Acta Chemica Scandinavica B 36 (1982) 685 – 688

Antibodies to Ornithine Decarboxylase. Immunochemical Cross-reactivity

LO PERSSON

Department of Physiology and Biophysics, University of Lund, Sölvegatan 19, S-223 62 Lund, Sweden

L-Ornithine decarboxylase was purified to apparent homogeneity from the kidneys of testosterone-treated mice. Antibodies to ornithine decarboxylase were raised in a rabbit using the purified enzyme. Ouchterlony double diffusion technique revealed a single precipitin line between the antiserum and purified mouse kidney ornithine decarboxylase. The antibodies inhibited ornithine decarboxylase from various tissues of mice and rats to the same extent, indicating a close immunological relationship. *S*-Adenosyl-L-methionine decarboxylase and L-histidine decarboxylase from mouse kidney as well as ornithine decarboxylase from *Escherichia coli* were unaffected by the antibodies.

L-Ornithine decarboxylase (ODC) (EC 4.1.1.17) catalyzes the first and rate-limiting step in the biosynthesis of polyamines. This enzyme has been intensely studied since an increase in ornithine decarboxylase activity has been observed in response to growth stimuli in various tissues.[1,2]

Ornithine decarboxylase of rat liver has been purified to what was considered to be homogeneity.[3,4] However, using radioactive α-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase, the specific activity of pure rat liver ornithine decarboxylase was calculated to be about 100-fold higher than the value reported for purified rat liver ornithine decarboxylase.[5] Recently, ornithine decarboxylase was purified to apparent homogeneity from kidneys of testosterone-treated mice.[6] The purified enzyme exhibited a specific activity in agreement with the theoretical value for the pure enzyme.[7] Similar results were obtained for rat liver ornithine decarboxylase by Kameji *et al.*,[8] who succeeded in purifying the enzyme 350 000 times to the same high specific activity.

Antibodies to rat liver ornithine decarboxylase have been prepared in several laboratories.[9-12] Since the enzyme preparations used for immunization must have contained considerable amounts of protein impurities, the antisera to ornithine decarboxylase so far reported should be considered nonspecific. Using purified ornithine decarboxylase from kidneys of testosterone-treated mice, the present work describes the generation of what apparently are monospecific antibodies to ornithine decarboxylase.

METHODS

Ornithine decarboxylase was purified from kidneys of testosterone-treated NMRI mice as described previously.[6] The purification resulted in an enzyme with a specific activity of 1.4 mmol/mg h.[7] To stabilize the enzyme 0.3 % Tween 80 was added to the purified ornithine decarboxylase.[13] The enzyme preparation was concentrated, using an Amicon YM 10 membrane, and resuspended in a small volume of 0.1 M phosphate buffer (pH 7.2) containing 0.1 mM EDTA, 2.5 mM dithiothreitol and 0.3 % Tween 80. The purified enzyme (0.15 mg) was emulsified in Freund's complete adjuvant and given to a rabbit by the multiple site, intradermal technique as described by Vaitukaitis *et al.*[14] A booster injection (0.1 mg) in complete adjuvant was given after 3 months. Antiserum to ornithine decarboxylase (code No. 8111) was collected every two weeks and stored at −20 °C. The specificity of the antiserum was tested by the Ouchterlony double diffusion technique.[15]

To examine the cross-reactivity of the antiserum, ornithine decarboxylase obtained from kidney of

0302-4369/82/100685-04$02.50
© 1982 Acta Chemica Scandinavica

testosterone-treated mice (200 μg daily for 7 days), liver of carbon tetrachloride-treated mice (1.5 ml/kg; 14 h before death), kidney of carbon tetrachloride-treated rats (1.5 ml/kg; 14 h before death), liver of thioacetamide-treated rats (150 mg/kg; 18 h before death), ovary of female rats treated with human chorionic gonadotrophin (400 IU/kg; 5 h before death) and ventral prostate of male rats were used. The mice and rats were of the NMRI and Sprague-Dawley strains, respectively.

