Vol. 151, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 29, 1988 Pages 450-457

STRAIN-SPECIFIC OCCURRENCE OF TWO ORNITHINE DECARBOXYLASE SPECIES IN MOUSE KIDNEY

Takako Kitani* and Hitoshi Fujisawa

Department of Biochemistry, Asahikawa Medical College
Asahikawa 078, Japan

Received January 4, 1988

Ornithine decarboxylase in the crude extract from the kidney of androgen-treated mice was labeled by reaction with radioactive α-difluoromethylornithine and analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis followed by autoradiography. Two species of ornithine decarboxylase with molecular weights of 54,000 and 52,000 were observed in the crude extracts from BALB, C57BL, C58, SJL, and dd mice but only the species with a molecular weight of 54,000 was observed in the crude extracts from AKR and C3H mice, indicating strain-specific occurrence of at least two species of mouse ornithine decarboxylase.

ODC (EC 4.1.1.17) is the first and rate-limiting enzyme in polyamine biosynthesis (1-4) and therefore the regulatory mechanism of the enzyme activity is very important. We purified ODC to homogeneity from the liver of thioacetamide-treated rat (5) but it was difficult to obtain the amounts of the enzyme sufficient for studying the molecular properties of the enzyme in detail, because the content of the enzyme in the rat liver was very low. Since the content of the enzyme in the kidney of androgen-treated mouse was known to be very much higher than that in the rat liver (6,7), we purified the enzyme from the mouse kidney to further define the molecular properties of the enzyme (8). Our purified preparation from the mouse kidney showed two protein staining bands on SDS-polyacrylamide gel electrophoresis,

*Affiliated with Laboratory for Radio-active Isotope Research, Asahikawa Medical College.

The abbreviations used are: ODC, ornithine decarboxylase; SDS, sodium dodecylsulfate; DFMO, α-difluoromethylornithine.

0006-291X/88 $1.50

both of which were labeled by reaction with radioactive DFMO, an enzyme-activated irreversible inhibitor (9,10). In contrast, Seely *et al.* (11) reported that the enzyme purified from the mouse kidney gave a single protein band on isoelectric focusing and on SDS-polyacrylamide gel electrophoresis. Isomaa *et al.* (12) demonstrated that the purified mouse kidney ODC gave a single protein band on SDS-polyacrylamide gel electrophoresis with an apparent molecular weight of 54,000 but showed charge heterogeneity with isoelectric points in the pI range of 4.7-4.9. On the other hand, Pulkka *et al.* (13) reported that the purified mouse kidney ODC gave two bands on SDS-polyacrylamide gel electrophoresis with molecular weights of 53,000 and 51,000 and discussed the possibility that 51-kDa species was produced from 53-kDa species by proteolysis. Persson *et al.* (14) also demonstrated the possibility that mouse kidney ODC (55 kDa) was degraded to a smaller size (53 kDa).

The present communication provides evidence that size heterogeneity of mouse kidney ODC did not occur as a result of proteolysis of the enzyme but occurred depending upon the strains of the mouse.

EXPERIMENTAL PROCEDURES

DL-[1-^{14}C]Ornithine (57 Ci/mol) was purchased from Amersham International. [5-^{3}H]DFMO (11.1 Ci/mmol) was obtained from New England Nuclear. Microbial protease inhibitors (antipain, leupeptin, chymostatin, and pepstatin) were from the Peptide Institute, Osaka, Japan. Ampholine was from LKB. Rabbit muscle phosphorylase *b*, bovine serum albumin, and ovalbumin were from Sigma Chemical Co. Rabbit muscle lactate dehydrogenase was from Boehringer Mannheim. Fuji X ray film (RX) was from Fuji Photo Film Co.

Adult male ddY and dd mice (outbred strains) were obtained from Shizuoka Laboratory Animal Center, Shizuoka, Japan, and Animal Laboratory, Asahikawa Medical College, respectively. Adult male AKR/J, C3H/HeJ, BALB/c, C57BL/6, C58/J, and SJL/J mice (inbred strains) were obtained from Animal Laboratory, Asahikawa Medical College.

