibited by these antibodies.

In the present paper we report the immunological reactivity between different pairs of amine oxidases and antibodies as followed by laser nephelometry. By this method, which has a much greater sensitivity with respect to immunodiffusion, we were able to rule out any immunological cross-reactivity of the mammalian amine oxidases studied.

MATERIALS AND METHODS

All the chemicals used were reagent grade and used without further purification.

The amine oxidase studied were the following. Pig kidney diamine oxidase was purified according to Bardsley et al. (4) with minor modifications. Pig and beef plasma amine oxidases were purified according to Buffoni et al. (5) and to Turini et al. (6) respectively. Antibodies against these three enzymes were raised in New Zealand rabbits by injection of 10 mg of enzyme with complete Freund's adjuvant. One month later boosters of 0.5 mg of enzyme were weekly injected and the animals bled. Immunoglobulins were then purified from sera by precipitating thrice with 33% saturated ammonium sulfate, followed by extensive dialysis against NaCl-borate buffer pH 8.4. Antigen-antibody reaction was routinely tested by double diffusion according to Outcherlony (7). The diffusion plates were either stained for proteins with amidoschwarz or for enzymic activity incubating the plates in a buffer solution containing the appropriate substrate, o-dianisidine and horseradish peroxidase (2). Light-scattering measures were carried out with a Behring Laser Nephelometer (Behringwerke AG, Marburg/Lane, G.F.R.) equipped with a helium-neon laser operating at 632.8 nm as the light source. The diffused light was measured at an angle of 5°-12° to the incident radiation with a photodiode and expressed in volts.

RESULTS AND DISCUSSION

Specificity of reaction between amine oxidases and their antibodies.

Antibodies raised against pig plasma benzylamine oxidase gave a sharp precipitation line against the specific antigen on double immunodiffusion plates. Beef plasma and pig kidney amine oxidases gave instead two and three precipitation lines respectively when

tested with the specific antisera. A staining technique based on the detection of hydrogen peroxide produced in the course of oxidation of their substrates by amine oxidases allowed us to determine whether these precipitation lines showed enzymic activity. It was found that both lines obtained with beef plasma amine oxidase preparations showed activity. Thus two forms of beef plasma amine oxidase are present which give rise to antibodies. Disc-gel electrophoresis of the purified enzyme also showed two active bands. Only one out of the three bands seen with the pig kidney enzyme was instead producing H_2O_2 in the presence of putrescine (2). This fact indicates that the protein preparation used for eliciting antibodies was inhomogeneous. The major contaminant was found to be DOPA-decarboxylase (8).

What appears most interesting is that no precipitation whatsoever between heterologous antigen-antibody pairs could be detected with the Outcherlony's technique. Thus anti-pig kidney diamine oxidase antibodies do not precipitate with crude human, beef or pig serum (2) nor with purified pig or beef plasma amine oxidases. Similarly neither anti-plasma enzyme antibodies do react with kidney diamine oxidase nor with the heterologous crude or purified plasma amine oxidase. However the sensitivity of the double immunodiffusion technique is not enough to exclude partial antigen-antibody reactions which does not lead to a precipitation. Therefore these reactions were also studied by light-scattering, which allows detection of even the first aggregation steps. Figure 1 shows the progress curve for the reaction between pig plasma amine oxidase (curve a), pig kidney diamine oxidase (curve b) or beef plasma amine oxidase (curve c) and their specific antibodies. When heterologous antigen-antibody pairs are mixed, no turbidity was developped even after more than 100 minutes indicating the absence of immunological reaction. This finding rules out any immunological similarity in the protein moieties of these enzymes, despite large analogies in their spectroscopic and catalytic properties. Thus the chromophoric and active sites regions of these proteins do not have antigenic properties, at least in the

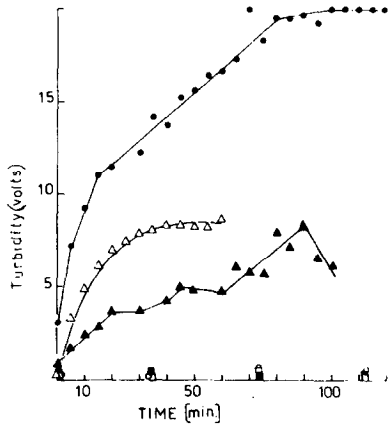

Figure 1. Nephelometric measure of reaction between amine oxidases and their antibodies.
The time course of turbidity development was studied in a final volume of 0.4 ml of 0.1 M phosphate buffer pH 7. Anti-pig-plasma amineoxidase antibody (3 mg/ml) were mixed with 0.3 mg/ml purified pig plasma amine oxidase (●——●) or with 0.2 mg/ml purified pig plasma amine oxidase (o——o). Anti-pig kidney diamine oxidase antibody (1.35 mg/ml) was mixed with 0.2 mg/ml purified pig kidney diamine oxidase (△——△) or 0.2 mg/ml beef plasma amine oxidase (□——□). Anti-beef plasma amine oxidase (0.6 mg/ml) was mixed with 0.2 mg/ml purified amine oxidase from beef (▲——▲) or 0.3 mg/ml pig plasma (■——■).

native conformation. Particularly interesting is the absolute immunological unrelatedness of pig kidney versus pig plasma enzyme and of the latter with respect to other mammalian plasma amine oxidases.