Immunoprecipitation of ornithine decarboxylase was carried out in 0.5 ml of 0.1 M Tris-HCl (pH 7.5), 0.1 mM EDTA and 2.5 mM dithiothreitol (buffer A) containing 0.25 % of bovine serum albumin at 4 °C overnight. To precipitate the antigen-antibody complexes 0.25 ml of buffer A containing 0.25 % of bovine serum albumin and 10 μl of goat anti-rabbit IgG was added and incubated overnight at 4 °C. The solution was then centrifuged at 20000 × g at 4 °C for 20 min. Remaining ornithine decarboxylase activity was measured in aliquots of the supernatant.

Ornithine decarboxylase activity was measured by determining the release of $^{14}CO_2$ from carboxyl-labelled ^{14}C-ornithine in a mixture containing 0.1 mM pyridoxal 5′-phosphate and 0.5 mM L-1-^{14}C-ornithine (0.4 or 4.0 MBq/mmol) in buffer A as described earlier.[6,7] One unit of ornithine decarboxylase was defined as the amount of enzyme giving rise to 1 nmol CO_2 per h.

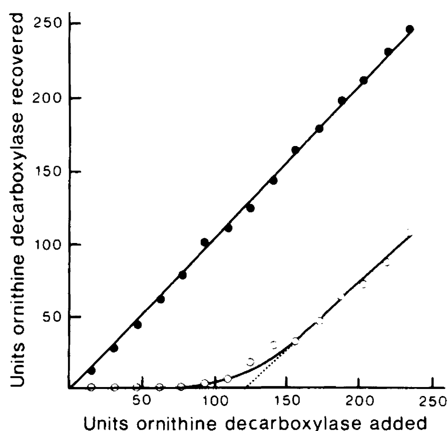

Fig. 1. Immunotitration of mouse kidney ornithine decarboxylase. Various amounts of ornithine decarboxylase from kidneys of testosterone-treated mice were incubated with 0.2 μl of control (●) or anti-ornithine decarboxylase (○) serum as described in Methods.

RESULTS

The antibodies raised against purified mouse kidney ornithine decarboxylase were shown to inhibit completely the enzyme in extracts from kidneys of testosterone-treated mice (Fig. 1). One μl of the antiserum was estimated to inhibit nearly 600 units of ornithine decarboxylase. The addition of normal rabbit serum to the enzyme extracts did not affect the enzyme activity. Utilizing the Ouchterlony double diffusion technique it was shown that the antibodies gave a sharp single precipitin line against purified mouse kidney ornithine decarboxylase (Fig. 2).

The immunochemical cross-reactivity of the antibodies with ornithine decarboxylase from various tissues of mice and rats was also studied. Ornithine decarboxylase from kidney and liver of mice and from kidney, liver, ovary and ventral prostate of rats was diluted to about the same activity and then incubated with different amounts of antiserum against the enzyme. After precipitating the antigen-antibody complexes with goat anti-rabbit IgG the solution was centrifuged and the residual ornithine decarboxylase activity determined. As seen in Fig. 3 the antibodies against purified mouse kidney ornithine decarboxylase inhibited ornithine decarboxylase from liver of mice and from kidney, liver, ovary and ventral prostate of rats.

To compare the capability of the antibodies to inhibit ornithine decarboxylase from these tissues the amounts of antiserum required to inactivate 50 % of 1 unit of enzyme (Ab^{50}) were calculated from Fig. 3. As shown in Table 1 the antiserum was found to inhibit ornithine decarboxylase from each of the tissues examined to the same extent, indicating a

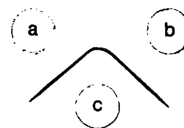

Fig. 2. Ouchterlony double diffusion precipitin analysis of ornithine decarboxylase antiserum and purified mouse kidney ornithine decarboxylase. The immunoplate (3 % agar) was incubated for 48 h in a moist chamber at room temperature and then washed with saline and stained with 0.1 % Coomassie brilliant blue. Wells a and b contained 4 μg of purified enzyme. Well c contained 5 μl of ornithine decarboxylase antiserum.

