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INDUCTION BY CHLOROFORM OF TWO FORMS OF ORNITHINE DECARBOXYLASE IN RAT LIVER

HALF-LIFE OF ISOZYMES

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Abstract—Ornithine decarboxylase (ODC) in rat liver was separated into two species by DEAE-Sepharose CL-6B column chromatography. The activity of both species of ODC was increased at least 20-fold by chloroform treatment of the rats. The major species, Peak A, contained 65% of the ODC activity and possessed a half-life of 11 min. The second species, Peak B, accounted for 35% of the activity and possessed a half-life of 50 min. The long-lived species of ODC activity, induced in rat liver by chloroform, has not been reported previously and might be related to the prolonged induction of ODC activity by chloroform and to tumor promotion and growth.

Ornithine decarboxylase (ODC, EC 4.1.1.17) catalyzes the first step in polyamine biosynthesis, the conversion of ornithine to putrescine. This is the rate-limiting step in the biosynthesis of spermidine and spermine [1]. Polyamines have been implicated in the regulation of cell proliferation and nucleic acid and protein synthesis [1–3]. ODC is induced by many agents including hormones, drugs, mitogens, tumor promoters and carcinogens, prior to the stimulation by these agents of cell proliferation and tissue growth [2–8]. The induction of ODC by these regulatory agents is preceded immediately by an increase in polyamine biosynthesis. Increased ODC activity and polyamine accumulation have also been reported in chemically induced animal tumors and in human tumours [2, 9–11].

The level of ODC activity in rat liver has been reported to increase very rapidly following the treatment of rats by a variety of agents [2]. The rapid and large rise in ODC activity can be attributed in part to the extremely short half-life of cytoplasmic ODC, 10–15 min which is one of the shortest half-lives of any enzyme [2, 3, 12]. The very low endogenous level of ODC activity in normal rat liver also indicates that the level of ODC in a cell might be an important site for regulation of polyamine levels. Since the amounts of ODC activity in a cell can be modulated very rapidly, it has been proposed that the level of ODC in a cell might serve an important function in the regulation of cell proliferation and macromolecular biosynthesis [2, 3].

We have reported previously that chloroform is one of the most potent inducers of ODC activity in rat liver, resulting in up to a 25-fold increase in activity [13]. Multiple species of ODC have been reported in the livers of thioacetamide-treated or partial hepatectomized rats [14, 15], dexamethasone-induced thymus and kidney [16], and 3T3 cells [17]; however, it has yet to be determined if these different species possess the same rapid half-life of 10–15 min. In this communication, we report that chloroform induces at least two species of ODC and that one of the species possesses a long half-life of approximately 50 min.

MATERIALS AND METHODS

Materials. Glass distilled chloroform without preservative was purchased from Burdick & Jackson Laboratories (Muskegon, MI), Tricaprylin from ICN Nutritional Biochemicals (Cleveland, OH), DEAE-Sepharose CL-6B from Pharmacia (Uppsala, Sweden), DL-[1-^{14}C]ornithine hydrochloride (51.3 mCi/mmole) from the New England Nuclear Corp. (Boston, MA), and cycloheximide, dithiothreitol and pyridoxal-5'-phosphate from the Sigma Chemical Co. (St. Louis, MO). Alpha-difluoromethylornithine (DFMO) was provided by Dr. P. P. McCann of the Merrell-Dow Research Center, Cincinnati, OH.

Animals. Male Fisher 344 albino rats were purchased from the Charles River Co. (Portage, MI) and weighed 200–250 g when used in these studies. They were fed Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO) and given distilled water *ad lib.* The animals were maintained in accordance with the guidance set forth in "The Maintenance and Care of Laboratory Animals" by the National Research Council, U.S.A.

Half-life of chloroform-induced ODC. The rats received intraperitoneally 6.0 mmoles/kg chloroform (0.5 ml/kg) and were killed 18 hr later, between 9:00 and 10:00 a.m., by decapitation. Cycloheximide

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(25 mg/kg) was administered intraperitoneally between 0 and 2 hr prior to sacrifice.

