Purification and Properties of Argininosuccinate Synthetase from Normal and Canavanine-Resistant Human Lymphoblasts†

Margaret E. Kimball and Lee B. Jacoby*

ABSTRACT: The specific activity of argininosuccinate synthetase in the canavanine-resistant human lymphoblast line MGL8D1 is 200-fold higher than in the parental lymphoblast line MGL8B2 grown in arginine-supplemented medium. Argininosuccinate synthetase was purifed from normal (MGL8B2) and overproducer (MGL8D1) lymphoblast lines to compare the physical, catalytic, and immunologic properties of enzymes from the two cell lines. The purification procedure included $(NH_4)_2SO_4$ fractionation, ion-exchange chromatography on DEAE-cellulose and CM-cellulose, and repeated gel filtration on Sephadex G-200. The overall yield of enzyme from both cell lines was 10–20%. The final products from both lines comigrated on sodium dodecyl sulfate gel electrophoresis at a position corresponding to a subunit molecular weight of 45 000. Catalytically active argininosuccinate synthetase from both MGL8B2 and MGL8D1 had a molecular weight of 180 000, indicating a tetrameric structure. The kinetic parameters of the two enzymes were essentially identical. Argininosuccinate synthetases from both MGL8B2 and MGL8D1 exhibited negative homotropic interactions in the binding of ATP but not of citrulline or aspartic acid. Rabbit antibody raised in response to MGL8D1 argininosuccinate synthetase inactivated enzymes from the two cell lines at the same rate. Ouchterlony double diffusion showed a reaction of immunologic identity for enzymes from the two cell lines. Thus, the argininosuccinate synthetase produced at high level by the canavanine-resistant human lymphoblast line MGL8D1 appears identical in all these respects with the enzyme produced by the parental line MGL8B2, consistent with the hypothesis that MGL8D1 is a constitutive mutant synthesizing argininosuccinate synthetase at a high rate and unresponsive to the normal repression by arginine.

Argininosuccinate synthetase (EC 6.3.4.5) catalyzes the ATP-dependent condensation of aspartic acid and citrulline to form argininosuccinic acid in the pathway converting ammonia to arginine and urea. In man it is probably the rate-limiting enzyme of the urea cycle, based on the relative specific activities in liver (Nuzum & Snodgrass, 1971). Argininosuccinate synthetase has been purified to homogeneity from rat liver (Saheki et al., 1975), bovine liver (Rochovansky et al., 1977), and yeast (Hilger et al., 1979). All three of these enzymes have native molecular weights of ~190 000 and are tetramers consisting of four identical subunits.

Purified argininosuccinate synthetase from bovine liver exhibits autoregulatory properties. At low substrate concentrations the affinity of argininosuccinate synthetase increases for all three substrates, providing a large increase in enzyme activity (Rochovansky et al., 1977). Schimke (1962) showed that the levels of all five urea cycle enzymes in rat liver increase in response to increased dietary nitrogen. In cultured human and mouse cell lines Schimke (1964) demonstrated that argininosuccinate synthetase and argininosuccinate lyase activities increase on limiting the arginine concentration in the growth medium. Similarly, in cultured human lymphoblasts the activity of argininosuccinate synthetase increases 50-fold when arginine is replaced by citrulline in the medium (Irr & Jacoby, 1978). Enzyme turnover studies suggest that this dramatic increase in activity is due to new synthesis of argininosuccinate synthetase rather than activation of preexisting enzyme or some other mechanism (Irr & Jacoby, 1978).

An apparent constitutive mutant affecting argininosuccinate synthetase has been isolated from the normal human lymphoblast line MGL8B2 by selection in the presence of canavanine, an arginine analogue (Jacoby, 1978). The activity of argininosuccinate synthetase in the overproducer line, MGL8D1, is 200-fold higher than that in normal cells grown in arginine and is refractory to repression by arginine. The high level of argininosuccinate synthetase in the overproducer line leads to increased synthesis of arginine (L. B. Jacoby, unpublished experiments), which presumably competes with canavanine thus providing resistance to the toxic analogue.