Rat liver ODC was purified from the liver of thioacetamide-treated rats as described previously (5). Mouse kidney ODC was purified from the kidney of androgen-treated mice as described previously (8). The activity of ODC was determined by measure-

ment of rate of $^{14}CO_2$ evolution from L-[1-^{14}C]ornithine as described previously (5). One unit of the enzyme was defined as the amount of enzyme which catalyzed the release of 1 nmol of CO_2 from L-ornithine/min at 37°C.

Analytical polyacrylamide gel electrophoresis in the presence of SDS was performed on 10% acrylamide gels essentially according to the procedure of Weber and Osborn (15) in the presence of 8 M urea. After electrophoresis, the gel was stained for protein with Coomassie brilliant blue R-250.

Polyacrylamide gel electrofocusing was performed on 5% acrylamide gels containing pH 3.5-10 Ampholine (2%) and 8 M urea essentially according to the procedure described by Wrigley (16). Electrophoresis was carried out at 0.25 watt per gel for 5 h in a cold room. Protein samples were electrophoresed from cathode to anode. After electrofocusing a gel was sliced into 2-mm sections and each piece was placed into a test tube containing 0.5 ml of water. After standing for 1 h at 4°C with shaking, the pH of water extract of each piece was measured. A parallel gel was stained for protein with Coomassie brilliant blue G-250 (17).

[5-^{3}H]DFMO-labeled samples were analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography. The binding of [5-^{3}H]DFMO to ODC was carried out as described previously (18), except that the concentration of ethyleneglycol was lowered to 5% and microbial protease inhibitors (antipain, leupeptin, pepstatin, and chymostatin) were added to a final concentration of 20 µg/ml each, and the sample was subjected to SDS-polyacrylamide gel electrophoresis according to the method of Laemmli (19) on a 10% polyacrylamide gel. After electrophoresis, the gel was stained for protein with 0.01% Coomassie brilliant blue R-250, destained, soaked for 30 min in 1 M sodium salicylate (20), dried *in vacuo* on filter paper for 2 h at 70°C and then exposed to Fuji X ray film (medical) for 5 days at -80°C.

Protein was determined spectrophotometrically by the method of Lowry *et al.* (21) as modified by Peterson (22) with bovine serum albumin as a standard.

RESULTS AND DISCUSSION

When the crude extracts from kidneys of a variety of mouse strains were allowed to react with radioactive DFMO and analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography, two radioactive bands with apparent molecular weights of 54,000 and 52,000 were observed with the crude extracts from BALB, C57BL, C58, SJL, and dd mouse strains but a single band with an apparent molecular weight of 54,000 was observed with the crude extracts from the other strains, AKR and C3H, as shown in Fig. 1. These results suggest that size heterogeneity observed

