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CARBAMOYL PHOSPHATE SYNTHETASE I OF HUMAN LIVER

PURIFICATION, SOME PROPERTIES AND IMMUNOLOGICAL CROSS-REACTIVITY WITH THE RAT LIVER ENZYME *

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Summary

The purification of mitochondrial carbamoyl phosphate synthetase I (carbon-dioxide ammonia ligase (ADP-forming, carbamate-phosphorylating), EC 6.3 4.16) from small samples of human liver is described The enzyme is composed of a single polypeptide of M_r 160 000 ± 500 as shown by SDS-polyacrylamide gel electrophoresis in the presence of reducing agents. The synthetase migrates in polyacrylamide gradient gels in the absence of detergents at a rate corresponding to a M_r of 165 000. Estimates of the molecular weight of the native enzyme by gel filtration and density gradient centrifugation yield a value of 178 000 These results indicate that the enzyme exists predominantly as monomers

Amino acid composition, isoelectric point, stability, K_m values and the ability to catalyze partial reactions have been measured and compared with known properties of carbamoyl phosphate synthetases from other sources From the available data a high degree of evolutionary conservation of the ammonia-dependent synthetase is suggested. This is also supported by the demonstration of extensive immunological cross-reactivity between the human and rat enzymes.

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Abbreviation SDS, sodium dodecyl sulphate

Introduction

Carbamoyl phosphate synthetase I (carbon-dioxide ammonia ligase (ADP-forming, carbamate-phosphorylating), EC 6 3.4.16) is located in the mitochondrial matrix of hepatocytes of ureotelic animals where it represents a large percentage of the protein [1,2]. Congenital deficiency of carbamoyl phosphate synthetase in man results in hyperammonemia which is related to the degree of the enzyme defect [3]. Low enzyme levels have been associated with the hyperammonemia observed in Reye's Syndrome [4] and with the reduction in the maximal rate of urea synthesis found in cirrhotic patients [5]. A detailed characterization of the human enzyme is of interest. Isolation of the human enzyme is a prerequisite to the study of potential variants of the synthetase.

The purification of carbamoyl phosphate synthetase from the livers of mammals has been hampered by its unstability. Only after the demonstration by Novoa and Grisolía [6] that glycerol and KCN stabilize the enzyme was it possible to isolate essentially pure rat liver synthetase. We modified the procedure of Guthorlein and Knappe [7] to process small samples (approx. 5 g) of rat liver [8]. We report here a further modification for small samples (approx. 5 g) of human liver. Some of the properties of the human enzyme are also presented.

Materials and Methods

Materials Purification of rat liver carbamoyl phosphate synthetase I, immunization of rabbits, and obtention of antisera specific for rat carbamoyl phosphate synthetase were as described [8,9]. γ-Globulin fractions from immune and control serum [10] were a gift from R. Wallace. Ferritin conjugated with IgG was from Miles. Other marker proteins were from Sigma and Boehringer. Complement, haemolytic antibody and sheep erythrocytes were from Materiales y Reactivos, S.A (Madrid). Ampholytes were from Serva (Servalyt AG 5—7, 7—9) and from Pharmacia (Pharmalyte 5—8).

Enzyme assays Marker enzymes were assayed by standard techniques [11]. Rat liver ornithine transcarbamylase (carbamoyltransferase EC 2 1.3.3.) was obtained and assayed by the method of Caravaca and Grisolía [12], 1 unit catalyzes the production of 1 μmol citrulline/min at 37°C. Except where indicated, carbamoyl phosphate synthetase was assayed at 37°C using the system coupled to NADH oxidation [7], 1 U corresponds to 1 μmol carbamoyl phosphate produced in 15 min.

Electrophoretic methods SDS electrophoresis in polyacrylamide gels was carried out as described [13].

Electrophoresis in polyacrylamide gradient gel slabs (Pharmacia, PAA 4/30) in the absence of detergents was carried out at 160 V for 12.5—16 h Cooling was done using tap water. The temperature of the buffer (0.09 M Tris/0.08 M boric acid/Na_2EDTA 0.93 g/l, pH 8.4) at the end of the electrophoresis was 20°C. Protein standards used were: thyroglobulin (M_r 669 000), ferritin (M_r 440 000), catalase (M_r 232 000), lactate dehydrogenase (M_r 140 000) and bovine serum albumin (M_r 67 000).