In this study we describe the purification of argininosuccinate synthetase from parental and overproducer lymphoblast lines and compare the physical and catalytic properties of enzymes from these two sources. Rabbit antibody to argininosuccinate synthetase from MGL8D1 was prepared and showed a reaction of identity between enzymes from MGL8B2 and MGL8D1. The antibody can thus be used as a specific probe for this enzyme in further studies of the regulation of argininosuccinate synthetase in normal and overproducer human lymphoblast lines.

Experimental Procedures

Materials. CM-cellulose, dithiothreitol, and sodium dodecyl sulfate ($NaDodSO_4$)[1] were purchased from Sigma. Fetal bovine serum was purchased from Microbiological Associates, Eagle's minimum essential medium was from Grand Island Biological, DEAE-cellulose (DE-52) was from Whatman, enzyme grade $(NH_4)_2SO_4$ was from Schwarz/Mann, flake poly(ethylene glycol) (Aquacide III) was from Calbiochem, Spectropor 1 dialysis tubing was from Fisher Scientific, electrophoretic grade acrylamide and *N,N'*-methylenebis-(acrylamide) were from Bio-Rad, and bromophenol blue and Coomassie brilliant blue R-250 were from Eastman.

Cell Lines and Culture. An established human lymphoblast line, MGL8B2, and its canavanine-resistant variant, MGL8D1, have been described previously (Jacoby, 1978; Irr & Jacoby, 1978). Cells were grown in suspension cultures in Eagle's minimum essential medium with Earle's salts plus 10% fetal bovine serum. MGL8B2 was grown under derepression conditions in arginine-deficient citrulline-supplemented medium.

† From the Genetics Unit, Massachusetts General Hospital and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02114. *Received August 10, 1979.* This work was supported by U.S. Public Health Service Grants CA14534, GM25758, T32 HD07092, and 5 S07 RR05486.

[1] Abbreviation used: $NaDodSO_4$, sodium dodecyl sulfate.

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MGL8D1 cells used in the enzyme purification were grown at the Massachusetts Institute of Technology Cell Culture Center.

Argininosuccinate Synthetase Assay. The activity of argininosuccinate synthetase was measured by the two-step radioactive assay described by Irr & Jacoby (1978), which measures the conversion of [^{14}C]citrulline to $^{14}CO_2$ at 37 °C in the presence of excess coupling enzymes. One unit of enzyme produces 1 nmol of $^{14}CO_2$ per h under the specified reaction conditions. The concentration of $MgCl_2$ in the initial reaction was changed from 2.4 to 6.0 mM for the kinetic and immunologic inactivation studies. The specific activity of the [^{14}C]citrulline substrate was corrected for unlabeled citrulline in assays where the enzyme solution contained citrulline.

Protein was measured spectrophotometrically (Lowry et al., 1951) after precipitation of the protein sample in cold 10% trichloroacetic acid. Crystalline ovalbumin or bovine serum albumin was used as the standard.

Molecular Weight Determination. The subunit molecular weight of the enzyme was determined by $NaDodSO_4$–polyacrylamide gel electrophoresis using 10% polyacrylamide containing 0.1% $NaDodSO_4$ in a 14 × 28 cm Bio-Rad slab gel apparatus using conditions adapted from Laemmli (1970). The gel was calibrated with rabbit muscle phosphorylase A, bovine serum albumin, catalase, ovalbumin, and chymotrypsinogen A of molecular weights of 92 500, 68 000, 57 500, 45 000, and 25 000, respectively. The gels were stained with Coomassie blue (Weber & Osborn, 1969) and dried onto Whatman 3MM paper in a Bio-Rad slab gel drier for storage.

The molecular weight of native argininosuccinate synthetase was determined by gel filtration as described by Andrews (1965). The Sephadex G-200 column (0.9 × 95 cm) was calibrated with hog kidney argininosuccinate lyase, rabbit muscle aldolase, bovine serum albumin, ovalbumin, chymotrypsinogen A, and ribonuclease A with molecular weights of 202 000, 158 000, 68 000, 45 000, 25 000, and 13 700, respectively.

