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Arginine as Competitive Inhibitor of Arginosuccinate Synthetase from Guinea Pig Liver*

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The influence of arginine on arginosuccinate synthetase (E.C. 6.3.4.5) from guinea pig liver has been investigated. The results show a competitive inhibition by arginine with respect to the three substrates of the enzime: L-citrulline, L-aspartate and ATP.

Arginosuccinate synthetase (E.C. 6.3.4.5) is a key catalyst of urea cycle and of arginine biosynthetic pathway in most organisms. This enzyme catalyzes the reaction of citrulline with aspartate in the presence of ATP, as energy source, to give arginosuccinate (5). It is known that pyrophosphate, a reaction product, inhibits the enzyme (4), and that the presence of pyrophosphatase prevents this inhibition and favours the displacement of the equilibrium of the reaction towards arginosuccinate formation. In addition several authors have reported inhibition of this enzyme by analogous structural substrates (10, 11) as well as inhibition by an excess of reaction substrates (6, 7, 12).

In this paper evidence is presented of the inhibition by arginine of arginosuccinate synthetase of guinea pig liver. This inhibition is competitive with respect to the three substrates of the enzyme, citrulline, aspartate and ATP.

In order to prevent interference by ATP-ase activity in the spectrophotometric measurement of the activity of arginosuccinate synthetase a partial purification of the enzyme has been carried out following the RATNER purification method (6,8) for the bovine liver enzyme.

The inhibition by arginine of arginosuccinate synthetase of guinea pig liver, described in this paper, could be explained, from the kinetic point of view, by chemical structural analogy between arginine functional groups and those of the substrates. On the other hand the arginine inhibition could also have a regulatory

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significance in the biosynthetic pathway of this aminoacid.

Materials and Methods

The enzymes and coenzymes used in the assays of activity or arginosuccinate synthetase were obtained from Boehringer Mannheim and Sigma. Other Chemicals were of reagent quality.

Crude preparation of arginosuccinate synthetase were obtained from livers of male guinea pigs weighing between 550 and 600 g. The animals were killed by cutting the carotides. The livers were extirpated immediately.

Homogenates were prepared in Braun Potter homogenizator. The partial purification of arginosuccinate synthetase was carried out according to the method of RATNER (6, 8) for bovine liver, but performing only the three first steps of the procedure, because this was sufficient to achieve an enzymatic purification grade for our purposes as the ATP-ase activity present in crude extracts was practically removed.

These purification steps were successively: extraction of acetone powder with 0.02 M potassium phosphate buffer pH 7.5; precipitation with ammonium sulfate in the range of 45 to 55 % of saturation and finally negative absorption in DEAE-cellulose equilibrated with 5 mM phosphate buffer, pH 7.9, followed by the separation of the enzyme, in the liquid phase, by gravity filtration.

The estimation of activity of arginosuccinate synthetase was carried out according to the method of RATNER (8) measuring the AMP formed in the enzymatic reaction by coupling with the systems myokinase-ATP, pyruvate kinase-PEP and NADH-lactate dehydrogenase.

The optimal reaction mixture contained: L-citrulline, 6 μmoles; L-aspartate, 15 μmoles; Tris-HCl pH 7.5, 150 μmoles; $MgCl_2$, 15 μmoles; KCl, 60 μmoles; ATP, 7.5 μmoles; PEP, 10 μmoles; myokinase, 2 units; pyruvate kinase, 3 units; lactate dehidrogenase, 4 units; pyrophosphatase, 2 units and NADH, 0.5 μmoles in a final volume of 3 ml.

The reaction rate was measured by the decrease in optical density (absorbancy) at 340 nm following the oxidation of NADH. The reaction starts by the addition of L-citrulline.

The amount of protein was determined by the method of LOWRY *et al.* (3).

Results

As shown in figure 1, arginosuccinate synthetase from guinea pig liver is inhibited by L-arginine. This inhibition is competitive with each of the reaction substrates. The inhibition constant (K_i), calculated from the double reciprocal plots of Lineweaver and Burk, were 0.75 mM with respect to L-citrulline, 1.8 mM in relation to L-aspartate and 6.34 mM against ATP. The apparent Michaelis constants (K_m) of arginosuccinate synthetase corresponding to L-citrulline, L-aspartate and ATP are respectively the

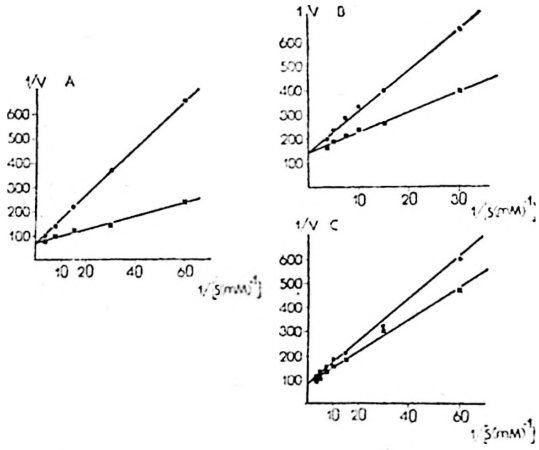

Fig. 1. *Competitive inhibition by arginine (2 mM) of arginosuccinate synthetase of guinea pig liver, with respect to L-citrulline (A), L-aspartate (B) and ATP (C).*

In all cases the points plotted represent the mean values of three assays.