Isoelectric focusing was performed in polyacrylamide gel rods (80 × 5 mm,

4.75% acrylamide/0.19% bisacrylamide/12.5% glycerol/1 7% Servalyte (5—9) or 6 25% Pharmalyte (5—8)) Anode and cathode solutions were 0 01 M H_3PO_4 or 0.01 M L-glutamic acid and 0.01 M NaOH or 0.01 M ethanolamine. 0.125 W/gel tube were applied at a maximum current of 0.5 mA/tube and a maximum voltage of 400 V (which was reached in less than 1 h). Running time was 6 h. The system was cooled with tap water.

Centrifugation in the airfuge (Beckman) This was performed at room temperature in standard cellulose nitrate tubes, using the 18° fixed-angle rotor, at 3 lb/inch^2 (25 000 rev /min nominal velocity, 10 300 × g_{max}) for 24 h The tubes contained 100 μl of a solution of 50 mM Tris-HCl, pH 7.2, 50 mM KCl, 1 mM dithioerythritol, 20% (v/v) glycerol, 5 mg/ml of bovine serum albumin (to provide a stabilizing density gradient [14,15]) and 35 μg of the enzyme and/or of bovine catalase. After centrifugation the top 40 μl were removed and the enzymes were assayed.

Density gradient centrifugation 0 2 ml samples containing 26 μg carbamoyl phosphate synthetase, 30 μg pig heart malate dehydrogenase ($S_{20,w}$ 4.35) 120 μg rabbit muscle lactate dehydrogenase ($S_{20,w}$ 6.95) and 350 μg rabbit muscle pyruvate kinase ($S_{20,w}$ 10.04) were layered onto 4.6 ml linear 10—30% (v/v) glycerol gradients in 50 mM Tris-HCl, pH 7.2/50 mM KCl/1 mM dithioerythritol. Centrifugation (20°C, 60 000 rev./min) was done using a Beckman VTI-65 Titanium vertical tube rotor. Twenty-four fractions were collected and assayed. $S_{20,w}$ value was estimated by the procedure of Martin and Ames [16]

Gel filtration estimate of Stokes' radius 1 6 mg horse heart cytochrome *c* (Stokes' radius 1.65 nm), 0 1 mg pig heart malate dehydrogenase (3 51), 1 mg rabbit muscle lactate dehydrogenase (4 12), 1 5 mg rabbit muscle aldolase (4 72), 1.3 mg rabbit muscle pyruvate kinase (5.41), 0 15 mg cow milk xanthine oxidase (5 84), 0.28 mg human carbamoyl phosphate synthetase and 0.66 mg of ferritin-IgG conjugate (molecular weight of the protein part of the conjugate was 930 000) were applied in 0.75 ml of a solution of 50 mM Tris-HCl, pH 7 2/ 50 mM KCl/1 mM dithioerythritol/20% glycerol, to a column (0.9 × 56 cm) of Sephadex G-200 equilibrated and eluted with the same solution at 23°C 12 five-drop fractions were collected hourly and the enzymes were assayed

Amino acid analysis Performed essentially as described by Cappugi et al [17] Tryptophan was measured according to Edelhoch [18]

Immunological reactions Precipitin reactions were carried out in 0 5 ml of a solution of 150 mM NaCl, 37 mM potassium glycylglycine, 5 mM mercaptoethanol, 1 mM potassium phosphate, 10 mM Tris-HCl, 4% (v/v) glycerol, 8 mM KCl, 0.2 mM dithioerythritol, pH 7.4, containing 860 μg of γ-globulin from immunized or control rabbits and the antigen. The tubes were incubated for 15 min at 37°C and overnight at 4°C. The precipitates were washed three times with 0.5 ml of 0.15 M NaCl, then dissolved with 10 μl of 0.2 M NaOH. After neutralization with 10 μl of 0.2 M HCl, protein was measured with the BioRad protein assay, using bovine IgG as standard.