Purification of Argininosuccinate Synthetase. All steps were performed at 0–4 °C. The stock solution of 1 M Tris-HCl was extracted with 1 μg/mL dithizone in CCl_4 followed by repeated extraction with CCl_4 alone to remove contaminating heavy-metal ions. CM-cellulose and DEAE-cellulose were precycled in NaOH and HCl as described by Peterson (1970). All buffer solutions contained 1 mM dithiothreitol, 0.1 mM EDTA, and 1 mM citrulline unless noted otherwise.

Step 1: Enzyme Extraction. Cells (10 g) were suspended in 60 mL of 50 mM Tris-HCl, pH 8.5, and sonicated in two batches in a Raytheon sonic oscillator for 8 min with three pauses for cooling. The sonicated cells were centrifuged for 1 h at 100000*g*, and the high-speed supernatant was adjusted to 100 mL with 50 mM Tris-HCl, pH 8.5.

Step 2: 35–55% $(NH_4)_2SO_4$ Fractionation. Crystalline enzyme grade $(NH_4)_2SO_4$ (24.7 g) was added with continuous stirring to the diluted supernatant over a 30-min period. After an additional 90 min, the suspension was centrifuged for 15 min in a Sorvall SS-34 rotor at 12 000 rpm and the pellet discarded. An additional 14.1 g of crystalline $(NH_4)_2SO_4$ was added to the supernatant, and the suspension was equilibrated and centrifuged as before. The pellet was carefully drained and dissolved in 10 mL of 50 mM Tris-HCl, pH 8.5, containing 10 mM KCl.

Step 3: Sephadex G-200 Gel Filtration. The 35–55% $(NH_4)_2SO_4$ fraction was applied to a 2.5 × 60 cm column of Sephadex G-200 equilibrated with 50 mM Tris-HCl, pH 8.5, containing 10 mM KCl and eluted with the same buffer. Fractions containing enzyme activity were pooled and concentrated by the addition of crystalline $(NH_4)_2SO_4$, equilibrated for 1 h, and centrifuged. The pellet was dissolved in 10 mL of 10 mM Tris-HCl, pH 7.2, and dialyzed overnight against a 100-fold excess volume of this buffer.

Step 4: CM-cellulose Chromatography. The concentrated pool from the first gel filtration was applied to a 2.6 × 10 cm column of CM-cellulose previously equilibrated with 10 mM Tris-HCl, pH 7.2. The column was washed with 100 mL of 10 mM Tris-HCl followed by a 400-mL linear gradient of 10–100 mM Tris-HCl, pH 7.2. The bulk of the protein washed through the column in the low ionic strength buffer, while the enzyme activity eluted near the middle of the gradient. Fractions containing enzyme activity were pooled and concentrated 10-fold by packing a Spectrapor 1 dialysis bag (molecular weight cutoff 6000–8000) containing the pooled fractions in flake poly(ethylene glycol) (Aquacide III, average molecular weight 40 000). The concentrated material was then dialyzed overnight against a 100-fold excess of 5 mM Tris-HCl, pH 8.5.

Step 5: DEAE-cellulose Chromatography. The concentrated pool of enzyme from step 4 was applied to a 0.9 × 10 cm column of DEAE-cellulose (Whatman DE-52) equilibrated with 5 mM Tris-HCl, pH 8.5. The enzyme passed through the column in the 5 mM Tris-HCl, pH 8.5, wash. The fractions containing enzyme activity were pooled and concentrated to 3 mL by dialysis against dry poly(ethylene glycol) as described above or by vacuum dialysis.

