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Molecular Characteristics of Chicken Liver Arginase

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A purification procedure for the preparation of chicken liver arginase in a homogeneous form is presented. The enzyme hydrolyses both arginine and argininic acid. Kinetic analysis reveals that the enzyme binds arginine when the amino group is protonated or unprotonated; however, the unprotonated form seems to be hydrolysed more rapidly. The enzyme exchanges with the medium approx. 1.6 Mn^{2+} ions per molecule.

Mora *et al.* (1965*b*) have classified liver arginases into two types, the ureotelic and the uricotelic ones. The ureotelic arginases have mol.wts. of approx. 130000 and a K_m for arginine that is lower than 10mM; the uricotelic arginases have mol.wts. of approx. 280000 and a K_m for the substrate that is of the order of 100mM.

This large difference in the K_m for the substrate must have a metabolic significance, since it may affect the efficiency of the enzyme in the hydrolysis of endogenous arginine. In fact ureotelic arginases have their main function in the urea cycle, whereas the function of the uricotelic arginases is unknown and their efficiency in hydrolysing endogenous arginine is dubious. They have been considered as vestigial enzymes that are a remnant of an ureotelic stage in the development of uricotelic animals (Mora *et al.*, 1965*a*). Chickens normally possess an uricotelic liver arginase; however, we have found that some starved strains develop, beside the old one, a new liver arginase which, from the properties mentioned above, can be classified as of the ureotelic type (Rossi & Grazi, 1969; Grazi *et al.*, 1969). We have therefore started a study on the relationships between these two arginases to establish whether the protein that is formed in the mutant starved animal is synthesized *de novo* or is produced through a modification of the wild-type arginase.

In the present paper we describe a procedure for the purification of the uricotelic arginase in a homogeneous form and some of its properties.

Materials and Methods

Animals

White Leghorn chickens 2–3 weeks old were purchased from the Stazione Sperimentale di Pollicultura, Rovigo, Italy.

Methods

Arginase assay. The assays were performed at 37°C and pH9.5 (Rossi & Grazi, 1969). With arginine as substrate no buffer was employed; with argininic acid 0.05M-triethylamine–HCl buffer was added. The incubation time was 10min. Ornithine and urea that formed in the course of the reaction were determined by the colorimetric methods of Chinard (1952) and Archibald (1945) respectively. One enzyme unit was defined as the amount of the enzyme that catalyses the formation of 1μmol of either ornithine or urea/h at 37°C. The specific activity is expressed as units/mg of protein. All the rates are expressed as the $V_{max.}$ obtained by extrapolation to infinite substrate concentration.

Protein determination. Throughout the purification procedure protein was measured by the turbidimetric method of Bücher (1947). With the pure enzyme, protein concentration was usually determined by measuring the E_{210} in 0.05M-tris–HCl buffer, pH7.5. Under the above conditions (light-path 1cm) the extinction of 1mg of the pure enzyme/ml was 26 at 210nm and 2.2 at 340nm from the method of Bücher (1947).

Electrophoresis. Disc-gel electrophoresis by the method of Ornstein & Davis (1961) was used both for analytical and for preparative purposes. Electrophoresis was done for 2.5h at 2°C in 7.5% cross-linked polyacrylamide gel polymerized in 13mm × 0.4mm tubes. The current was 5mA/tube. For analytical purposes 20–100μg of protein was used. Gels were prepared at pH9.0; the buffer was 0.2M-glycine–0.025M-tris–HCl, pH9.0. Protein bands were detected by staining with Amido Schwarz purchased from Carlo Erba, Milan, Italy.

For preparative purposes, before the addition of the protein samples, the tubes were submitted to electrophoresis for 30min in 0.05M-tris–HCl buffer, pH7.0, followed by 30min in 0.05M-maleate–0.05M-$MnCl_2$ buffer, pH7.0. Protein (0.5–1mg/tube) was then added and the electrophoresis was performed for 2.5h with a new solution of 0.05M-maleate–0.05M-$MnCl_2$ buffer, pH7.0. At the end of the run, the gels were sliced (every slice was 0.4cm wide), and the protein was eluted with 0.05M-tris–HCl buffer,

pH6.8, by homogenization in a Potter–Elvehjem apparatus.