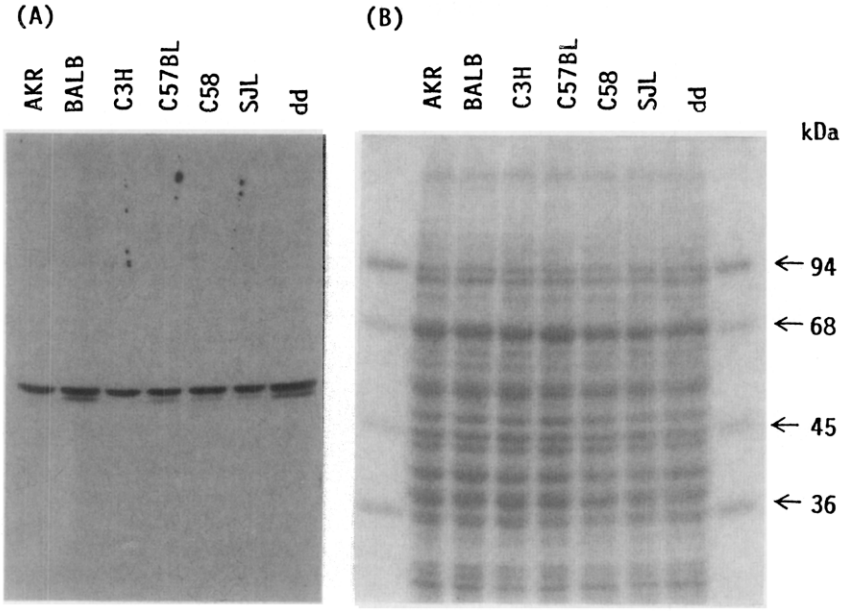

Fig. 1. **SDS-polyacrylamide gel electrophoresis of [^{3}H]DFMO-labeled ODC of the crude extract from the kidney of various strains of the mouse.** The fresh kidney from AKR, BALB, C3H, C57BL, C58, SJL, and dd mice, which were given testosterone as described previously (8), was homogenized in 3 volumes of a solution consisting of 0.25 M sucrose, 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.1 mM pyridoxal phosphate, 5 μg each/ml of microbial protease inhibitors such as antipain, leupeptin, pepstatin, and chymostatin, and 10 mM sodium phosphate buffer (pH 7.0) in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 105,000 X *g* for 1 h. The supernatant (crude extract) containing 0.6-1.0 unit of ODC was incubated with [^{3}H]DFMO as described under "Experimental Procedures" and then analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography. The radioactive bands (**A**) and protein bands (**B**) were visualized by fluorography and Coomassie brilliant blue, respectively. Molecular weight markers are phosphorylase *b* (Mr = 94,000), bovine serum albumin (Mr = 68,000), ovalbumin (Mr = 45,000) and lactate dehydrogenase (Mr = 36,000).

in mouse kidney ODC may occur depending upon the strains of the mouse, since it has been established that DFMO is an irreversible inhibitor specific for ODC acting via an enzyme-activated 'suicide' mechanism (9,10). On the other hand, there are some reports (13,14) demonstrating the possibility that the heterogeneity is due to the proteolytic degradation of ODC. To rule out this possibility, the crude extract from the kidney of androgen-treated dd mice was incubated at 37°C in the presence or absence of protease inhibitors and the electrophoretic behavior of the species reacting with DFMO was examined as shown in Fig.2. The

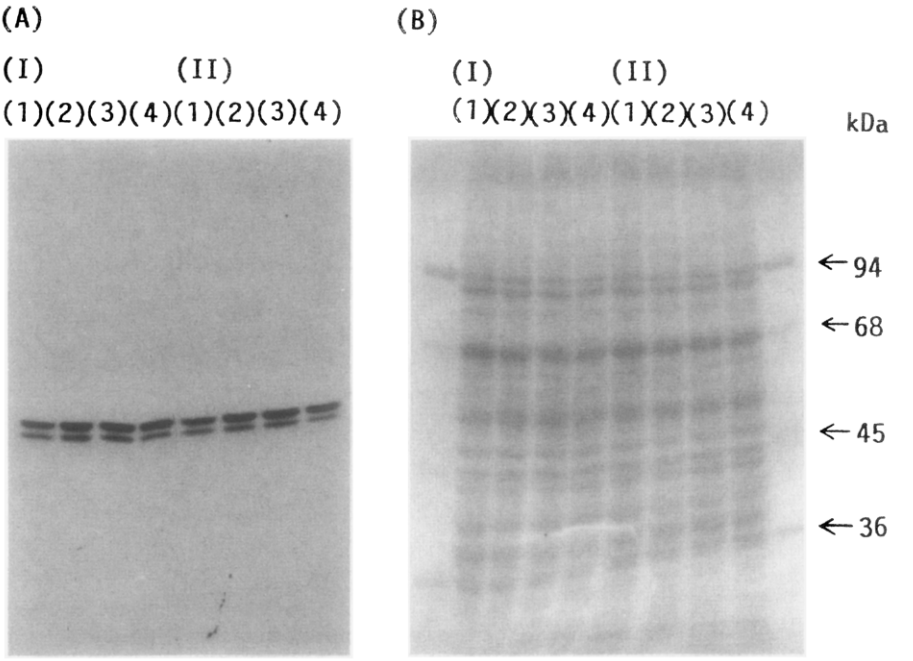

Fig. 2. **SDS-polyacrylamide gel electrophoresis of [^{3}H]DFMO-labeled ODC of the crude extract which was incubated in the presence and absence of protease inhibitors.** The fresh kidney from androgen-treated dd mice was homogenized as described in the legend for Fig. 1 in the presence (**I**) or absence (**II**) of the microbial protease inhibitors. The crude extract obtained by centrifugation for 1 h at 105,000 X *g* was incubated for 0 (**1**) 0.5 (**2**), 1 (**3**), and 3 h (**4**) at 37°C, then incubated with [^{3}H]-DFMO, and then analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography. The radioactive bands (**A**) and protein bands (**B**) were visualized by fluorography and Coomassie brilliant blue, respectively.

electrophoretic behavior of [^{3}H]DFMO-labeled species did not vary before and after incubation as well as in the presence and absence of protease inhibitors, suggesting that the 52-kDa species was not produced from the 54-kDa species by proteolysis.