Step 6: Sephadex G-200 Gel Filtration. The partially purified enzyme from the previous step was applied to a 0.9 × 95 cm column of Sephadex G-200 equilibrated with 50 mM Tris-HCl containing 10 mM KCl and eluted with the same buffer. The last few fractions containing enzyme activity always had a lower specific activity, indicating contamination by a low molecular weight protein. Therefore, the peak of enzyme activity was pooled into two fractions. The first pooled fraction was concentrated by vacuum dialysis against 50 mM Tris-HCl, pH 8.5. The second pooled fraction was concentrated by dialysis against dry poly(ethylene glycol) as described in step 4 and again applied to the smaller Sephadex G-200 column. The fractions containing enzyme activity were pooled and concentrated by vacuum dialysis against 50 mM Tris-HCl, pH 8.5.

Preparation of Antibody. The 270-fold purified MGL8D1 argininosuccinate synthetase (0.2 mg/mL in 50 mM Tris-HCl, pH 8.5) was emulsified with an equal volume of Freund's complete adjuvant. Two white New Zealand rabbits were injected with 1 mL of the emulsion each at 5–10 subcutaneous sites. The rabbits were given an additional intramuscular (80 μg each) and subcutaneous (200 μg each) boost at 2-week intervals. The rabbits were bled, and sera were tested by radial immunodiffusion and double diffusion. The γ-globulin fraction of rabbit serum was isolated by fractionation with Na_2SO_4 as described by Thurston et al. (1957).

Results

Purification of Argininosuccinate Synthetase. Argininosuccinate synthetase from both normal and overproducer human lymphoblast lines was purified in order to compare the two enzymes and to produce antibody for studies of the regulation of synthesis of argininosuccinate synthetase. The purification protocol consisted of $(NH_4)_2SO_4$ fractionation, ion-exchange chromatography on CM-cellulose and DEAE-cellulose, and repeated gel filtration on Sephadex G-200. The overall yield from either cell line was 10–20%. In parallel purification procedures, argininosuccinate synthetase from the

Table I: Purification of Argininosuccinate Synthetase from Normal MGL8B2 and Overproducer MGL8D1 Lymphoblasts

step	protein (mg)	enzyme act. (μmol/h)	sp act. [μmol/(h mg)]	purifn (x-fold)	yield (%)
		MGL8B2			
(1) S-100	298	7.14	0.024	1	100
(2) $(NH_4)_2SO_4$	67	(4.22)[a]			
(3) Sephadex G-200	54	4.90	0.091	4	69
(4) CM-cellulose	9.4	4.02	0.427	18	56
(5) DEAE-cellulose	0.42	2.80	6.72	286	39
(6) Sephadex G-200[b]	0.025	1.33	53.3	2220	19
		MGL8D1			
(1) S-100	550	116	0.21	1	100
(2) $(NH_4)_2SO_4$	127	(45)[a]			
(3) Sephadex G-200	64.2	87	1.36	6.5	75
(4) CM-cellulose	2.5	37.1	14.8	70.7	32
(5) DEAE-cellulose	0.64	29.0	45.3	216	25
(6) Sephadex G-200[b]	0.34	19.7	57.2	272	17

[a] $(NH_4)_2SO_4$ inhibits assay. [b] Combined from two runs.

normal line MGL8B2 and the overproducer MGL8D1 eluted at the same relative position in each fractional step with similar yields (Table I), suggesting that the molecular species being purified from the two cell lines were the same. The final products from both cell lines migrated as single bands on $NaDodSO_4$–polyacrylamide gel electrophoresis, indicating a purity of 90% or greater. The subunit of argininosuccinate synthetase from the normal cell line MGL8B2 had the same mobility as the subunit from the overproducer line MGL8D1, again suggesting that the two enzymes are identical. Argininosuccinate synthetase from both cell lines appeared to have primarily positively charged groups at the molecular surface even at high pH since it bound only to the cationic ion exchanger.

