PREFERENTIAL HYDROLYSIS OF ENDOGENOUS ARGININE BY RAT LIVER ARGINASE.

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SUMMARY. It has been previously postulated that the enzymes that participate in urea biosynthesis should be physically linked. The possibility that arginase keeps a close functional relation with the arginine formation sites has been explored in rat liver homogenate by generating arginine from two sources: I) labelled citrulline and aspartic acid, and II) hippuryl arginine and carboxypeptidase B. The results obtained show that the former is preferentially hydrolysed, which is in accordance with the stated postulate.

Several instances of the integration of enzymes engaged in a multienzyme system in the form of protein aggregates have been well substantiated (1,2,). We have postulated that, in order to function efficiently, the urea cycle enzymes should be physically linked (3,4). Such assumption is based on the following considerations: a) the pools of arginine and ornithine are rather low in the liver of ureotelic animals (5), b) dietary arginine is required for some metabolic functions in ureotelic animals capable of arginine synthesis (6,7), c) the administration of radioactive citrulline to the rat causes significant incorporation of radioactive arginine into kidney proteins but not into liver proteins (8), d) the existence of arginase in addition to the enzymes responsible for arginine synthesis does not necessarily mean that the urea cycle is operating (9,10,11). Relevant to the situation stated in d) is the description of two molecular forms of arginase, one present in the liver of ureotelic animals and the other present in the liver of uricotelic animals and in N. crassa (12, 13).

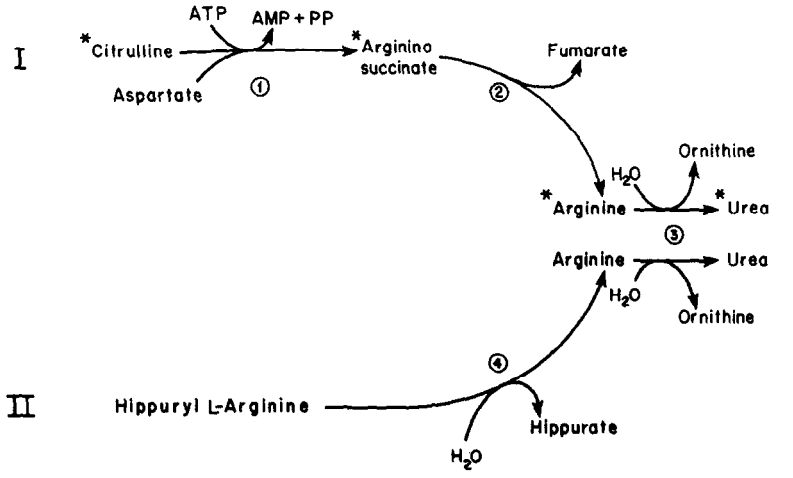
The most recent efforts of our laboratory have been devoted to the demonstration of the integration between arginase and the arginine formation sites. For this purpose we have studied the concomitant synthesis and hydrolysis of arginine in rat liver homogenates.

In order to learn if rat liver arginase is functionally coupled with the arginine formation sites, the hydrolysis of endogenous arginine, that is, the one generated from citrulline and aspartic acid (source I) has been explored in a system also containing "artificially nascent arginine", that is, generated by the carboxypeptidase B hydrolysis of hippuryl L-arginine (source II). To distinguish arginine produced by source I from that originated in source II, labelled citrulline was used (see scheme 1).

To carry out the experiment 1.2 ml. of 10% rat liver homogenate in water, 12 μmoles of ureido-C^{14} L-citrulline (specific activity 29,000 cpm/μmole), 20 μmoles of L-aspartic acid, 20 μmoles of ATP, 20 μmoles of $MgSO_4$, 12 μmoles of hippuryl L-arginine, 4 μg of carboxypeptidase B and 200 μmoles of potassium phosphate buffer, pH 7.0, in a final volume of 4 ml. were incubated at 37^0 during 45 minutes. Six tubes were simultaneously started by the addition of homogenate and carboxypeptidase and incubated during different periods of time. The reaction was stopped by the addition of 10 ml. of a saturated solution of picric acid.