One-third of the supernatant was tested for the presence of antigen by the addition of 50 μl of γ-globulin solution (860 μg protein). Enzyme assays and SDS-polyacrylamide gel electrophoresis were also used. To detect excess antibody, 2 μg of rat or human antigens were added to 0.2 ml of the supernatants

The micro-complement fixation technique was performed as described by Levine [19], but the volumes of all components were reduced to one-fifth. 34 μg of rabbit γ-globulin fraction and 0.2 ml of a 1 50 solution of guinea-pig complement were used. After addition of the antigen, the mixture (1 2 ml) was incubated for 60 min at 37°C Then, 0.2 ml of sensitized sheep erythrocyte suspension ($5 \cdot 10^7$ cells/ml) were added and the incubation continued for 28 min at 37°C.

Other methods P_i, citrulline, ADP and ATP were assayed by standard methods [11]. Protein was measured by the Lowry technique with bovine serum albumin (Sigma) as standard. For purified samples of the enzyme, the absorbance at 280 nm was used.

Results

Purification of human liver carbamoyl phosphate synthetase I Unless specified, all operations were at 4°C.

Extraction with cetyltrimethyl ammonium bromide About 5 g of neonatal human liver * were homogenized with 9 vols. of 0.25 M sucrose in a Potter homogenizer (seven strokes). The homogenate was centrifuged for 5 min at 500 × *g*, the precipitate discarded and the supernatant centrifuged for 5 min at 10 000 × *g*. The pellet was suspended in 14 ml of 0.1% (w/v) cetyltrimethyl ammonium bromide, 20% (v/v) glycerol, 1 mM dithioerythritol, 50 mM Tris-HCl, pH 7.4, and homogenized with ten strokes in a Potter homogenizer. After centrifugation for 20 min at 12 000 × *g* at 10°C, the residue was extracted a second time in the same way. The combined supernatants (28 ml) were filtered through glass wool.

Precipitation with ammonium sulphate 9 g ammonium sulphate were dissolved into the extract. The mixture was then stirred at low speed for 15 min and centrifuged for 10 min at 25 000 × *g*. Five g ammonium sulphate were added to the supernatant as in the previous step and after centrifugation (10 min, 25 000 × *g*), the precipitate (ammonium sulphate precipitate) was suspended in 1 ml of 50 mM potassium glycylglycine (pH 7.2)/20% (v/v) glycerol/2.95 M ammonium sulphate and stored overnight at −20°C.

Chromatography in DEAE-Sephadex The preparation was centrifuged at 35 000 × *g* for 15 min. The precipitate was dissolved in about 1.5 ml of 20% (v/v) glycerol/50 mM Tris-HCl, pH 7.2/1 mM dithioerythritol/5 mM KCl (buffer A). This solution was freed from ammonium sulphate by gel filtration (Sephadex G-25) and applied to a column of DEAE-Sephadex A-50 (0.7 × 10 cm) equilibrated with buffer A. The column was washed with 12 ml of this buffer and eluted with a linear gradient of KCl (5—75 mM, 36 ml) in the same buffer. The synthetase activity was eluted by the gradient after 15 ml; the enzyme collected between 20 and 30 ml was used. The results of the purification are summarized in Table I. Although the bulk of the protein had an average specific activity of 22—23 U/mg, individual tubes reached specific

* Liver tissue was obtained less than 8 h after death from neonates with no signs of liver disease and was processed immediately

TABLE I

PURIFICATION OF HUMAN CARBAMOYL-PHOSPHATE SYNTHETASE (AMMONIA)

Fraction	Total activity (units)	Protein/ (mg)	Specific activity (U/mg)	Recovery (%)
Cetyltrimethyl ammonium bromide extract *	193	147	1 3	100
Ammonium sulphate precipitate	157	59	2 7	81
DEAE-Sephadex	72	3 2	22 5	37

* From 4 6 g of liver

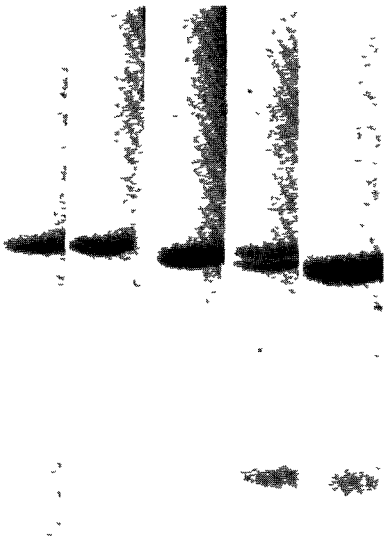
activities of 25. Protein from these tubes were used for amino acid analysis (see below)