Molecular Weight of Argininosuccinate Synthetase. The molecular weight of the enzyme subunits from the two lines was determined by electrophoresis on a 10% polyacrylamide gel containing 0.1% $NaDodSO_4$, after reducing and denaturing the purified enzymes by using 2-mercaptoethanol and $NaDodSO_4$. The bands corresponding to argininosuccinate synthetase from the two cell lines had the same minimum molecular weight of 45 000 (±10%). The molecular weight of catalytically active argininosuccinate synthetase was determined by gel filtration on Sephadex G-200. Argininosuccinate synthetase activity from both lines eluted in a sharp peak at a position corresponding to a molecular weight of 180 000 (±10%) (Figure 1) by using centrifuged lysates of MGL8B2 and MGL8D1 cells. These results indicate that the native enzyme from both cell lines is a tetramer and that the four subunits are probably identical. When pure argininosuccinate synthetase was chromatographed on Sephadex G-200, there was a minor peak of enzyme activity corresponding to a molecular weight of 90 000 in addition to the major peak of enzyme activity at 180 000, suggesting that the enzyme is catalytically active both as a tetramer and dimer.

Kinetic Properties of Argininosuccinate Synthetase. Affinity constants were determined for each of the three substrates of argininosuccinate synthetase by using saturating amounts of the other two substrates. With aspartic acid as the variable substrate, linear double-reciprocal plots yielded apparent K_m values of 0.078 mM for MGL8B2 and MGL8D1 argininosuccinate synthetases (Figure 2, panel A). With citrulline as the variable substrate, linear double-reciprocal plots yielded apparent K_m values of 0.061 and 0.089 mM, respectively, for the MGL8B2 and MGL8D1 argininosuccinate

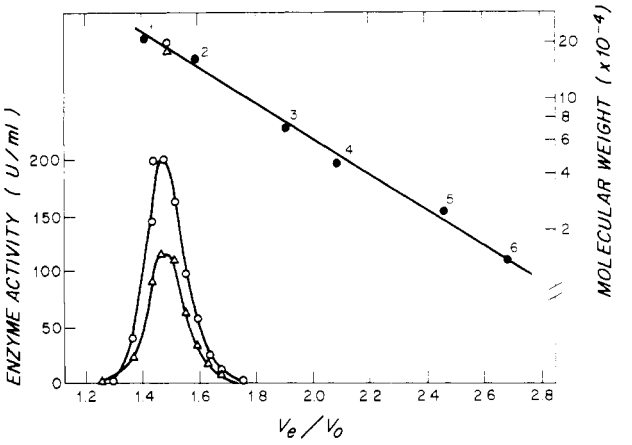

FIGURE 1: Gel filtration of MGL8B2 argininosuccinate synthetase (Δ) and MGL8D1 argininosuccinate synthetase (○) on a column (0.9 × 95 cm) of Sephadex G-200 in 50 mM Tris-HCl, pH 8.5, 10 mM KCl, 1 mM dithiothreitol, and 0.1 mM EDTA. The abscissa (V_e/V_o) is the elution volume of the protein divided by the void volume of the column. The protein standards (●) used to calibrate the column are the following: 1, hog kidney argininosuccinate lyase; 2, muscle aldolase; 3, bovine serum albumin; 4, ovalbumin, 5, chymotrypsinogen A; 6, ribonuclease A.

synthetases (Figure 2, panel B). When the fixed substrates were reduced to limiting levels, no homotropic interactions were apparent with either aspartic acid or citrulline as the variable substrate.

In contrast, when ATP was the variable substrate, a biphasic double-reciprocal plot was obtained even at saturating levels of aspartic acid and citrulline (Figure 2, panel C). At high ATP concentrations apparent K_m values of 0.47 mM were obtained for both MGL8D1 and MGL8B2 argininosuccinate synthetases. By extrapolation, apparent K_m values of 0.094 mM were obtained for MGL8D1 and MGL8B2 enzymes at low ATP concentrations. Negative cooperative behavior was indicated by a Hill coefficient of 0.74 for the low ATP concentration region of the plot. The Hill plot was calculated from the kinetic data and V_m shown in Figure 2, panel C, with the break point occurring at the same substrate concentration.