Amino acid analysis. Protein was hydrolysed in 6M-HCl for 24h at 110°C in evacuated and sealed tubes. The hydrolysate was evaporated to dryness five times from water and finally dissolved in citrate buffer, pH2.2 (Moore & Stein, 1954). Cysteine was determined as cysteic acid after performic acid oxidation (Schram *et al.*, 1954). The amino acid analysis was performed in a Beckman model 120 B analyser (Spackman *et al.*, 1958).

Tryptophan was determined by the bromosuccinimide method (Funatsu *et al.*, 1964) and by the method of Goodwin & Morton (1946). The contribution of light-scattering to the E_{280} was determined by linear extrapolation of the base-line in the 380–320nm region into the 280nm region.

The dry-weight protein content of the pure enzyme solutions was calculated by relating the E_{210} and the tyrosine content (evaluated by the method of Goodwin & Morton, 1946) to the tyrosine content of 100000g of protein as determined from amino acid analysis. With this procedure the E_{210}/mg of protein is 26 with light-path 1cm.

$^{54}Mn^{2+}$-binding experiments. The enzyme to be used for binding experiments with $^{54}Mn^{2+}$ was dialysed for 3h against two changes of 5mM-Na_2CO_3–$NaHCO_3$ buffer, pH9.8. At this pH dialysis of arginase in the absence of Mn^{2+} does not decrease the catalytic activity of the enzyme. Further, dialysis under the conditions described above allows at least a 10000-fold dilution of unlabelled unbound Mn^{2+} present in the enzyme solution, as was established by using $^{54}MnCl_2$.

Radioactivity determinations. These were done in a Packard Tri-Carb liquid-scintillation counter in 10ml of Bray's (1960) solution. To the samples (usually 0.1–0.2ml) to be counted for radioactivity an equal volume of 99% formic acid was added.

Sucrose-density-gradient centrifugation. This was done as described by Martin & Ames (1961). The sucrose solutions used were prepared in 0.02M-triethanolamine–HCl buffer, pH7.5. The hydrolytic activity on argininic acid in the sucrose-density-gradient fractions was followed by measuring the formation of α-hydroxy-δ-aminovalerianic acid with the ninhydrin test (Rosen, 1957). DEAE-cellulose (Whatman DE11, nominal capacity 1.0mequiv./g) was purchased from W. and R. Balston, Maidstone, Kent, U.K.

Results

Purification procedure (*Table* 1)

Purification was performed at 2°C unless otherwise indicated.

First 20 chicken livers (59g) were homogenized for 3min at 2°C with 200ml of 0.15M-KCl–5mM-$MnCl_2$. The homogenate (230ml) was centrifuged for 1h at 12000*g*. The supernatant was discarded. The precipitate was homogenized in a Waring Blendor for 3min with 300ml of acetone chilled to −20°C. The resulting suspension was filtered on a large Buchner funnel. The residue was again homogenized in the Waring Blendor for 3min as before. The acetone-dried powder was extracted for 30min at 2°C with 200ml of 0.1M-triethanolamine–HCl buffer, pH7.5, containing 5mM-$MnCl_2$. The suspension was centrifuged for 15min at 12000*g* and the supernatant was collected (vol. of extract, 195ml).

Table 1. *Purification procedure of the uricotelic arginase from chicken liver*

Experimental details are given in the text.

			Enzyme activity				
			Arginine as substrate		Argininic acid as substrate		
Fraction	Volume (ml)	Protein (mg)	(units)	(units/mg) (A)	(units)	(units/mg) (B)	Ratio (B)/(A)
Homogenate	230	11000	5700	0.52	8550	0.78	1.50
Acetone-dried powder extract	195	3000	2800	0.93	5460	1.82	2.00
DEAE-cellulose chromatography	109	120	2240	18.6	5040	42	2.26
Methanol precipitation	25	29	2100	70	3820	131	1.87
Heat step	25	21	2040	98	3700	180	1.83
Ammonium sulphate and dialysis	1.1	9.1	2100	230	3920	430	1.86
Disc-gel electrophoresis	3	1.3	1050	810	1000	760	0.94