Molecular properties 91% of the protein (as integrated A_{520nm} of Coomassie blue in the densitometric trace) migrated as a single band in polyacrylamide gels in the presence of SDS (Fig. 1). Seven minor contaminants accounted for the remaining of the protein. The mobility of the main band was identical to that of rat liver carbamoyl phosphate synthetase monomer, as shown by co-electrophoresis. From the mobility of protein standards of known M_r, an M_r of 160 000 ± 5000 was calculated for the protein migrating at this band. Thus the enzyme appears to be composed of a single subunit with respect to M_r.

The human enzyme was subjected to electrophoresis in polyacrylamide gradient gels in the absence of detergents (see Methods). There was a linear relationship between the migration distance of globular proteins and the logarithm of their M_r. Most of the protein in the enzyme preparation (approx. 90%) migrated at a rate corresponding to a M_r of 165 000. This is to be expected if, under the conditions of the experiment, the enzyme is present as a monomer.

A qualitative confirmation that the monomer predominates were obtained by centrifugation in the Airfuge under conditions at which the enzyme is stable and at which the fraction of a protein remaining in the upper 40 μl of the tube and the M_r of the protein are related, with higher M_r less protein remains. When carbamoyl phosphate synthetase and bovine catalase were centrifuged, the fraction of carbamoyl phosphate synthetase in the top 40 μl of the tubes was 0.17 while that of the catalase was 0.09, indicating a lower M_r for the human enzyme than for the catalase (M_r = 232 000).

Gel filtration in a buffer where the enzyme activity is preserved was used to estimate the Stokes' radius of the human synthetase. The enzyme was eluted at a position corresponding to a Stokes' radius of 5.1 nm. Glycerol density gradient centrifugation (in the presence of 1 mM dithioerythritol to prevent enzyme inactivation) revealed that the enzyme sedimented between lactate dehydrogenase and pyruvate kinase giving an s value of 8.6 S. The frictional ratio and M_r of the enzyme were calculated from the Stokes' radius and s value by the method of Siegel and Monty [20] (assuming a value for $\bar{v}$ of 0.721, see below). The enzyme has an estimated frictional ratio of 1.36 and a M_r of 178 000. The close agreement between this value and the M_r obtained after denaturation with SDS confirms that the enzyme exists essentially as a monomer under conditions at which loss of activity is prevented.



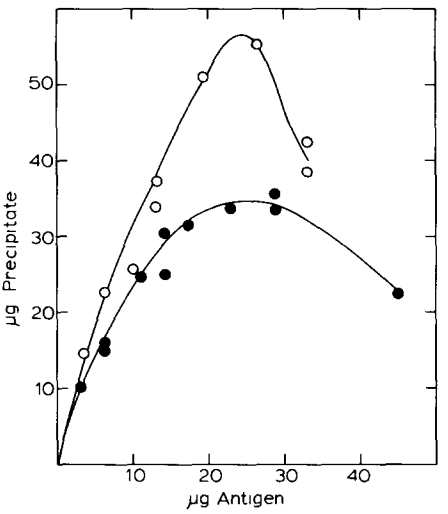


Fig 1 Electrophoresis of human and rat liver carbamoyl phosphate synthetase I and of protein standards on sodium dodecyl sulphate polyacrylamide gels From left to right 8 µg of human enzyme, 10 µg of rat liver enzyme, 5 µg of human plus 5 µg of rat liver enzyme, 4 µg of bovine serum albumin (monomer M_r 68 000) plus 4 µg of RNA polymerase (α-chain M_r 39 000, β-chain M_r 155 000, β'-chain M_r 165 000), 4 µg of bovine serum albumin, 4 µg of RNA polymerase and 4 µg of human liver carbamoyl phosphate synthetase

Fig 2 Precipitin reaction of human (•) and rat liver (○) carbamoyl phosphate synthetase with rabbit antibodies to the rat enzyme For details, see Materials and Methods

A high degree of homology is suggested by the comparison of the amino acid compositions of the human and rat liver enzymes (Table II). The proportion of acidic groups in the amide form is not known for the human enzyme From the amino acid composition a partial specific volume can be calculated [21] of 0.723 to 0.720 depending on the degree of amidation of the acidic residues in the human enzyme. If only 35% are in the amide form (as is the case with the rat liver enzyme [13]) a value of 0.721 results.