Nonlinear kinetics are not necessarily due to allosteric interactions of enzyme with ATP. Nonlinearity may result from multiple enzymes metabolizing ATP or from the Mg^{2+}/ATP ratio. It is unlikely that the nonlinear kinetics are due to other enzymes metabolizing ATP, since there was no evidence of any other protein copurifying with argininosuccinate synthetase from either cell line. The Mg^{2+}/ATP ratio was varied over a 2.5-fold range, and the kinetics remained nonlinear, with the same slopes and break points. Thus, it is likely that the change in slope at different ATP concentrations reflects a discrete change in enzyme conformation affecting the substrate affinity, with high affinity at low ATP concentrations and low affinity at high ATP concentrations.

Immunologic Characterization of Argininosuccinate Synthetase from MGL8B2 amd MGL8D1. Antibody to MGL8D1 argininosuccinate synthetase was prepared by injection of purified enzyme into rabbits. The resulting antiserum reacted strongly with argininosuccinate synthetase from both MGL8D1 and MGL8B2, causing complete loss of catalytic activity for equal amounts of enzyme from each cell line at similar rates (Figure 3). Preimmune serum from the same rabbit had no effect on argininosuccinate synthetase activity from either cell line. The immunologic relationship between MGL8B2 and MGL8D1 argininosuccinate synthetases was studied by Ouchterlony double diffusion (Figure 4). A single precipitin line formed between pure argininosuccinate

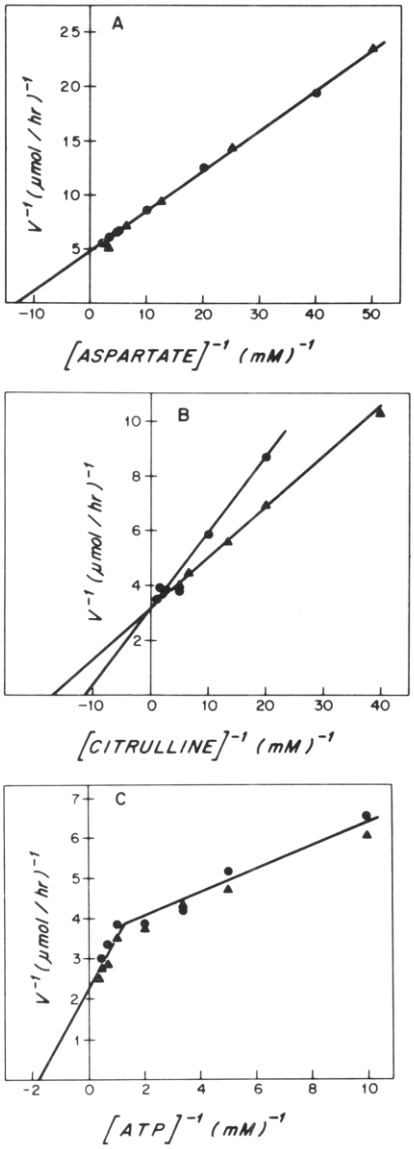

FIGURE 2: Determination of the apparent K_m values of human lymphoblast argininosuccinate synthetase for aspartic acid (panel A), citrulline (panel B), and ATP (panel C). Double-reciprocal plots of initial reaction velocity with substrate concentration for pure argininosuccinate synthetase derived from MGL8D1 (●) (panels A, B, and C) and argininosuccinate synthetase derived from MGL8B2 (▲) (step 3 partially purified enzyme in panels A and B and pure enzyme in panel C). For the kinetic analysis with citrulline, MGL8B2 and MGL8D1 enzymes were purified by using buffer solutions lacking citrulline throughout. The intercepts and slopes were calculated by unweighted least-squares analysis.

synthetase from MGL8B2 and anti-MGL8D1 argininosuccinate synthetase. This precipitin line fused in a reaction of identity with the lines that formed with unfractionated extract and partially purified enzyme from MGL8D1. Thus, anti-MGL8D1 argininosuccinate synthetase selects a single protein from the unfractionated MGL8D1 extract which reacts with identity to pure argininosuccinate synthetase from MGL8B2. These immunologic tests thus support the identity of argininosuccinate synthetase from the normal MGL8B2 and overproducer MGL8D1 lines.