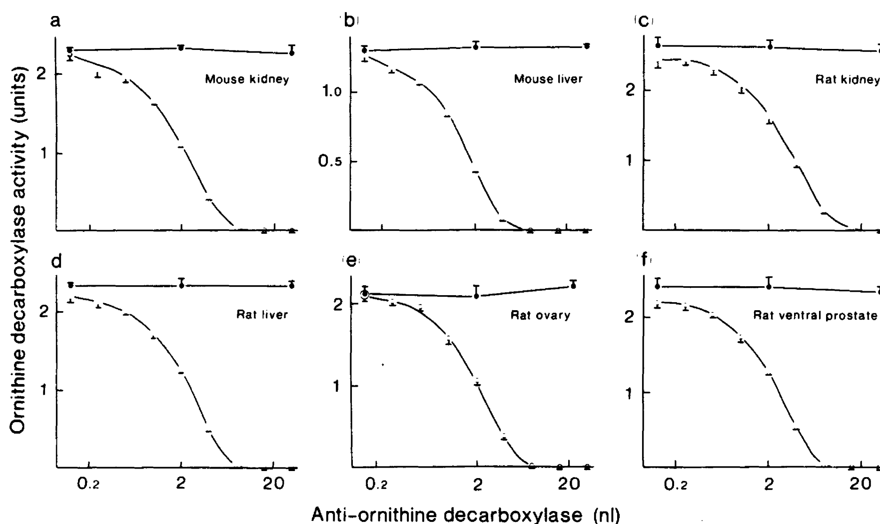

Fig. 3. Immunoprecipitation of ornithine decarboxylase from various tissues with mouse kidney ornithine decarboxylase antiserum. Increasing amounts of control (●) or anti-ornithine decarboxylase (○) serum were added to a fixed quantity of enzyme activity. After incubation, as described in Methods, remaining ornithine decarboxylase activity was determined in aliquots of the supernatant. Ornithine decarboxylase from mouse kidney (a), mouse liver (b), rat kidney (c), rat liver (d), rat ovary (e) and rat ventral prostate (f). Each point represents the mean ±S.E. of the mean, $n=5$.

close, if not identical, immunological relationship. Because of the low amounts of ornithine decarboxylase in these tissues the immunological relationship could not be determined by the Ouchterlony double diffusion technique. It should be mentioned that the antibodies did not inhibit ornithine decarboxylase from *Escherichia coli*, nor did they affect *S*-adenosylmethionine decarboxylase or histidine decarboxylase from mouse kidney (results not shown).

Table 1. Immunochemical cross-reactivity of antiserum to mouse kidney ornithine decarboxylase.

Origin of ornithine decarboxylase	Ab^{50} [a] (nl/unit)
Mouse kidney	0.85
Mouse liver	1.12
Rat kidney	1.13
Rat liver	0.96
Rat ovary	0.98
Rat ventral prostate	0.93

[a] Ab^{50} is defined as the amount of antiserum needed for 50% inactivation of 1 unit of ornithine decarboxylase. Ab^{50} is calculated from data of Fig. 3.

DISCUSSION

The antiserum against ornithine decarboxylase was shown to have a much higher titre than those reported previously.[10,12] It would appear that this is due to the fact that the preparations used for immunization until now have been impure. Using the calculated value of the specific activity of a pure ornithine decarboxylase[5] as confirmed for the purified enzyme,[7,8] it can be concluded that previous preparations used for immunization must have contained less than 2% ornithine decarboxylase. The kidneys of testosterone-treated mice seem to be most useful for purification of ornithine decarboxylase since they contain 100-fold more ornithine decarboxylase activity than reported for the rat liver.[16-18] In fact, it even seems to be difficult to obtain adequate amounts of the pure enzyme for immunization from stimulated rat livers.[8] Until monoclonal antibodies to ornithine decarboxylase are available, the generation of ornithine decarboxylase antisera could easily be achieved using purified mouse kidney enzyme.