DEAE-cellulose chromatography. The extract was diluted with an equal volume of water and passed through a DEAE-cellulose column (2.6cm×12cm; 60ml) equilibrated with 10mM-triethanolamine–HCl buffer, pH6.8, containing 2mM-$MnCl_2$. The column was washed with 100ml of the same buffer solution, followed by 100ml of 60mM-NaCl–5mM-$MnCl_2$–50mM-triethanolamine–HCl buffer, pH6.8. The elution was continued with a linear gradient from 0.1M-NaCl – 5mM-$MnCl_2$ – 0.05M-triethanolamine – HCl buffer, pH6.8 (140ml) to 0.34M-NaCl–5mM-$MnCl_2$–0.05M-triethanolamine–HCl buffer, pH6.8 (140ml). The flow rate was 2ml/min; 5ml fractions were collected. Most of the activity was eluted approx. between 0.20M- and 0.28M-NaCl (vol. of DEAE fraction, 109ml).

Methanol fractionation. To the eluate (109ml) was added 5ml of 1M-$MnCl_2$ followed by 11ml of 0.1M-tris–HCl buffer, pH9.5 to adjust the pH to 7.5. The solution was treated with 100ml of a cold (−20°C) solution of methanol–1M-$MnCl_2$ (19:1, v/v). The precipitate was collected by centrifugation at −14°C and extracted for 30min with 25ml of 0.1M-maleate–0.1M-$MnCl_2$ buffer, pH7.0. The supernatant was collected by centrifugation (vol. of methanol fraction, 25ml).

Heat step. The protein solution was heated for 10min at 45°C. The supernatant was collected by centrifugation. Before the heat step the protein concentration should not exceed 5mg/ml; if dilution was necessary it was done with water (vol. of heat-step fraction, 25ml).

Ammonium sulphate fractionation and dialysis. The heat-step fraction (25ml) was treated with 5.65g of solid $(NH_4)_2SO_4$. The turbid suspension was centrifuged; the precipitate was discarded and to the supernatant was added 3g of solid $(NH_4)_2SO_4$. The precipitate was collected by centrifugation and dissolved in the minimum amount of cold 5mM-maleate–5mM-$MnCl_2$ buffer, pH7.0. The solution was then dialysed for 3h against the same buffer (vol. of ammonium sulphate fraction, 1.1ml).

Disc-gel electrophoresis. The ammonium sulphate fraction was subjected to electrophoresis for 2.5h at pH7.0 in 18 tubes as described in the Materials and Methods section. At the end of the run the gels were sliced, the slices from corresponding positions were pooled, and to each fraction 3ml of 0.05M-tris–HCl buffer, pH6.8, was added. Extraction was done by homogenization in a Potter–Elvehjem apparatus, then the gel was separated by centrifugation and the supernatant was collected. Enzymic activity was usually found between fractions 5 and 6, with an overall recovery of 80% (vol. of disc-gel fraction, 3ml).

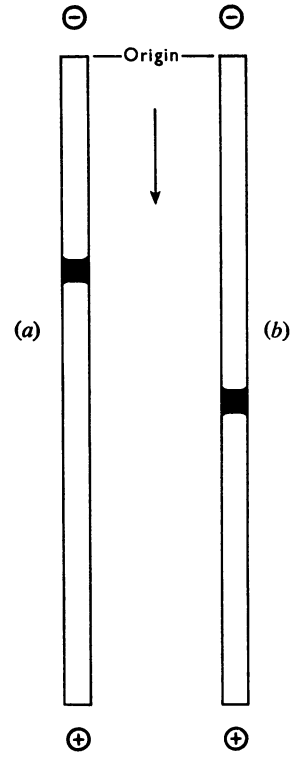

Fig. 1. *Polyacrylamide-gel electrophoresis of the uricotelic chicken liver arginase*

(*a*) At pH7.0; (*b*) at pH9.0. Electrophoresis was performed as described in the Materials and Methods section; 50μg of pure enzyme was employed.

Enzyme properties

Criteria of purity. All the enzyme preparations used in the experiments reported below showed a single protein band on disc-gel electrophoresis at both pH7.0 and 9.0 (Fig. 1).

Argininic acid as substrate for arginase. Chicken liver homogenate catalyses the hydrolysis of both argininic acid and arginine, the ratio between the two activities under these assay conditions being about 1.5. During the purification procedure the ratio rises to 2.26. After disc-gel electrophoresis the two activities are still linked, the ratio being 0.94 (Table 1).