Discussion

Argininosuccinate synthetase from cultured human lymphoblasts closely resembles the rat liver, bovine liver, and yeast enzymes in size and quaternary structure (Saheki et al., 1975; Rochovansky et al., 1977; Hilger et al., 1979). All four argininosuccinate synthetases are tetrameric with subunits of

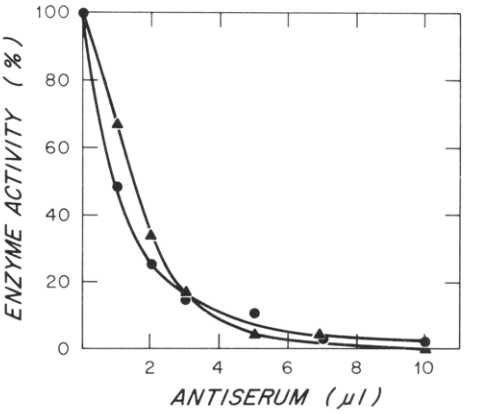

FIGURE 3: Antibody inactivation of argininosuccinate synthetase activity by rabbit anti-MGL8D1 lymphoblast argininosuccinate synthetase. Centrifuged extract of MGL8B2 (9.9 units) (▲); centrifuged extract of MGL8D1 (10.6 units) (●). Extracts were incubated for 2 h at 25 °C in 5 mM Tris-HCl, pH 8.5, 0.1 mM EDTA, and 1 mM dithiothreitol with the indicated amount of antibody in a total volume of 0.15 mL and assayed for argininosuccinate synthetase activity.

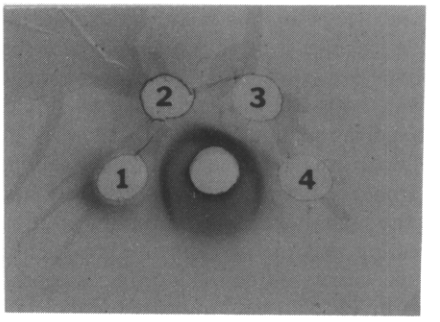

FIGURE 4: Ouchterlony double diffusion with argininosuccinate synthetase from MGL8B2 and MGL8D1. The diffusion plate contained 1% agarose in 0.05 M sodium barbital with 30-μL wells. The plate was stored for 24 h at room temperature and then washed extensively with phosphate-buffered saline and water. The plate was covered with lint-free paper, pressed under a uniform weight, and dried under a stream of warm air. The plate was stained with 5 g/L Coomassie brilliant blue R-250 in ethanol–acetic acid–water (4:1:5) and destained with the ethanol–acetic acid solution. Center well: rabbit antihuman lymphoblast argininosuccinate synthetase γ-globulin. Well 1, pure argininosuccinate synthetase from MGL8B2; well 2, unfractionated centrifuged extract of MGL8D1; well 3, partially purified argininosuccinate synthetase from MGL8D1 (step 4); well 4, partially purified argininosuccinate synthetase from MGL8D1 (step 5).

~45 000 molecular weight. The major difference between the human lymphoblast enzyme and the other enzymes is in surface charge. The yeast, bovine, and rat enzymes adsorb to DEAE ion exchangers in the pH range 6.5–8.5, while human lymphoblast argininosuccinate synthetase does not, even at low ionic strength (5 mM Tris-HCl) and high pH (8.5), indicating no accessible areas of negative charge.