The picric acid was removed by a small column of AG2X8 resin. The eluate was divided in two fractions one for the assay of arginine and the other for the assay of urea. The former was adjusted to pH 5.0 with NaOH and passed through a Dowex 50X2 column equilibrated with sodium citrate buffer, pH 5.0 More of this buffer was added to collect 25 ml. which were followed by the addition of 60 ml. of 0.001 N NaOH and then 20 ml. of 0.2 N NaOH. Urea and citrulline are eluted with the citrate buffer, hippuryl L-

SCHEME 1



Enzymes present in the liver homogenate: ①Arginino succinate synthetase ②Arginino succinase ③ arginase

Enzyme added: ④ Carboxypeptidase B

arginine is removed with the less concentrated NaOH and arginine is collected after the addition of the more concentrated NaOH. The absolute amount of arginine was measured by the Sakaguchi reaction (14), the radioactivity was determined by adjusting the sample to pH 7.0 with HCl, then the guanidino C of arginine was converted to CO_2 (by the action of purified arginase and urease) which was trapped in hyamine and counted in a liquid scintillation spectrometer. To the aliquot for urea determination 1.0 N HCl was added to give a final concentration of 0.1N; it was passed through a column of Dowex 50X8 resin equilibrated with 0.1N HCl. Elution was carried out with 4.0 N NaCl in 0.1N HCl. Under these conditions urea is eluted and citrulline, arginine and hippuryl L-arginine remain behind. Urea was measured as described by Koritz and Cohen (15), the radioactivity was appraised by adjusting the sample to pH 5.0 with NaOH, and converting the urea to CO_2 (by means of purified urease) which was handled as already indicated. Recoveries of arginine and urea were carried out

TABLE 1

Time (min.)	a) Citrulline source		b) Hippuryl L-arginine source		c) Distribution of arginine produced		d) Distribution of arginine not hydrolyzed.		e) Specific activity cpm/μmole	
	Arginine	Urea	Arginine	Urea	% from a)	%from b)	% from a)	%from b)	Arginine	Urea
7.5	0.049	0.10	0.196	0.47	18	82	20	80	5500	5150
15	0.061	0.27	0.205	1.05	20	80	23	77	6650	6150
25	0.035	0.40	0.160	1.55	20	80	18	82	5150	5790
35	0.012	0.52	0.136	1.95	20	80	8	92	2300	6300
45	0.003	0.47	0.090	1.85	20	80	3	97	1050	5950

The amount of arginine and urea originated from citrulline and from hippuryl L-arginine (columns a) and b) expressed as μmoles/ml produced from zero time), was inferred from the following data: amount of each metabolite at any given time, their specific activity (column e) and the specific activity of citrulline (see text).

and proved to be quite satisfactory.

The results, presented in table 1, show that during the first 35 minutes the production of arginine from both sources was linear as a function of time, arginine being generated from hippuryl L-arginine approximately four times faster; after 35 minutes the rates from both sources leveled off, but the ratio of arginine contributed by them was not altered. The rate of production of arginine is obtained from the arginine remaining and that converted to urea at any given time. During the first 25 minutes the hydrolysis of the arginine derived from citrulline was about 25% of that from the other source. However, after this time the arginine originated from citrulline was more rapidly hydrolyzed which causes a drop in the specific activity of arginine. It should be borne in mind that the contribution of labelled urea from the radioactive arginine hydrolysed from 25 to 45 minutes is not enough to be reflected in a corresponding increase in the specific activity of urea because of the amount of this metabolite already accumulated. When the convertion of citrulline to arginine and urea was studied in the absence of the hippuryl L-arginine source, the specific activity of the three metabolites was the same.

These results indicated that the arginine generated from citrulline and aspartic acid is preferentially hydrolyzed to that produced from a different source, which can be interpreted as meaning that arginase is more readily available for the disposal of the arginine generated in the arginine formation system (arginine succinate synthetase and arginine succinase).

The above interpretation is in accordance with the postulated physical integration of arginase with the arginine formation sites in the liver of ureotelic animals (3,4).

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