This slight variation in the ratio between the two catalytic activities could be an indication that they are linked to two different proteins. This conclusion, however, is not supported by the observation that the ratio is fairly constant throughout all the fractionation procedures of high resolving power that were employed, such as DEAE-cellulose chromatography, sucrose-density-gradient centrifugation and disc-gel electrophoresis (Fig. 2). The different ratios between

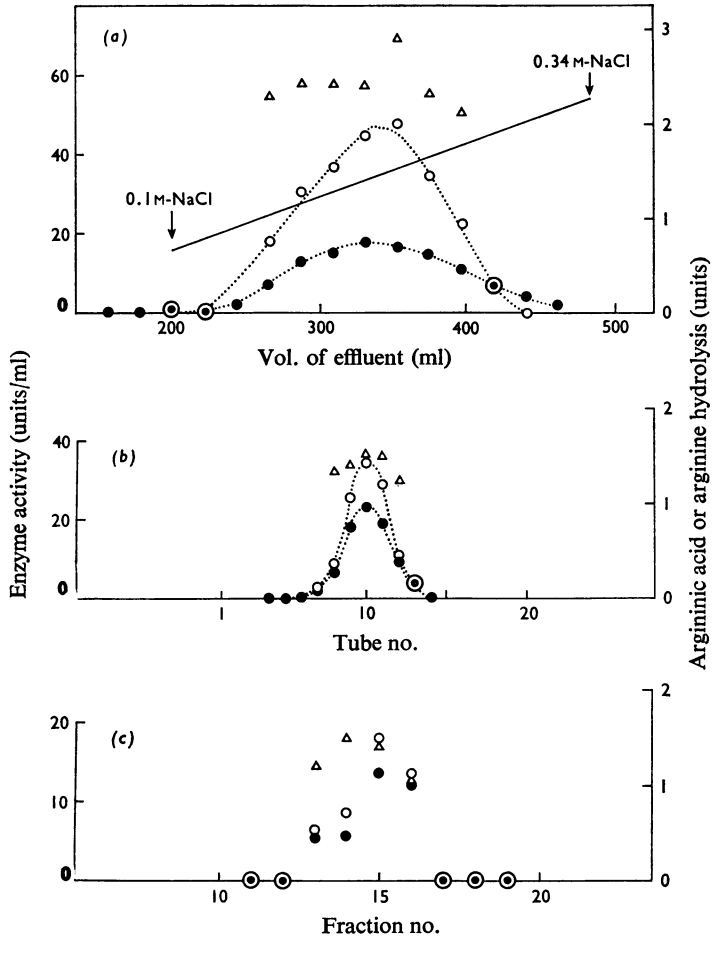

Fig. 2. *Ratios of the rate of argininic acid hydrolysis to the rate of arginine hydrolysis*

Arginase activity (units/ml) with arginine as substrate (●) and with argininic acid as substrate (○); rate of argininic acid hydrolysis/rate of arginine hydrolysis (△). (*a*) Elution pattern of arginase from DEAE-cellulose column (for the experimental details see 'Purification procedure'). (*b*) Sedimentation pattern of arginase on sucrose-density-gradient centrifugation. Ammonium sulphate fraction (0.1 mg; specific activity 230 units/mg of protein) in 0.1 ml of 5 mM-maleate–5 mM-$MnCl_2$ buffer, pH 7.0, was layered on a sucrose gradient. After 14 h of centrifugation at 36000 rev./min at 10°C the gradient was fractionated into 0.16 ml fractions (meniscus at tube no. 28) and analysed. (*c*) Electrophoretic pattern of arginase in polyacrylamide gel, pH 9.0. Ammonium sulphate fraction (0.1 mg; specific activity 230 units/mg of protein) was subjected to disc-gel electrophoresis at pH 9.0 as described in the Materials and Methods section. After 2 h (current 2 mA), the gel was fractionated and analysed.

the two activities in the various steps of the purification procedure can therefore most probably be explained by partial inactivation rather than by separation of two different enzymic proteins. This conclusion is also substantiated by the fact that during preparative disc-gel electrophoresis at pH 7.0 (Table 1), a step in which the ratio falls from 1.86 to 0.94, both activities are superimposed to form a single peak of two fractions, and no other fraction is catalytically active.

