Acta Chemica Scandinavica B 35 (1981) 737–745

Short Communications

The Specific Activity of Purified Mammalian Ornithine Decarboxylase is in Accordance with the Theoretical Value of a Pure Enzyme

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Mammalian ornithine decarboxylase (EC 4.1.1.17) (ODC*) catalyzes the first and rate-limiting step in the biosynthesis of polyamines. The purification of ODC from rat liver has been reported to result in apparently homogenous preparations of the enzyme.[1,2] Recently it was shown that ODC purified from mouse kidney exhibited a specific activity 50-fold higher than that reported for purified rat liver ODC.[3] This discrepancy was believed to be due to differences in the enzyme protein. However, in a recent report[4] using radioactive DL-α-difluoro methylornithine (DFMO*), a specific irreversible inhibitor of ODC, Pritchard *et al.* estimated the specific activity of pure rat liver ODC to a value more than 80 times that reported thus far. Since the assay conditions for ODC used by Pritchard *et al.*[4] were different from those used in the report on the purification of mouse kidney ODC,[3] no direct comparison can be made. In the present study it is shown than the specific activity of ODC purified from mouse kidney is in good agreement with the theoretical value for a pure mammalian enzyme, when assayed under identical conditions.

Experimental. ODC was purified from kidneys of testosterone-treated male mice as described earlier.[3] The purification procedure consisted of acid precipitation, gel-filtration on Sephadex G150 Superfine, ion-exchange chromatography using DEAE-Sephadex A-50 and affinity chromatography on pyridoxamine 5'-phosphate linked to activated agarose gel. ODC activity was measured in two different assay mixtures by determining the release of $^{14}CO_2$ from carboxyl-labeled ^{14}C-ornithine. One

*Abbreviations: ODC, ornithine decarboxylase; DFMO, D,L-α-difluoromethylornithine.

Table 1. Purification of ornithine decarboxylase from testosterone propionate-stimulated mouse kidney.

Purification step	Assay mixture[a]	Total protein (mg)	Total activity (μmol/h)	Specific activity (μmol/mg h)	Purification (-fold)	Recovery (%)
1. 20000 × *g* supernatant	A	3830	1565	0.41	1	100
	B		2348	0.61	1	100
2. Acid treatment (pH 4.6)	A	1050	1056	1.01	2.5	67
	B		1634	1.56	2.5	70
3. Sephadex G-150 Superfine	A	116.6	765	6.56	16.0	49
	B		1097	9.41	15.3	47
4. DEAE-Sephadex	A	11.44	529	46.2	113	34
	B		799	69.8	114	34
5. Affinity chromatography	A	0.445	355	797	1949	23
	B		618	1389	2266	26

[a] As described in Experimental.

0302-4369/81/100737-02$02.50

assay mixture (A), routinely used in our laboratory,[3] contained 0.1 M sodium phosphate buffer (pH 7.2), 0.5 mM dithiothreitol, 0.1 mM EDTA, 0.01 mM pyridoxal 5'-phosphate and 0.5 mM L-[1-^{14}C]ornithine (3.7 MBq/mmol). In agreement with Pritchard *et al.*[4] the other assay mixture (B) contained 0.1 M Tris-HCl (pH 7.5), 2.5 mM dithiothreitol, 0.1 mM EDTA, 0.1 mM pyridoxal 5'-phosphate and 0.5 mM L-[1-^{14}C]ornithine (3.7 MBq/mmol). Protein was measured by the methods of Lowry *et al.*[5] and Bradford.[6]

Results and discussion. Table 1 summarizes the results of the purification of the enzyme. As shown in this Table the activity of ODC was regularly about 50% higher when measured with the assay conditions used by Pritchard *et al.*[4] As further seen in the Table the purification of mouse kidney ODC resulted in a highly purified enzyme with a specific activity of 1.4 mmol/mg h. Using ^{14}C-DFMO, Pritchard *et al.*[4] predicted the specific activity of a pure mammalian ODC to be a value between 1.0 to 1.5 mmol/mg h. Hence the specific activity of the purified enzyme is in good accordance with the theoretical value. Recently this was also shown for rat liver ODC. Kameji *et al.*[7] were able to purify ODC from livers of thioacetamide treated rats 350 000-fold to the same high specific activity. However, due to the low enzyme activity in stimulated rat livers, compared to the kidneys of testosterone-treated mice, only 27 μg of pure ODC was obtained.[7] Hence, the mouse kidney seems to be most favourable when large amounts of pure ODC are needed, *e.g.* for immunization or protein analysis.

Acknowledgements. This 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.

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Received September 9, 1981.