Separation of two species of ODC activity. Multiple species of ODC were separated by a modification of the procedure of Richards *et al.* [16]. Briefly, with all subsequent procedures performed at 0–4°, the livers were homogenized at a concentration of 1 g/ml in a buffer containing 50 mM Tris, 5 mM dithiothreitol and 0.1 mM EDTA (final pH 7.3). The homogenate was sequentially centrifuged at 10,000 *g* for 10 min and at 144,000 *g* for 60 min. A 10-ml aliquot of the supernatant fraction was applied to a DEAE-Sepharose CL-6B solumn (1.6 × 40 cm) previously equilibrated with Buffer A containing 50 mM Tris, 5 mM dithiothreitol, 0.1 mM EDTA, 0.125 M NaCl and 50 μM pyridoxal phosphate (final pH 8.0). The column was eluted with 150 ml of Buffer A followed by a 600-ml linear gradient of 0.125 to 0.3 M NaCl in Buffer A at a flow rate of 0.5 ml/min. Prior to assay of the unfractionated cytoplasm, the buffer of the cytoplasm was changed to Buffer A by chromatography on Pharmacia PD-2 disposable columns equilbrated with Buffer A.

ODC assay. The cytoplasmic sample or fractions collected from the eluate of the DEAE-Sepharose CL-6B column (500 μl) were added to Kontes incubation flasks containing 500 μl of the incubation medium at a final concentration of 50 mM sodium phosphate (pH 7.5), 3.5 mM dithiothreitol, 2.0 mM EDTA, 8 mM DL-ornithine monohydrochloride, and 1.0 μCi of DL-[1-^{14}C]ornithine (51.3 mCi/mmole). After incubation for 30 min at 37°, the reaction was terminated by addition of 0.3 ml of 5 M H_2SO_4 and allowed to stand for an additional 45 min while the liberated $^{14}CO_2$ was absorbed by filter paper saturated with hyamine hydroxide. The filter paper, with the $^{14}CO_2$ trapped onto it, was transferred to a liquid scintillation counting vial containing 15.0 ml of ACS and 0.41 ml of 0.5 HCl. The radioactivity in the samples was measured in a Beckman model 8000 liquid scintillation counter (Beckman Instruments, Fullerton, CA). ODC activity was expressed as dpm $^{14}CO_2$ liberated/30 min of incubation at 37°.

RESULTS

Separation of two species of ODC activity. The elution profiles from DEAE-Sepharose CL-6B column chromatography of a cytoplasmic fraction from the liver of control and chloroform-treated rats are shown in Fig. 1. Two peaks of activity were separated by the chromatographic procedure. Peak A accounted for more than 65% of the recovered activity, with the rest found in Peak B. The elution profile of ODC activity from liver of control rats also exhibited two peaks of activity that appeared to correspond to the two peaks found in chloroform-treated animals. The activity eluted from control rats contained less than 5% of the activity found in either peak A or B of chloroform-treated rats. Therefore, chloroform treatment resulted in a large (at least 20-fold) induction of ODC activity in both Peaks A and B.

Measurement of the half-lives of ODC activity. The half-life of ODC activity in a cytoplasmic fraction obtained from chloroform-treated rats was measured using cycloheximide to inhibit the synthesis of ODC. The half-life curve was biphasic which indicated there were two species of cytoplasmic ODC with half-lives of approximately 12 and 90 min (Fig. 2). The rapidly turning over species of ODC activity accounted for approximately 75% of the cytoplasmic ODC activity.

Figure 3 depicts the measurement of the half-life of two species (Peaks A and B) of cytoplasmic ODC activity separated by DEAE-Sepharose CL-6B column chromatography. Peak A, the major species of ODC activity, contained 65% of the recovered