When we previously purified ODC from the kidney of androgen-treated ddY mouse, a strain very similar to dd mouse, the purified ODC showed two protein staining bands molecular weights of 54,000 and 52,000 on SDS-polyacrylamide gel electrophoresis (8). In contrast, ODC purified from the kidney of androgen-treated AKR mouse, the crude extract of which showed a single radioactive DFMO-labeled band as shown in Fig. 1, according to the same procedures as described previously (8) showed a single

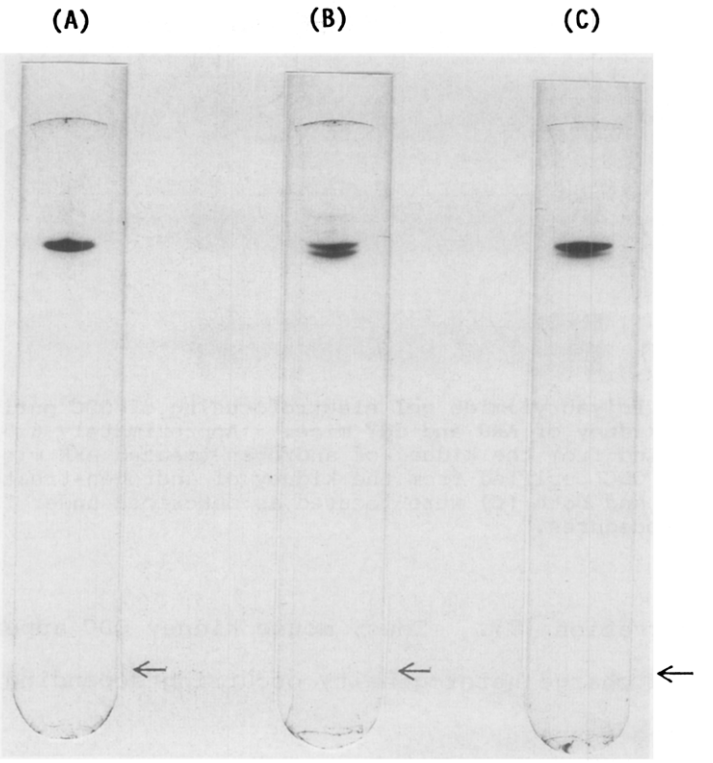

Fig. 3. **SDS-polyacrylamide gel electrophoresis of ODC purified from the kidney of AKR and ddY mice.** Approximately 0.64 µg of ODC purified from the kidney of androgen-treated AKR mice (**A**), 0.69 µg of ODC purified from the kidney of androgen-treated ddY mice (**B**), and both (**C**) were applied to each gel. The *arrows* indicate the bromphenol blue dye front.

protein staining band as shown in Fig. 3A, the mobility of which was the same as that of the DFMO-labeled band of the crude extract and also the same as that of the slower migrating one (54 kDa) of the two protein bands of the purified ODC from ddY mice as shown in Fig. 3C. Thus, the electrophoretic behavior of ODC on SDS-polyacrylamide gel electrophoresis appeared to be the same before and after purification of the enzyme.