Kinetic properties. The K_m of arginase for arginine and argininic acid at pH 9.5 and 37°C varies from preparation to preparation between 30 and 100 mM, the K_m values for the two substrates being very close to each other.

A study on the influence of pH on both $V_{max.}$ and

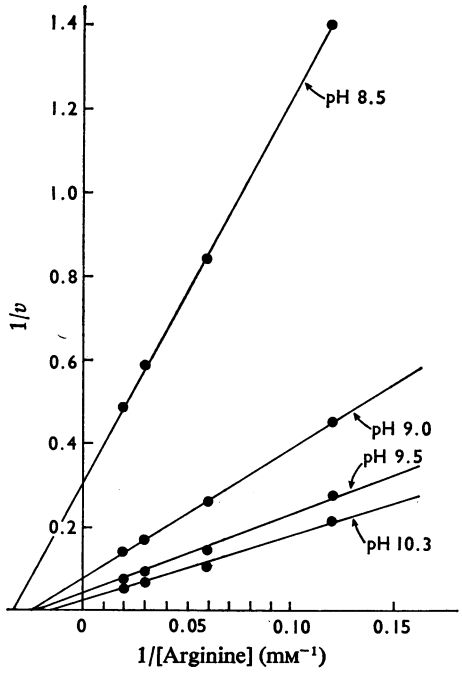

Fig. 3. *Double-reciprocal plot of initial velocity against arginine concentration at a series of different pH values*

The samples (0.6ml) contained 2μg of arginase (specific activity 800units/mg of protein), 0.02M-tris–HCl buffer and arginine as indicated. The temperature was 37°C. Initial velocity (v) was expressed as nmol of ornithine formed/min.

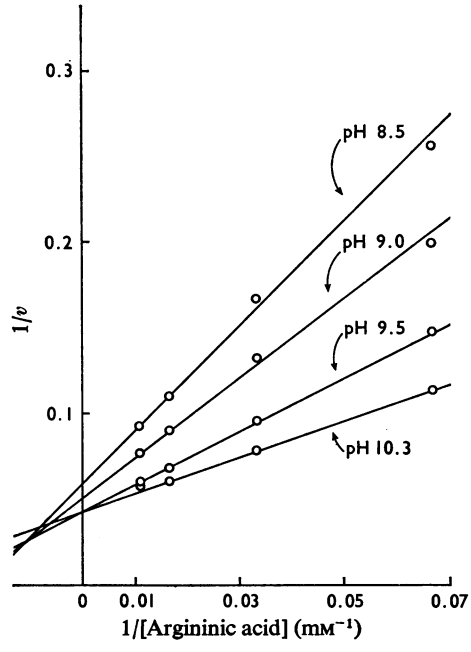

Fig. 4. *Double-reciprocal plot of initial velocity against argininic acid concentration at a series of different pH values*

The samples (0.6ml) contained 2μg of arginase (specific activity 800units/mg of protein), and 0.01M-tris–HCl plus 0.01M-Na_2CO_3–$NaHCO_3$ buffer and argininic acid as indicated. The temperature was 37°C. Initial velocity (v) was expressed as nmol of urea formed/min.

the K_m was performed and the results are reported in Figs. 3, 4, and 5. As Fig. 5 shows, with arginine as substrate the $V_{max.}$ increases by a factor of 14 from pH8.5 to 10.3, and the pK_m decreases from 1.52 to 1.16 in the same pH range. With argininic acid as substrate the situation is completely different: the $V_{max.}$ increases only by a factor of 1.5 between pH8.5 and 10, whereas the pK_m increases from 1.28 to 1.62 in the same pH range.

Amino acid analysis. The results of the amino acid analysis of the uricotelic chicken liver arginase are reported in Table 2. The values are expressed as mol of amino acid/100000g of protein. The tryptophan content was determined as described in the Materials and Methods section and is referred to the content of other amino acids by making use of the tyrosine/tryptophan ratio calculated by the method of Goodwin & Morton (1946). In Table 2 is also reported for comparison the amino acid composition of rat liver arginase (Hirsch-Kolb & Greenberg, 1968) which is a basic protein (Schimke, 1964) in contrast with chicken liver arginase, which is a very acidic protein as shown by its behaviour on DEAE-cellulose chromatography.