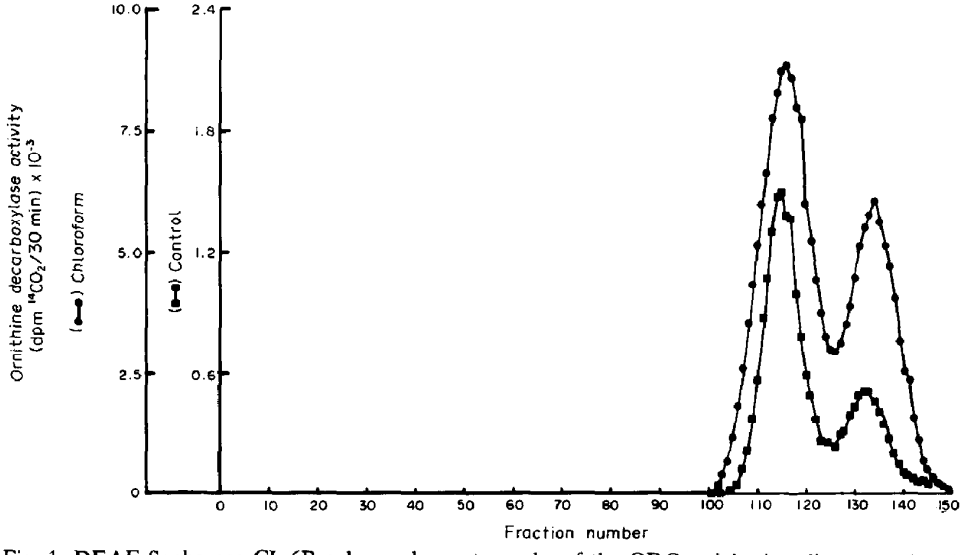

Fig. 1. DEAE-Sepharose CL-6B column chromatography of the ODC activity in a liver cytoplasmic fraction. The livers of three rats were pooled and homogenized. A 10-ml aliquot of the cytoplasmic fraction was applied to a DEAE-Sepharose CL-6B column (1.6 × 40 cm) and chromatographed as described in Materials and Methods. Key: (■) control rats; and (●) rats treated with 6.0 mmoles/kg chloroform and killed 18 hr later.

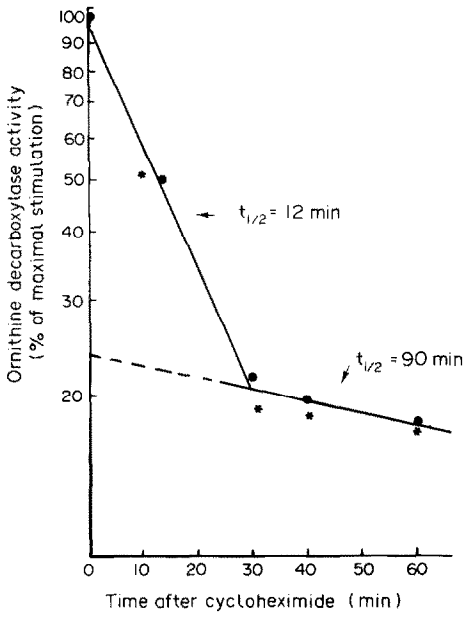

Fig. 2. Half-life of ODC activity in the hepatic cytoplasm from chloroform-treated rats. Rats received intraperitoneally 6.0 mmoles/kg chloroform 18 hr prior to termination and 25 mg/kg cycloheximide at the indicated times prior to termination. ODC activity was determined in a 144,000 *g* cytoplasmic fraction. Each point represents the results from the livers pooled from three rats. The closed circles (●) and asterisks (*) are results from two separate experiments performed on different days using saturating and unsaturating amounts, respectively, of ornithine in the ODC assay. The lines depicted are the least-squares estimates of the linear relationship between log activity and time.

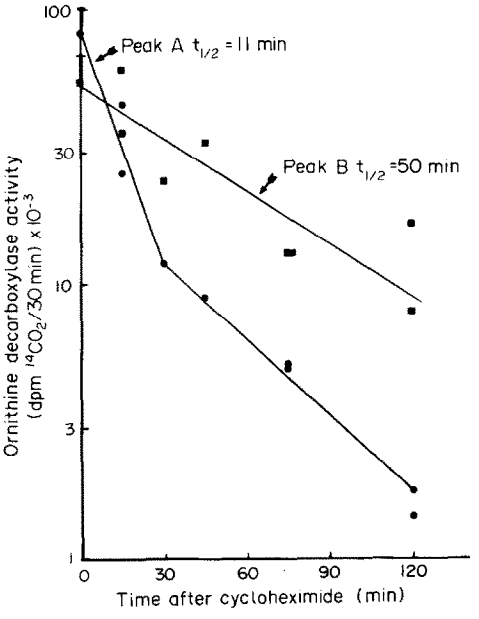

Fig. 3. Half-lives of ODC activity in Peaks A and B from chloroform-treated rats. The rats received intraperitoneally 6.0 mmoles/kg chloroform and were killed 18 hr later. Cycloheximide (25 mg/kg) was administered at the indicated time prior to termination. The rats killed at 75 and 120 min after the cycloheximide received a second dose of cycloheximide at 30 and 60 min prior to termination respectively. The livers from three rats were pooled and homogenized, and the ODC activity of the 144,000 *g* cytoplasmic fraction was separated by DEAE-Sepharose CL-6B column chromatography. The results at zero time after cycloheximide represent the mean ± standard error from four different groups of rats. ODC activities in Peak A are depicted by closed circles (●) and in Peak B by closed squares (■). The lines depicted are the least-squares estimates of the linear relationship between log activity and time.

activity and exhibited a biphasic turnover curve. This suggests the presence in Peak A of two forms of ODC activity. Eighty percent of the activity in Peak A decreased with a half-life of 11 min. The remainder of the activity, which probably represents contamination by peak B, possessed a half-life of 50 min. Peak B possessed a half-life of 50 min.