The kinetic properties of argininosuccinate synthetase purified from human lymphoblasts are similar to those reported for unfractionated extracts of normal human lumphoblasts (Kennaway et al., 1975) and for purified enzyme from yeast, bovine liver, and rat liver (Hilger et al., 1979; Rochovansky et al., 1977; Saheki et al., 1975). Negative cooperative substrate binding by argininosuccinate synthetase was reported for both bovine liver argininosuccinate synthetase (Rochovansky et al., 1977) and yeast argininosuccinate synthetase (Hilger et al., 1979). Unlike the human lymphoblast enzyme, bovine liver argininosuccinate synthetase yields biphasic double-reciprocal plots for citrulline and aspartic acid when another substrate is limiting. However, human lymphoblast,

yeast, and bovine liver argininosuccinate synthetases yield biphasic plots for ATP regardless of the concentrations of the other substrates. The negative homotropic interactions of argininosuccinate synthetase under conditions of limiting substrate may be valuable in maintaining a steady flow of nitrogen through the urea cycle. At the times when ATP may be limiting in vivo, the allosteric properties of the enzyme would provide an enhanced affinity for ATP in response to the limitation in energy supply.

We could detect no difference between the argininosuccinate synthetases purified from the normal and overproducer lymphoblast lines. Enzymes from the two cell lines had the same subunit molecular weight and tetrameric structure. The two enzymes copurified at each step with similar yields, and the final specific activities were very close. Furthermore, the kinetic parameters of the two enzymes were essentially identical. Finally, antibody produced in response to argininosuccinate synthetase from MGL8D1 reacted with enzyme from MGL8B2 with apparent identity. Thus, the argininosuccinate synthetase produced at high level by the canavanine-resistant line MGL8D1 appears identical in all respects with the enzyme produced by its arginine-repressible parent MGL8B2 as would be expected were MGL8D1 a constitutive mutant synthesizing argininosuccinate synthetase independent of arginine repression. Whether canavanine resistance is due to an increased number of argininosuccinate synthetase gene copies, as recently observed for dihydrofolate reductase in methotrexate-resistant cells (Alt et al., 1978), awaits further study.

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Inactivation of 3-(3,4-Dihydroxyphenyl)alanine Decarboxylase by 2-(Fluoromethyl)-3-(3,4-dihydroxyphenyl)alanine†

A. L. Maycock,* S. D. Aster, and A. A. Patchett

ABSTRACT: 2-(Fluoromethyl)-3-(3,4-dihydroxyphenyl)alanine [α-FM-Dopa (I)] causes rapid, time-dependent, stereospecific, and irreversible inhibition of hog kidney aromatic amino acid (Dopa) decarboxylase. The inactivation occurs with loss of both the carboxyl carbon and fluoride from I and results in the stoichimetric formation of a covalent enzyme–inhibitor adduct. The data are consistent with I being a suicide inactivator of the enzyme, and a plausible mechanism for the inactivation process is presented. The inactivation is highly efficient in that there is essentially no enzymatic turnover of I to produce the corresponding amine, 1-(fluoromethyl)-2-(3,4-dihydroxyphenyl)ethylamine [α-FM-dopamine (II)]. Amine II is also a potent inactivator of the enzyme. In vivo compound I is found to inactivate both brain and peripheral (liver) Dopa decarboxylase activity. The possible significance of these data with respect to the known antihypertensive effect of I is discussed.

It was recently reported by workers at Merck (Kollonitsch et al., 1978) and independently by others (Metcalf et al., 1978; Palfreyman et al., 1978) that a number of α-fluoromethyl amino acids are potent, time-dependent inhibitors of α-amino acid decarboxylases. The investigations by the Merck group were prompted by earlier observations that fluoroalanine is bactericidal (Kollonitsch et al., 1973) as a consequence of irreversible inhibition (inactivation) of the pyridoxal phosphate dependent enzyme alanine racemase (Kahan & Kropp, 1975). Although it was only recently verified (Wang & Walsh, 1978) that D-fluoroalanine is acting as a suicide inactivator (Morisaki & Bloch, 1972; Rando, 1974; Abeles & Maycock, 1976; Walsh, 1977; Rando, 1978) of the enzyme, that possibility was recognized earlier by one of us (A.A.P.) and by others (Kollonitsch, 1978; Bey et al., 1977). This inhibitor design principle was considered extendable to other pyridoxal phosphate dependent enzymes. As part of this development, α-fluoromethyl amino acids, that is, 2-substituted fluoroalanines,

† From Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065. *Received July 6, 1979; revised manuscript received November 15, 1979.*

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