Mn^{2+} binding experiments. The binding of $^{54}Mn^{2+}$ to the enzyme was studied at pH7.5 and 20°C by allowing radioactive Mn^{2+} of the medium to equilibrate with the enzyme. Preliminary experiments (Fig. 6) had shown that 12h of incubation at 20°C were sufficient to allow complete equilibration and that during this time no changes in the catalytic activity occurred. In the experiments reported in Table 3 the number of Mn^{2+} ions bound to the enzyme at equilibrium was measured and found to reach the average value of 0.73 Mn^{2+} ion/100000g of protein. In the same experiments we have also shown that increasing the concentration of $^{54}MnCl_2$ from 0.1 to 0.4mM does not increase the extent of the binding significantly. This means that the dilution of radioactive Mn^{2+} by the unlabelled Mn^{2+} that is eventually left in the medium after the dialysis is a negligible factor.

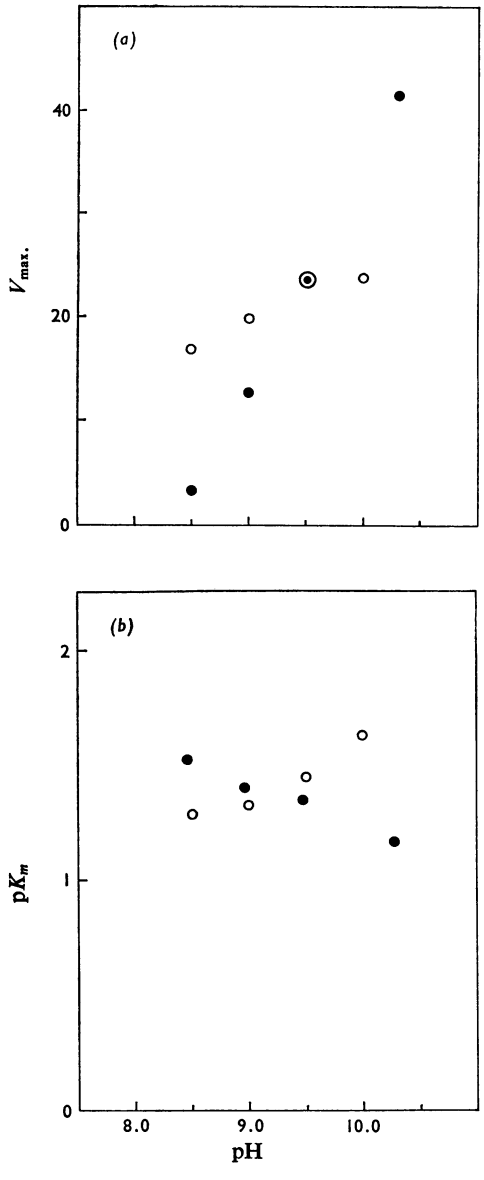

Fig. 5. *Secondary plots from the results in Figs. 4 and 5*

(*a*) $V_{max.}$ as a function of the pH; (*b*) pK_m as a function of the pH. ●, Arginine as substrate; ○, argininic acid as substrate.

Discussion

The uricotelic arginase from chicken liver has been purified 1600-fold. The preparation is homogeneous in polyacrylamide-gel electrophoresis at both pH7 and 9. Liver arginase activity is quite variable; the specific activity of the acetone-dried powder extracts ranges from 0.4 to 2 in different preparations. Treatment of the acetone-dried powder with 0.1M-triethanolamine–HCl buffer, pH7.5, containing 5mM-$MnCl_2$ allows the extraction of about 50% of the arginase activity. Higher recoveries are obtained when extraction is performed at higher pH values and higher ionic strength; however, in this case, protein is extracted that aggregates when the ionic strength is lowered, causing the precipitation of arginase and serious losses of the enzyme during the purification procedure.

After disc-gel electrophoresis more than 80% of the catalytic activity is recovered. However, since only the fraction with the highest activity is used for the experiments, the actual recovery is about 50%, as is shown in Table 1.

The enzyme is active with both arginine and argininic acid. The K_m for the two substrates is not significantly different and varies between 30 and 100mM from preparation to preparation, the K_m being independent of added Mn^{2+}. The reason for the phenomenon is unknown.

