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# EVIDENCE FOR COOPERATIVE EFFECTS IN HUMAN LIVER ARGINASE

NELSON CARVAJAL, MARINA ACORIA, JUAN PABLO RODRÍGUEZ, MIREYA FERNÁNDEZ and JORGE MARTÍNEZ

*Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Casilla 15138, Santiago 11 (Chile)*

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**The reaction kinetics of human liver arginase (L-arginine amidinohydrolase, EC 3.5.3.1) in terms of arginine concentration is strikingly altered by varying the pH. Lowering the pH from the optimum (9.5) toward a more physiological value (7.5) there is a transition from hyperbolic to sigmoidal kinetics. The cooperative effects are observed in the presence and absence of the product ornithine. Dimers of arginase exhibit typical Michaelis-Menten kinetics even in the presence of ornithine. Dimer-dimer interactions are suggested to explain the kinetic properties of arginase at pH 7.5.**

In the past, the reaction catalyzed by human liver arginase (L-arginine amidinohydrolase, EC 3.5.3.1) was studied at pH 9.5. At this pH, which is optimum for arginase, hyperbolic kinetics was observed even in the presence of inhibitory amino acids [1,2]. Moreover, studies utilizing matrix-bound species revealed that at pH 9.5 subunit interactions have no effect on the catalytic aspects of the enzyme [3,4]. In the present paper we wish to report that at pH 7.5 cooperative effects are apparent in the response of the enzyme to the substrate arginine.

The molecular form of human liver arginase designated enzyme II by Bascur et al. [1] was used throughout. Purification and attachment of this enzyme to strips of nylon mesh ($2 \times 1$ cm) were performed as described [3]. Protein was estimated by the method of Lowry et al. [5].

Dissociation of both soluble and immobilised arginase into dimers was performed by incubation with 1 mM *p*-hydroxymercuribenzoate in 50 mM Tris-HCl (pH 8.0) for 30 min at 37°C. To remove the blocking reagent, dissociated species were incubated with 1 mM 2-mercaptoethanol for 30 min at 15°C [4].

Arginase activity was determined by measuring the formation of urea [6] from arginine. The buffers were 20 mM potassium phosphate (pH 7.5) and 50 mM glycine (pH 9.5). All the incubation mixtures contained 100 mM KCl and the reactions were initiated by adding the enzyme. Other details pertinent to the matrix-bound enzyme were given in a previous paper [3]. Before the assays, the enzymes were activated with 1 mM $MnCl_2$.

L-Arginine, L-ornithine, 2-mercaptoethanol, *p*-hydroxymercuribenzoate and Trizma Base were purchased from Sigma Chemical Co. Type 66 nylon was obtained from a local industry and glutaraldehyde from Matheson Coleman and Bell. All other chemicals were of analytical grade.

Studies with soluble arginase. Fig. 1 shows the results obtained when the reaction catalysed by arginase was studied at pH 7.5 in the presence and absence of ornithine. It is seen that the lines in the double reciprocal plot are curved rather than linear and that all the lines shares a common intercept in the $1/v$ axis. The inhibition by ornithine is then competitive and positive cooperativity for the substrate is apparent at this pH. The Hill coefficient [7] changed from 1.21 in the absence of

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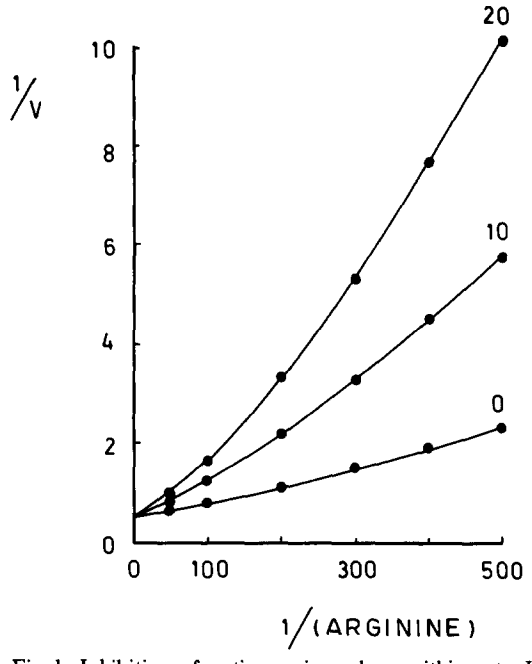


Fig. 1. Inhibition of native arginase by ornithine at pH 7.5. Velocity is expressed as μmol urea produced per min and arginine concentrations in molar units. The numbers on the lines express the concentrations of ornithine in millimolar units.