When subjected to electrofocusing, the purified human synthetase yielded two protein bands of decreasing intensities at positions corresponding to p*I* 6.26 and 6.23, respectively. Similar behavior has been observed with the purified rat [22] and frog [23] liver enzymes and has been attributed to progressive deamidation of the protein in the course of purification or during isoelectric focusing [23].

Enzymatic properties The enzyme was stable for at least 1 month at 4° C in buffer A. It lost about 30% activity when warmed for 4 h at 37°C in 50 mM

TABLE II

AMINO ACID COMPOSITION OF HUMAN AND RAT PHOSPHATE SYNTHETASES

Enclosed in brackets are reference numbers

Amino acid	Human (mol/160 000 g)	Residues per 100 residues		
		Human	Rat	
			[13]	[1]
Lys	103	6 98	6 94	6 41
His	36	2 45	2 00	1 87
Arg	48	3 29	3 80	3 75
Asx	169	11 47	10 08	9 83
Thr	95	6 43	5 81	5 28
Ser	123 *	8 35	6 54	7 41
Glx	139	9 42	9 88	10 23
Pro	74	5 04	4 13	5 13
Gly	129	8 73	7 81	7 30
Ala	114	7 71	8 28	7 60
Cys (half)	19	1 25	1 40	2 83
Val	101	6 85	7 88	7 48
Met	30	2 00	2 53	2 72
Ile	78	5 27	6 21	5 99
Leu	114	7 70	9 21	8 85
Tyr	36	2 44	2 34	2 41
Phe	51	3 44	3 80	4 00
Trp	18	1 18	1 33	0 93
Total	1477			

* Corrected for 15% loss during hydrolysis

glycylglycine, pH 7.4, and about 25 and 80% when heated for 5 min at 46 and 55°C, respectively. The rat liver enzyme exhibited identical stability.

At saturation of all substrates, synthesis of 1 mol of citrulline (in the system coupled with ornithine transcarbamylase, see legend to Table IV) released 2

TABLE III

COMPARISON OF SEVERAL PROPERTIES OF HUMAN, FROG AND RAT CARBAMOYL PHOSPHATE SYNTHETASES

References are given in brackets The asterisk indicates unpublished data from this laboratory The turnover number is given per enzyme monomer The immunological cross-reactivity is based on recognition of frog and rat enzymes by the antibody prepared to frog enzyme, and on recognition of the human and rat enzymes by the antibody to rat enzyme

Property	Species		
	Frog	Rat	Human
M_r subunit (10^3)	160 [35]	155—165 [13,22]	160
Turnover number at 37°C (min^{-1})	3 84 10^2 [35]	2 99 10^2 [1]	2 67 10^2
K_m NH_4^+ (mM)	2 0 [36]	2 0 [22]	1 3
K_m bicarbonate (mM)	1 5—4 5 [26]	2 0 [25]	2 2
K_m ATP (mM)	0 5 [36]	0 24 *	0 26
K_m acetylglutamate (mM)	0 19 [37]	0 21 [38]	0 15
K_m carbamylglutamate (mM)	2 94 [37]	1 6 [38]	2 0
Immunological cross-reactivity	+ [29]	+	+