The analysis of activity versus pH confirms, with arginine as substrate, the classical results of Roholt & Greenberg (1956) but the slight modification of

Table 2. *Amino acid composition of the uricotelic chicken liver arginase as compared with rat liver arginase*

The results for rat liver are quoted from Hirsch-Kolb & Greenberg (1968).

Amino acid	Composition (mol/100000g of arginase)	
	Chicken liver	Rat liver
Lysine	49.6	73.9
Histidine	15.5	19.5
Arginine	47.1	27.5
Aspartic acid	94.7	73.7
Threonine	41.6	57.7
Serine	50.1	50.8
Glutamic acid	123.5	73.7
Proline	47.0	60.2
Glycine	74.0	76.2
Alanine	74.1	54.2
Half-cystine	17.7	6.1
Valine	66.6	73.6
Methionine	15.5	10.8
Isoleucine	38.6	43.2
Leucine	80.1	60.2
Tyrosine	23.1	20.3
Phenylalanine	30.0	26.2
Tryptophan	10.6	10.3

the pK_m for arginine as a function of the pH does not allow, in our case, the inference that the active form of the substrate is the zwitterion form of arginine, as postulated by Greenberg (1960). It is helpful in this respect to study the behaviour of the enzyme with argininic acid as substrate. Kinetic analysis reveals that between pH8.5 and 10 the pK_m for argininic acid increases from 1.28 to 1.62. The increase must be exclusively enzyme-dependent as argininic acid possesses only the carboxylate (pK2.17) and the guanidinium (pK12.48) ions. In the same pH range, on the contrary, the pK_m for arginine decreases from 1.52 to 1.16. This pH effect must therefore be explained by two functionally opposite phenomena: (*a*) a change in the state of ionization of the enzyme; (*b*) a change in the state of ionization of the substrate. If we now assume that effect (*a*) is independent of the nature of the substrate employed, we should be able to estimate effect (*b*) by subtracting the positive increment of pK_m for argininic acid from the negative increment of the pK_m for arginine. The difference is $-0.68pK$ unit in the range pH8.5–10, whereas it should be $+0.94pK$ unit if only arginine with the unprotonated amino group could be bound to the enzyme. It is therefore likely that both forms of arginine can be bound to the enzyme, perhaps with different association constants.

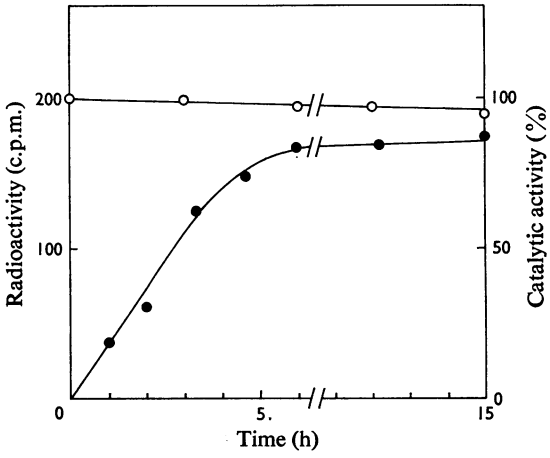

Fig. 6. *Rate of the equilibration process of $^{54}Mn^{2+}$ with arginase*

The incubation mixtures (1ml) contained arginase (0.3mg; specific activity 750units/mg of protein), dissolved in 0.7ml of 5mM-Na_2CO_3–$NaHCO_3$ buffer, pH9.8, 0.04M-triethanolamine–HCl buffer, pH7.4, and 0.1mM-$^{54}MnCl_2$ (specific radioactivity 900c.p.m./nmol). The final pH was 7.5. At the times indicated the mixtures were subjected to filtration through Sephadex G-25 columns (1.2cm×30cm) equilibrated with 10mM-tris–HCl buffer, pH7.5. Fractions (1ml) were collected. Enzymic activity, protein concentration and radioactivity were measured as described in the Materials and Methods section. ●, Radioactivity; ○, % of the original catalytic activity.