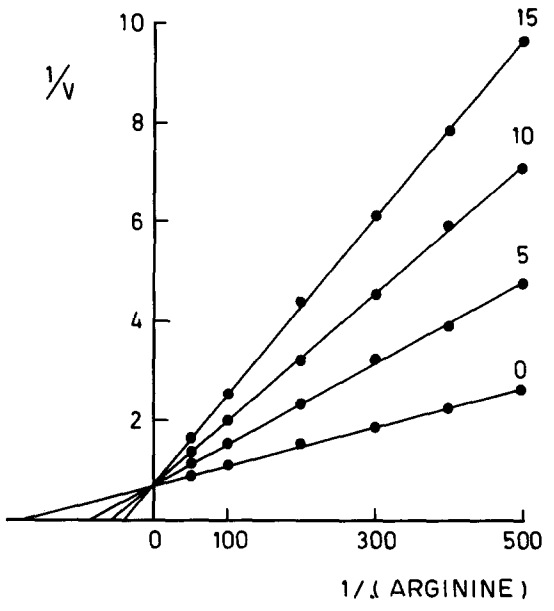


Fig. 2. Inhibition of *p*-hydroxymercuribenzoate-treated, dimeric arginase by ornithine at pH 7.5. Velocity is in μmol urea formed per min and arginine concentrations in molar units. The concentrations of ornithine (numbers on the lines) are in millimolar units.

ornithine to 1.34 in the presence of 20 mM ornithine. Confirming previous information [1,2], hyperbolic kinetics was observed at pH 9.5 even in the presence of ornithine.

We have shown that *p*-hydroxymercuribenzoate dissociates arginase into active dimers which reassociate when the reagent is removed with 2-mercaptoethanol [4]. Fig. 2 shows that the chemically modified, dimeric arginase exhibits hyperbolic kinetics even in the presence of the product. Moreover, the slopes of the lines in this figure were linear functions of inhibitor concentrations, as one would expect for a linear competitive inhibitor. As occurs with the native enzyme, the reassociated species showed sigmoidal kinetics.

Studies with immobilised arginase. Immobilised arginase was used to analyze the kinetic behavior of unmodified dimers. For this purpose, matrix-bound tetrameric arginase was dissociated with *p*-hydroxymercuribenzoate and then the blocking reagent was removed with 2-mercaptoethanol [4]. The transition from tetramers to dimers was associated with a change from sigmoidal to hyperbolic kinetics at pH 7.5. The kinetic behavior of the dimers was the same before and after the addition of 2-mercaptoethanol.

In contrast with the results obtained at pH 9.5 we have observed that a positive cooperativity is apparent in the action of arginase at pH 7.5. The kinetic behavior at pH 7.5 is related to the structural state of the enzyme. In fact, when arginase is dissociated into dimers there is a transition from sigmoidal to hyperbolic kinetics.

Studies of the control of oligomeric enzymes have emphasized the importance of subunit interactions [8,9]. A valuable piece of evidence to indicate that such effects are involved is the change from allosteric kinetics to the more usual hyperbolic function when structural changes are induced in the enzyme. The results obtained with arginase dissociated by *p*-hydroxymercuribenzoate are then an indication that subunit interactions would play a role in the functional aspects of the enzyme. The requirement of two dimers to associate in order to express cooperative effects for the substrate is of

interest in connection with the $D_2$ symmetry we have suggested for arginase [4]. This would explain the relatively low values we have found for the Hill coefficients.

Effects of the allosteric type were also suggested for arginase from other sources. Kaysen and Strecker [10] found that rat kidney arginase is activated by high concentrations of arginine and that the activation disappears in the presence of lysine. From these observations, they suggested a second binding site for arginine and certain amino acids which when occupied modifies the catalytic site and which is dependent on pH. Bedino [11] observed that in the presence of ornithine bovine liver arginase shows sigmoidal kinetics and suggested the allosteric regulation of the enzyme by the product. In contrast with human liver arginase, the enzyme from bovine liver shows sigmoidal kinetics at pH 9.5 and only in the presence of ornithine. The different sources of the enzymes would explain these differences and those found with arginase of rat kidney. In any case, it is clear that effects of the allosteric type would be significant in the catalytic aspects of arginase. The exact nature and the possible physiological significance of these effects have to be analyzed by further experiments. Studies addressed to this point are now being undertaken in this laboratory.

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