TABLE IV

RELATIVE RATES OF REACTIONS CATALYZED BY HUMAN LIVER CARBAMOYL PHOSPHATE SYNTHETASE

Assays were done at 37°C Carbamoyl phosphate synthesis was assayed in 1 ml of a solution containing 50 mM K glycylglycine, pH 7 4/15 mM $MgSO_4$/5 mM mercaptoethanol/2 5 mM phospho*enol*pyruvate/50 μg pyruvate kinase/5 mM ATP/5 mM ornithine/50 mM $KHCO_3$/35 mM ammonium sulphate/50 units ornithine transcarbamylase and where indicated 10 mM acetylglutamate After 15 min 0 1 ml 2 M $HClO_4$ were added and citrulline was measured ATP hydrolysis was assayed under the conditions used for carbamoyl phosphate synthesis except for the omission of ammonium sulphate Citrulline production from traces of NH_4^+ was less than 2% of the P_i released ATP synthesis from carbamoyl or acetyl phosphate was assayed in 1 ml of a solution of 50 mM potassium glycylglycine, pH 7 4, 15 mM $MgSO_4$, 5 mM mercaptoethanol, 5 mM ADP, 10 mM acetyl glutamate and 5 mM carbamoyl phosphate or acetyl phosphate After 15 min 0 1 ml of 2 M $HClO_4$ were added and the amount of ATP formed was measured Acetyl phosphate and formyl phosphate synthesis were assayed in conical tubes containing 0 3 ml of a solution of 0 5 M K acetate or potassium formate/5 mM ATP/10 mM manganese chloride/5 mM mercaptoethanol/113 mM hydroxylamine/16 mM acetylglutamate (when used)/10 mM KCl/2 4% (v/v) glycerol/ 0 24 mM dithioerythritol (final pH 6 8) After 30 min 150 μl of 2 M hydroxylamine, pH 7 4, were mixed in and the hydroxamate formed was measured [38] Results are μmol of product formed in 15 min per mg of enzyme

Reaction	Acetyl glutamate	
	added	not added
Carbamoyl phosphate synthesis	22 3	0 2
ATP hydrolysis	1 6	0 4
ATP synthesis from carbamoyl phosphate	4 1	—
ATP synthesis from acetyl phosphate	3 6	—
Acetyl phosphate synthesis	0 9	0 0
Formyl phosphate synthesis	1 9	0 2

mol of ADP and of P_i. Glutamine (up to 60 mM) could not substitute for ammonia, and did not inhibit carbamoyl phosphate synthesis in the presence of 60 mM ammonium NH_4^+ ions. Michaelis constants (see Table III) for carbamoyl phosphate synthesis were obtained at saturation of the non-varied substrates. Double reciprocal plots were linear for all substrates including ATP (concentration range 0.1—10 mM) under these conditions *N*-Carbamoyl glutamate was an effective activator of the enzyme.

The human synthetase catalyzed the hydrolysis of ATP in the absence of ammonium ions, the synthesis of ATP from carbamoyl phosphate or acetyl phosphate and ADP, and the phosphorylation of acetate and formate (Table IV). ATP hydrolysis (at 0.1 M K^+) and formyl phosphate synthesis (at 0.5 M K^+) proceeded to a limited extent in the absence of acetylglutamate.

In agreement with previous findings [24] indicating that HCO_3 and not CO_2 is the substrate for the enzyme, CS_2 could not substitute for bicarbonate in the ATPase or carbamoyl phosphate synthetase reactions of the enzyme.

Immunological cross-reactivity with the rat liver enzyme Rat and human carbamoyl phosphate synthetases were completely precipitated by rabbit antibodies to the rat enzyme (Fig. 2). Maximal precipitate was obtained at similar concentrations of enzyme for both species, but the maximal amount of antibody precipitated by the rat enzyme (about 1.9 mg antibody/mg) was approx. 1.5-times that precipitated by the human enzyme. Also, antibodies reacting with the rat but not with the human enzyme could be detected in supernatants

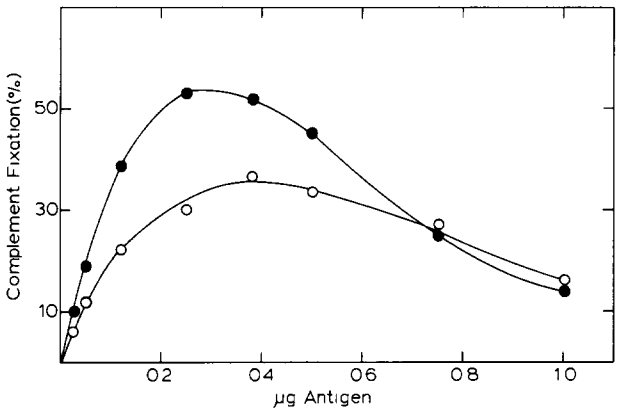


Fig 3 Microcomplement fixation with rat (•) and human (○) carbamoyl phosphate synthetase For details, see Materials and Methods