Influence of substrates on the antigen-antibody reaction.

The influence of protein conformation in the antigen-antibody reaction involving amine oxidases is furtherly indicated by the influence of substrates on this reaction. In a previous paper (2) it was shown that the antibody against pig kidney diamine oxidase was inhibitory to the enzyme activity in a pseudo-competitive way. The relatively high K_i' for inhibition by antibody left some enzymic activity in the immunoprecipitates present in double diffusion plates (2) and in tissue slices prepared for histological studies on diamine oxidase localization (9). The activities of pig and beef plasma amine oxidases were in contrast unaffected by reacting with

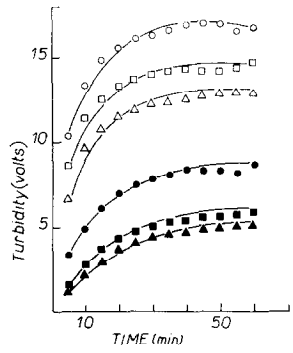

Figure 2. Effect of the presence of substrate or inhibitor on the diamine oxidase- anti diamine oxidase reaction.
a and a': 2.5 mg/ml or 1.25 mg/ml anti-pig kidney diamine oxidase antibody reacted with 0.2 mg/ml pure pig kidney diamine oxidase.
b and b': same as a and a' but in the presence of 20 mM putrescine.
c and c': same as a and a' but in the presence of 2.5 mM arcaine (1,1'-tetramethylene diguanidine). Total reaction volume: 0.4 ml. Temperature 25°C.

their antibodies. It seemed of interest to study the effect of substrate addition on the time course of the reaction between pig kidney diamine oxidase and its antibody. As shown in figure 2 the increase in turbidity of the solution due to the formation of antigen-antibody complexes is much less in the presence of putrescine. The diamine oxidase inhibitor arcaine (a diguanido analogue of putrescine) was also found to slow the antigen-antibody reaction, though to a lesser extent. Neither pig nor beef plasma amine oxidase were affected by the presence of their substrates in the reaction with their specific antibodies.

The inhibition of the diamine oxidase-antidiamine oxidase antibody complex formation by substrate or inhibitor (Fig. 2) supports the suggested competition between the antibody and the substrate (2). It should be recalled that a polymerization of diamine oxidase in the presence of substrates has been described (9, 10). This polymerization could slow down the reaction of the enzyme with its antibody.

ACKNOWLEDGEMENTS. This work was in part supported by the Italian C.N.R., special Project on the "Chimica fine" - Contract No. 80.02.107.95.

REFERENCES

1) Malmström, B.G., Andréasson, L.-E. and Reinhammar, B. (1975) in The Enzymes (P.D. Boyer, Ed.) pp. 511-527, Acad. Press, New York.
2) Oratore, A., Guerrieri, P., Ballini, A., Mondovì, B., and Finazzi-Agrò, A. (1979) FEBS Lett. 104, 154-156.
3) Buffoni, F., Della Corte, L. and Hope D.B. (1977) Proc. Roy. Soc. B. 195, 417-423.
4) Bardsley, W.G., Crabbe, M.J.C., Shindler, J.S., and Ashford J.S. (1972) Biochem. J. 127, 875-879.
5) Buffoni, F. and Blaschko, M. (1964) Proc. Roy. Soc. B. 161, 153-167.
6) Turini, P., Costa, M.T., Sabatini, S. and Mondovì, B. (1978) Ital. J. Biochem. 27, 365-366.
7) Outcherlony, Ö. (1956) Acta Pathol. Microbiol. Scand. 26, 507-515.
8) Rosei, M.A., D'Erme, M., Guerrieri, P., Aquilio, E. 26th National Congress of Italian Biochemical Society, Bologna, September 24-26, 1980, p. 170.
9) Argento-Cerù, M.P., Oratore, A., Beninati, S. and Finazzi-Agrò, A. (1980) Cell. Mol. Biol. , in press.
10) Guerrieri, P., Finazzi-Agrò, A., Costa, M.T., Rotilio, G. and Mondovì, B. (1976) Ital. J. Biochem. 25, 160-166.
11) Pionetti, J.-M. (1974) Biochem. Biophys. Res. Comm. 58, 495-498.