Inhibition by difluoromethylornithine. The inhibition of ODC activity in Peaks A and B by DFMO, an irreversible inhibitor of ODC, was investigated in an attempt to differentiate between the two forms of ODC. The inhibition of ODC activity in Peaks A and B by increasing concentrations of DFMO is shown in Table 1. DFMO inhibited both Peaks A and B to the same extent, with 50% inhibition ocurring at approximately 8×10^{-6} M DFMO. A similar K_i equal to 4×10^{-5} M was determined for DFMO by Metcalf *et al.* [18].

Table 1. Inhibition by DFMO of hepatic ODC activity in Peaks A and B*

DFMO concn (M)	Peak A		Peak B	
	Activity (dpm/60 min)	Inhibition (%)	Activity (dpm/60 min)	Inhibition (%)
0	4030 ± 413*		2130 ± 59	
3×10^{-6}	3110 ± 47	23	1660 ± 59	22
10^{-5}	1600 ± 45	60	887 ± 20	58
3×10^{-5}	662 ± 19	84	340 ± 29	84
10^{-4}	193 ± 25	95	92 ± 14	96
3×10^{-4}	5.6 ± 5.1	100	33 ± 12	98
10^{3}	1.7 ± 1.7	100	13 ± 8.2	100

* Results are means of four incubations ±S.E. The cytoplasm from three rats treated with 6.0 mmoles/kg chloroform 18 hr prior to termination was pooled and used to isolate Peaks A and B.

DISCUSSION

The presence of at least two species of ODC in the cytoplasm of the liver from chloroform-treated rats was indicated by the response in the cytoplasmic fraction of two half-lives of ODC activity as measured following protein synthesis inhibition by cycloheximide. Two species of ODC activity were subsequently separated from control and from chloroform-treated rats by DEAE-Sepharose CL-6B column chromatography. In chloroform-treated rats, both species of ODC activity were increased at least 20-fold compared to control rats. This 20-fold induction by chloroform in Peaks A and B is consistent with our previously reported 25-fold induction by chloroform of total cytoplasmic ODC activity [13]. Using a similar DEAE-Sepharose CL-6B column chromatography system, multiple species of ODC have been reported in the livers of thioacetamide-treated or partial hepatectomized male rats [14, 15], dexamethasone-induced thymus and kidney [16], and 3T3 cells [17]. Multiple forms of an enzyme can result from differences in amino acid composition, in post-translational modification of the amino acids, or in quaternary structure of monomers to form isozymes. ODC undergoes post-translational modifications by phosphorylation [19] and transamination [20]. Therefore, multiple forms of ODC could differ by post-translational modification though differences are also possible in the primary and quaternary structures.

Two major species of ODC activity present in the livers of chloroform-treated rats (Peaks A and B) possessed different half-lives. The species of ODC in Peak A possessed two half-lives of 10 and 50 min each. The short-lived form in Peak A accounted for approximately 80% of the activity in Peak A and 65% of the total cytoplasmic activity. This half-life is consistent with previously reported half-lives of cytoplasmic ODC in rat liver [2, 3, 12]. The long-lived species of ODC in Peaks A and B possessed half-lives of approximately 50 min and probably represent the same form of the enzyme. This long-lived form of the enzyme has not been reported previously. The half-lives of Peaks A and B roughly correspond to the two half-lives of 12 and 90 min found for unfractionated cytoplasm, indicating that Peaks A and B are not artefacts of the chromatography. The induction by chloroform of rat liver ODC resulted in a prolonged elevation of ODC activity, starting at 2 hr after treatment and lasting for at least 24 hr [13]. The relationship between the long-lived species of ODC in chloroform-treated rats and this prolonged elevation of ODC activity is only speculative at present.

Prolonged elevation of ODC activity and polyamine accumulation are associated with a high rate of tumor growth [3, 7, 21, 22] and are important components of tumor promotion [5, 6]. Chloroform has been shown to be a carcinogen [23] and has been proposed to exert carcinogenic activity by an epigenetic mechanism of tumor promotion [24, 25]. The increase in the cellular concentration of ODC caused by treatment with chloroform resulted from an increased concentration of both the short- and long-lived forms of ODC. The biological significance of the different species of ODC, especially the long-lived species, to tumor promotion and growth requires further investigation.

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