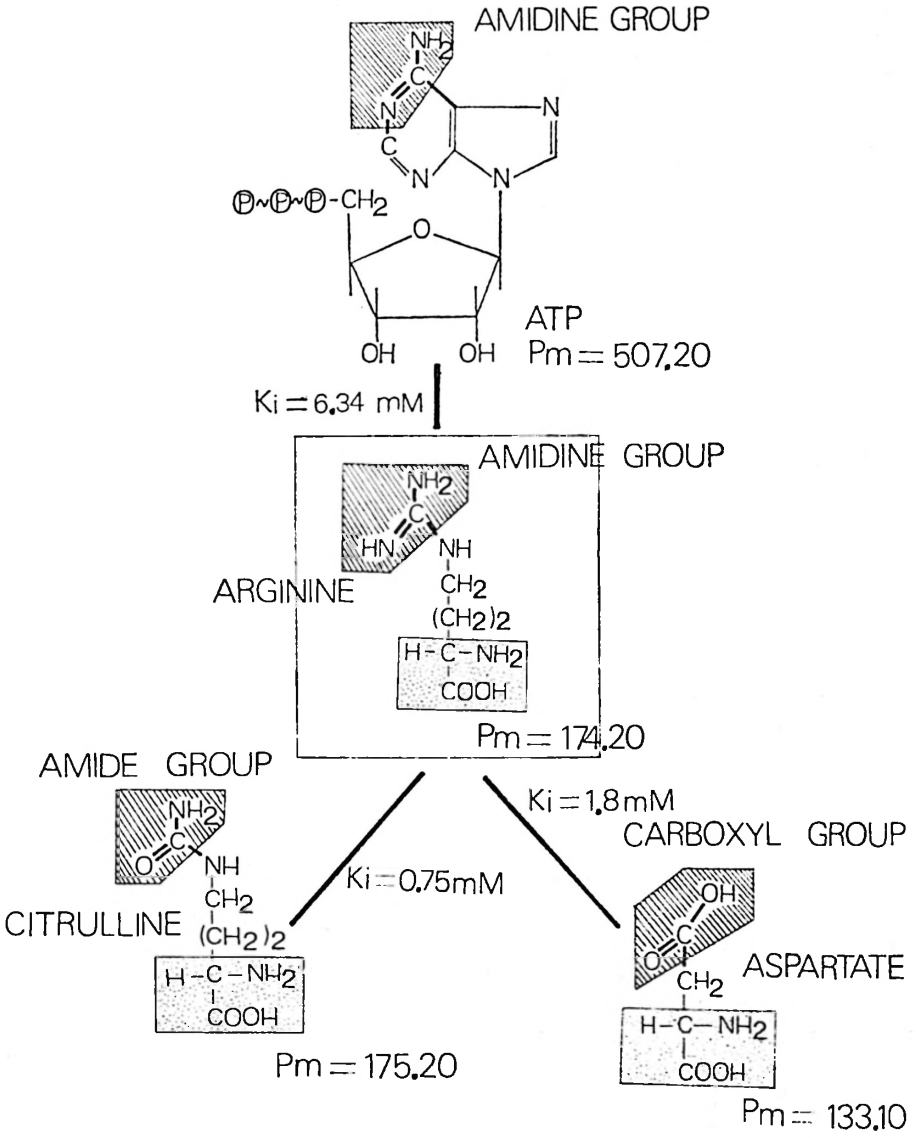

Fig. 2. *Structural relation between arginine functional groups and those of L-citruline, L-aspartate and ATP.*

following: 0.036 mM, 0.063 mM and 0.083 mM. These results have been adjusted by the method of least squares.

Discussion

The inhibition of the arginosuccinate synthetase by L-arginine could be explained, from a chemical point of view, by the structural analogy between arginine and some functional groups of the three substrate molecules. The scheme in figure 2 shows the possible similarity between some chemical groups of citrulline, aspartate and ATP which act as substrates for the enzyme and the inhibitor argi-

nine. This mentioned structural relation between inhibitor and the substrate molecules can be reinforced, due to the fact that the molecular weight of arginine is practically the same as that of citrulline and also of the same magnitude as aspartate. Taking into account the inhibition by arginine for the arginosuccinate synthetase from guinea pig liver described here, and also the high arginine K_m value for the arginase (9), we wonder whether L-arginine could be one of the main modulators of the urea cycle. This suggestion would be in agreement with the fact that in mammals the mentioned urea cycle has to be considered not only as a detoxication mechanism but also as a metabolic pathway of arginine biosynthesis. Since arginine is an important intermediate in the crossroad to both urea formation and protein biosynthesis, it can be accepted that the arginine concentration has to be accurately regulated in order to satisfy both metabolic possibilities and also to avoid the situation of arginine excess, known as argininemie (1, 2).

On the basis of the above mentioned, we can consider an extreme state in an animal with a high ammonia level. In this condition the arginine levels would be increased and this compound would inhibit the arginosuccinate synthetase activity, avoiding in this way an accumulation of this aminoacid. In addition, the high concentration of the accumulated arginine would also increase the velocity of the reaction catalysed by arginase, due to the rise of substrate concentration (considering the high arginine K_m value for arginase); this circumstance bringing about a fall in the arginine concentration to normal levels concomitantly with a decrease or a disappearance of the above mentioned inhibition. If the animal in this condition needs arginine for protein synthesis purposes, the concentration of this aminoacid could also diminish, with the rate of urea formation dropping and, in this way, a detoxication function takes place.

Resumen

Se investiga la influencia de la arginina sobre la arginosuccinato sintetasa (E.C. 6.3.4.5) de hígado de cobaya. Los resultados obtenidos ponen de manifiesto una inhibición por arginina competitiva con respecto a los tres sustratos de la enzima: L-citrulina, L-aspartato y ATP.

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