The finding that ornithine decarboxylase from mouse kidney and rat liver were inactivated to the same degree by the antibodies indicated that the molecules of ornithine decarboxylase in these

tissues are equally effective in decarboxylating ornithine. During the progress of the present work, this suggestion was confirmed by the reports of Pritchard *et al.*[5] and Kameji *et al.*,[8] who demonstrated that the specific activity of pure rat liver ornithine decarboxylase was in accordance with the specific activity of purified ornithine decarboxylase from mouse kidney. This seems to apply also to ornithine decarboxylase from mouse liver, rat kidney, rat ovary and rat ventral prostate since the enzymes from these tissues were equally inhibited by the ornithine decarboxylase antiserum.

The available antibodies to ornithine decarboxylase have successfully been employed in an immunofluorescent technique for the histochemical localization of ornithine decarboxylase in the kidneys of testosterone-treated mice.[19] The demonstrated capability of the antibodies to cross-react with ornithine decarboxylase from other tissues indicates that these antibodies may be of general use for the immunohistochemical localization of ornithine decarboxylase.

Acknowledgements. I am most grateful to Ingvar Larsson, Department of Clinical Chemistry, University of Lund, Malmö, Sweden, for his valuable help in the generation of the antibodies, and to Dr. Ragnhild Cigén, Department of Physiological Chemistry, University of Lund, Lund, Sweden, for her generous help in the Ouchterlony double diffusion technique. The study was supported by grants from the Swedish Medical Research Council (grant No. B81-04X-02212-14A) and from the Medical Faculty, University of Lund.

REFERENCES

1. Jänne, J., Pösö, H. and Raina, A. *Biochim. Biophys. Acta 473* (1978) 241.
2. Russell, D. H. *Pharmacology 20* (1980) 117.
3. Ono, M., Inoue, H., Suzuki, F. and Takeda, Y. *Biochim. Biophys. Acta 284* (1972) 285.
4. Obenrader, M. F. and Prouty, W. F. *J. Biol. Chem. 252* (1977) 2860.
5. Pritchard, M. L., Seely, J. E., Pösö, H., Jefferson, L. S. and Pegg, A. E. *Biochem. Biophys. Res. Commun. 100* (1981) 1597.
6. Persson, L. *Acta Chem. Scand. B 35* (1981) 451.
7. Persson, L. *Acta Chem. Scand. B 35* (1981) 737.
8. Kameji, T., Murakami, Y., Fujita, K., Noguchi, T. and Hayashi, S. *Med. Biol. 59* (1981) 296.
9. Hölttä, E. *Biochim. Biophys. Acta 399* (1975) 420.
10. Theoharides, T. C. and Canellakis, Z. N. *J. Biol. Chem. 251* (1976) 4436.
11. Kallio, A., Löfman, M., Pösö, H. and Jänne, J. *FEBS Lett. 79* (1977) 195.
12. Obenrader, M. F. and Prouty, W. F. *J. Biol. Chem. 252* (1977) 2866.
13. Kitani, T. and Fujisawa, H. *Eur. J. Biochem. 119* (1981) 177.
14. Vaitukaitis, J., Robbins, J. B., Nieschlag, E. and Ross, G. T. *J. Clin. Endocrinol. 33* (1971) 988.
15. Ouchterlony, Ö. *Acta Pathol. Microbiol. Scand. 26* (1949) 507.
16. Henningsson, S., Persson, L. and Rosengren, E. *Acta Physiol. Scand. 102* (1978) 385.
17. Jänne, J. and Raina, A. *Acta Chem. Scand. 22* (1968) 1349.
18. Raina, A. and Jänne, J. *Fed. Proc. 29* (1970) 1568.
19. Persson, L., Rosengren, E. and Sundler, F. *Biochem. Biophys. Res. Commun. 104* (1982) 1196.

Received March 16, 1982.