Polyacrylamide gel electrofocusing of the purified AKR mouse ODC revealed two protein staining bands with isoelectric points of around 6.0 and 5.8 as shown in Fig. 4A. On the other hand, electrofocusing of the purified ddY mouse ODC showed not only the two same bands but also two bands with very close isoelectric proteins around 5.7 as shown in Fig. 4B, in agreement with our

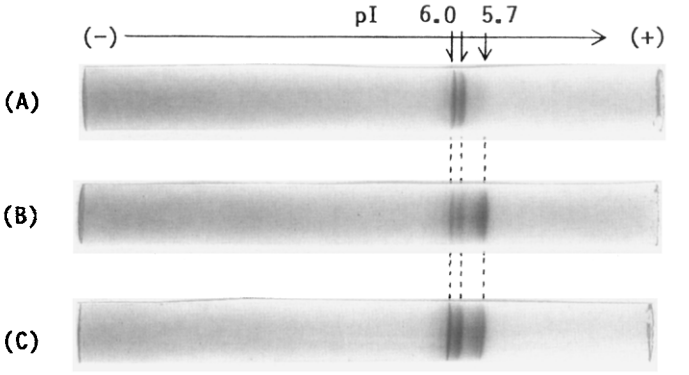

Fig. 4. **Polyacrylamide gel electrofocusing of ODC purified from the kidney of AKR and ddY mice.** Approximately 6.5 μg of ODC purified from the kidney of androgen-treated AKR mice (**A**), 7.2 μg of ODC purified from the kidney of androgen-treated ddY mice (**B**), and both (**C**) were focused as described under "Experimental Procedures."

earlier observation (8). Thus, mouse kidney ODC appeared to show size and charge heterogeneity occurring depending upon the strains of the mouse.

ACKNOWLEDGEMENT

This work has been supported in part by grants-in-aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan, the Naito Foundation, Japan Brain Foundation, and a Asahi Research Grant, the Daiwa Health Foundation, and the Byotai Taisha Research Foundation.

REFERENCES

1. Tabor, H., Rosenthal, S. M., and Tabor, C. W. (1956) Fed. Proc. **15**, 367.
2. Jänne, J. (1967) Acta Physiol. Scand. Suppl. **300**, 1-71.
3. Pegg, A. E., and Williams-Ashman, H. G. (1968) Biochem. J. **108**, 533-539.
4. Russell, D. H., and Snyder, S. H. (1968) Proc. Natl. Acad. Sci. U.S.A. **60**, 1420-1427.
5. Kitani, T., and Fujisawa, H. (1983) J. Biol. Chem. **258**, 235-239.
6. Grahn, B., Henningsson, S. S. G., Kahlson, G., and Rosengren, E. (1973) Br. J. Pharmacol. **48**, 113-120.
7. Henningsson, S., and Rosengren, E. (1975) J. Physiol. (London) **245**, 467-479.
8. Kitani, T., and Fujisawa, H. (1987) J. Biochem. (Tokyo) in press.
9. Metcalf, B. W., Bey, P., Danzin, C., Jung, M. J., Casara, P., and Vevert, J. P. (1978) J. Am. Chem. Soc. **100**, 2551-2553.
10. Bey, P. (1978) in Enzyme-activated Irreversible Inhibitors (Seiler, N., Jung, M. J., and Koch-Weser, J., eds) pp.27-41, Elsevier/North-Holland, Amsterdam.

11. Seely, J. E., Pösö, H., and Pegg, A. E. (1982) Biochemistry **21**, 3394-3399.
12. Isomaa, V. V., Pajunen, A. E. I., Bardin, C. W., and Jänne, O. A. (1983) J. Biol. Chem. **258**, 6735-6740.
13. Pulkka, A., Taskinen, T., Aaltonen, H., Ramberg, J., and Pajunen, A. E. I. (1985) Biochem. Int. **11**, 845-851.
14. Persson, L., Seely, J. E., and Pegg, A. E. (1984) Biochemistry **23**, 3777-3783.
15. Weber, K., and Osborn, M. (1969) J. Biol. Chem. **244**, 4406-4412.
16. Wrigley, C. W. (1971) Methods Enzymol. **22**, 559-564.
17. Reisner, A. H., Nemes, P., and Bucholtz, C. (1975) Anal. Biochem. **64**, 509-516.
18. Kitani, T., and Fujisawa, H. (1986) Biog. Amines **3**, 279-285.
19. Laemmli, U. K. (1970) Nature **227**, 680-685.
20. Chamberlain, J. P. (1979) Anal. Biochem. **98**, 132-135.
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. **193**, 265-275.
22. Peterson, G. L. (1977) Anal. Biochem. **83**, 346-356.