from tubes in the region of excess human enzyme. In the supernatants from the same region of the precipitin curve for rat enzyme, no antibodies for rat or human enzymes could be detected. SDS-polyacrylamide gel electrophoresis of the immunoprecipitates of the rat and human enzymes gave three protein bands (M_r 160 000, 50 000 and 25 000) corresponding to the synthetase monomer and to the heavy and light chains of immunoglobulin.

Because of its high sensitivity to antigenic differences, the micro-complement fixation technique was also used. This technique employs very low concentrations of antigen and antiserum and thus, only the antibodies of highest affinity are measured. As shown in Fig. 3, a higher amount of complement was fixed with the rat than with human enzyme, but the differences were not large, indicating high immunological similarities. The vertical shift of the curve for the human enzyme suggests altered complementarity of one or a minority of antigenic determinants, the less marked lateral shift indicates slightly reduced affinity of most antigenic determinants of the human enzyme with respect to those of the rat enzyme [19].

Discussion

The procedure described here allows isolation of essentially pure human carbamoyl phosphate synthetase within 1—2 workdays. Liver samples obtained several hours after death are suitable. Although the smallest sample processed until now weighed 3.5 g, it should be possible to scale down the procedure to mg quantities of liver.

Early studies with rat liver carbamoyl phosphate synthetase I described a dimer-monomer transition induced by acetylglutamate [7]. More recent reports suggest that the enzyme monomer may be catalytically active [25,26] and have indicated that the rat liver enzyme exists predominantly as a monomer, although the presence of dimers (less than 20%) could be substantiated [13,22]. The results presented here are consistent with this conclusion, since the measured molecular weight of the human enzyme was only 11% larger than that of the polypeptide chain.

Table III documents some of the similarities between frog, rat and human carbamoyl phosphate synthetases. These enzymes also have similar amino acid compositions and p*I* values, cannot use glutamine, and catalyze identical partial reactions at similar rates. The enzyme from beef is composed of a polypeptide of M_r 165 000 and possesses amino acid composition [27] and kinetic properties similar to the rat enzyme [28] Enzyme preparations from beef, rat, rabbit and turtle liver cross-reacted with antibodies to the frog liver enzyme [29]. There seems to be considerable evolutionary conservation of the ammonia-dependent enzyme. Even the large subunit of the *Escherichia Coli* enzyme, which catalyzes carbamoyl phosphate synthesis from NH_3, is composed of one polypeptide of M_r 130 000 and has an amino acid composition remarkably similar to that of the rat liver enzyme [30].

Since 9% of the purified carbamoyl phosphate synthetase from man can be accounted for by contaminants (on the basis of SDS-polyacrylamide gel electrophoresis), maximal specific activity will be 25.2 U/mg. The activity in fresh human liver is about 0.5 U/mg protein [31], therefore, carbamoyl phosphate synthetase represents 2% of all liver protein and about 7% of the liver mitochondrial protein in man. This corresponds to 0.44 nmol of the M_r 160 000 monomer per mg of mitochondrial protein and to 0.22 mM monomer in the matrix of the resting (state IV) mitochondria (on the basis of 175 mg total protein, 50 mg mitochondrial protein and 0.1 ml volume of the mitochondrial matrix per g of liver [32]). The content of acetylglutamate in human liver is unknown, but values as low as 0.26 nmol/mg mitochondrial protein [33] have been found in rats, e.g., 0.18 mM in the water of the matrix space (assuming 0.7 ml water/ml matrix [34]). At this concentration, if each enzyme monomer can bind a molecule of the activator, there will be more binding sites than acetylglutamate molecules and about 40% of the enzyme will be in the active state (assuming a K_D of 0.15 mM for the enzyme-acetylglutamate complex). Since during state III respiration there is rapid extrusion of water from the mitochondrial matrix [34], the concentration of acetylglutamate in the water space may increase by two orders of magnitude. This may represent very extensive activation of the enzyme in short periods of time.