Analysis of $V_{max.}$ as a function of the pH reveals that, between pH8.5 and 10, with arginine as substrate $V_{max.}$ increases by a factor of 10 whereas with argininic acid as substrate it increases only by a factor of 1.5. Since, for the reasons explained above, the increase in $V_{max.}$ for argininic acid must reflect exclusively changes in the state of ionization of the protein, we should conclude that the much higher increase in the $V_{max.}$ for arginine is mainly dependent on changes in the state of ionization of the amino group of the arginine bound to the enzyme and therefore that the zwitterion form of arginine is the form that is preferentially hydrolysed by the enzyme.

Chicken liver arginase slowly exchanges with the medium 0.73g-atom of Mn^{2+}/100000g of protein,

Table 3. *Binding of $^{54}Mn^{2+}$ to uricotelic chicken liver arginase*

The incubation mixtures (1ml) contained: arginase (specific activity 780units/mg), 0.2–0.4mg dissolved in 0.7ml of 5mM-Na_2CO_3–$NaHCO_3$ buffer, pH9.8; 0.04M-triethanolamine–HCl buffer, pH7.4 and 0.1–0.4mM-$^{54}MnCl_2$ (specific radioactivity 850c.p.m./mol). The final pH was 7.5. After 12h of incubation at 20°C the mixtures were subjected to filtration through Sephadex G-25 columns (1.2cm×30cm) equilibrated with 0.01M-tris–HCl buffer, pH7.5. Fractions (1ml) were collected. Enzymic activity, protein concentration and radioactivity were measured as described in the Materials and Methods section.

Expt. no.	Arginase (mg)	Equilibrium concn. of $^{54}MnCl_2$ (mM)	Mn^{2+} bound to the enzyme (c.p.m.)	(ng-ion)	Mn^{2+}/100000g of enzyme
1	0.30	0.1	235	0.27	0.9
2	0.30	0.1	142	0.16	0.53
3	0.27	0.1	165	0.19	0.70
4	0.36	0.4	255	0.30	0.83
5	0.42	0.4	264	0.31	0.73

which corresponds approximately to 1.6g-atoms of Mn^{2+}/molecule of enzyme. This value is calculated by assuming a mol.wt. of 220000 as estimated by sucrose-density-gradient centrifugation with aldolase as a standard (Rossi & Grazi, 1969). Mn^{2+} ions are tightly bound to the enzyme and cannot be removed by filtration through a Sephadex G-25 column. The association constant for the Mn^{2+}–enzyme complex must therefore be larger than $10^6 M^{-1}$ by analogy with the behaviour of other ligand enzyme complexes (Pontremoli *et al.*, 1968). At variance with the report of Hirsch-Kolb *et al.* (1971) on rat liver arginase, we have not been able to demonstrate a dependence of the catalytic activity on loosely bound Mn^{2+} ions. Filtration through Sephadex G-25 columns in the absence of Mn^{2+} does not decrease the catalytic activity whereas treatment with 1mM-EDTA leads to irreversible inactivation.

It is useful to compare further the properties of the uricotelic chicken liver arginase with those of rat liver arginase, which is one of the most carefully characterized of the mammalian liver arginases. The two enzymes have different intracellular distribution. Rat liver arginase, which is a basic protein, at low ionic strength is apparently bound both to nuclei and the microsomal fraction, whereas at the intracellular ionic strength it is very likely present as a soluble form in the cytoplasm (Rosenthal *et al.*, 1956). Chicken liver arginase, which is a very acidic protein, is, on the contrary a particulate enzyme. It is bound to mitochondria and nuclei (Grazi *et al.*, 1969) and can be satisfactorily extracted only by non-ionic detergents such as Triton X-100. Chicken liver arginase, being restricted to mitochondria and nuclei, could perform a selective hydrolytic action perhaps limited to a particular arginine pool. This could be necessary to avoid an indiscriminate hydrolysis of arginine, which is an essential amino acid for the chicken (Albanese, 1959). However, concentrating the arginase activity into a small area could locally increase the efficiency of the enzyme. The need for such a concentration can be appreciated by considering that the turnover number of chicken liver arginase (2970mol/mol of enzyme per min at 37°C and pH9.5) is significantly lower than the turnover number of rat liver arginase (327600mol/mol of enzyme per min at 25°C and pH9.5) (Hirsch-Kolb & Greenberg, 1968).

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