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References

1 Rajman, L and Jones, M E (1976) Arch. Biochem Biophys 175, 270—278
2 Meijer, A J (1979) Trends Biochem Sci 4, 83—86
3 Shih, V E (1976) in The Urea Cycle (Grisolía, S , Báguena, R and Mayor, F , eds), pp 367—414, John Wiley & Sons, New York
4 Thaler, M M (1979) in Reye's Syndrome (Crocker, J F S , ed), pp 115—137, Grune and Stratton, New York
5 Khatra, B S , Smith, R B III, Millikan, W J , Sewell, C W , Warren, W D and Rudman, D (1974) J Lab Clin Med 84, 708—715
6 Novoa, W and Grisolía, S (1964) Biochim Biophys Acta 85, 274—282
7 Guthorlein, G and Knappe, J (1968) Eur J Biochem 7, 119—127
8 Nicoletti, M , Guerri, C. and Grisolía, S (1977) Eur J Biochem 75, 583—592

9 Knecht, E , Hernandez-Yago, J , Wallace, R and Grisolía, S (1979) J Histochem Cytochem , 27, 975—981
10 Schimke, R T (1975) Methods Enzymol , 40, 241—266
11 Bergmeyer, H U (ed), (1974) Methods of Enzymatic Analysis Vols II and IV, Academic Press, New York
12 Caravaca, J and Grisolía, S (1960) J Biol Chem., 235, 684—693
13 Clarke, S (1976) J Biol Chem , 251, 950—961
14 Clarke, R G and Howlett, G J (1979) Arch Biochem Biophys , 195, 235—242
15 Pollet, R.J., Haase, B.A and Standaert, M L (1979) J Biol Chem., 254, 30—33
16 Martin, R G and Ames, B N (1961) J Biol Chem , 236, 1372—1379
17 Cappugi, G , Manao, G , Camici, G and Ramponi, G (1980) J Biol Chem , 255, 6868—6874
18 Edelhoch, H (1967) Biochemistry, 6, 1948—1954
19 Levine, L (1978) in Handbook of Experimental Immunology (Weir, D M , ed), pp 5B 1—5B 8, Blackwell Scientific Publ , Oxford
20 Siegel, L M and Monty, K J (1966) Biochim Biophys Acta, 112, 346—362
21 Rowe, A J (1978) in Techniques in Protein and Enzyme Biochemistry, B105a, Elsevier/North-Holland, County Clare, Ireland
22 Lusty, C (1978) Eur J Biochem., 85, 373—383
23 Strahler, J (1974) Ph D Thesis, University of Wisconsin
24 Jones, M E and Spector, L (1960) J Biol Chem., 235, 2897—2901
25 Britton, H G , Rubio, V and Grisolia, S (1979) Eur J. Biochem , 102, 521—530
26 Rubio, V and Grisolia, S (1977) Biochemistry, 16, 321—329
27 Elliott, K R F and Tipton, K F (1973) FEBS Lett 37, 79—81
28 Elliott, K R F and Tipton, K F (1974) Biochem J , 141, 817—824
29 Marshall, M and Cohen, P P (1961) J Biol Chem , 236, 718—724
30 Trotta, P P , Pinkus, L M , Haschemeyer, R H and Meister, A (1974) J Biol Chem , 249, 492—499
31 Nuzum, C T and Snodgrass, P J (1976) in The Urea Cycle (Grisolía, S , Baguena, R and Mayor, F , eds), pp 325—349
32 Loud, A V (1968) J Cell Biol , 37, 27—46
33 Mc Givan, J D , Bradford, N M and Mendes-Mourao, J (1976) Biochem J , 154, 415—421
34 Srere, P A (1980) Trends Biochem. Sci 5, 120—121
35 Mori, M and Cohen, P P (1978) J Biol Chem , 253, 8337—8339
36 Marshall, M , Metzenberg, R L and Cohen, P P (1961) J Biol Chem , 236, 2229—2237
37 Kennedy, J and Grisolía, S (1965) Biochim Biophys Acta, 96, 102—113
38 Grisolia, S and Cohen, P P (1953) J Biol Chem , 204, 753—757