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Arginase inhibition by dialysable factor(s) from chick liver

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Chimica biologica. — *Arginase inhibition by dialysable factor(s) from chick liver* (*). Nota di GENNARO DELLA PIETRA, MARIO SOSCIA e RICCARDO RAVA, presentata (**) dal Corrisp. F. CEDRANGOLO.

RIASSUNTO. — Viene studiata la cinetica dell'inibizione dell'enzima arginasi da parte di un fattore, non ancora chimicamente definito, presente nel dializzato di fegato di pulcino. I risultati ottenuti dimostrano che l'inibizione è di tipo competitivo.

INTRODUCTION.

In previous papers [1, 2, 3] the inhibition by factor(s) from chick and pigeon liver on arginase activity has been studied: the inhibitor from chick liver was shown to be thermostable when heated for 10′ at 90° C, not extractable from chick liver acetone powder by organic solvents like chloroform or ethyl-ether and dialysable [2]. Further, the inhibitor obtained by dialysis from pigeon liver homogenate, was shown to be of competitive type [3].

This report demonstrates that also arginase inhibitor from chick liver is of competitive type.

EXPERIMENTAL.

Preparation of the inhibitor: Six chicks (20 ± 5 days old) were killed; the liver, rapidly removed in an ice bath, was diluted 1 : 4 with cold distilled water and homogenized in a Potter Elvehjem apparatus for 3′. The homogenate was dialysed overnight in cellulose tubing (Thomas Company, Philadelphia) against 3 volumes of distilled water.

The dialysate was concentrated in a rotevapor « R » at 37° C so as to obtain 1 ml dialysate for each g./liver (w.w).

The obtained preparation, frozen at — 20° C, was stable for more than 1 month. 12 preparations, obtained in the described conditions, have given comparable results.

Arginase activity determination: The incubation mixture contained the following: Veronal–Na buffer 0.1 M pH 9.4—0.5 ml; $MnCl_2$ I μmole; enzyme (acetone powder from cow liver—Sigma) 80 μgs in 0.5 ml veronal–Na buffer; inhibitor solution, when added, 0,3 ml; final volume 1.5 ml. The mixture was incubated in a Dubnoff metabolic shaking incubator for 10′ at 37° C. After addition of 0.5 ml arginine solution (containing from 4 to 10 μmoles)

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(**) Nella seduta del 19 aprile 1969.

incubation was carried for 10′; at the end the enzyme was inactivated by boiling the mixture for 2′. The enzyme activity is expressed as μmoles urea formed in the incubation mixture in 10′. Urea determination according to Archibald [4].

RESULTS

In fig. 1 preliminary results, obtained by plotting enzyme activity against time, are reported.

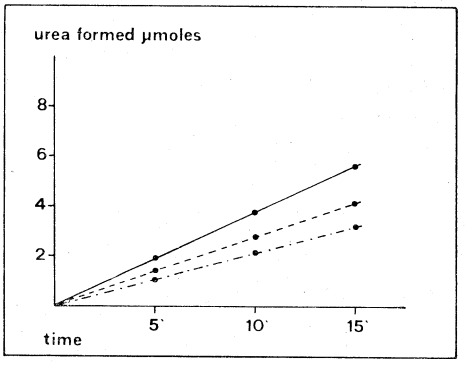

Fig. 1. – Arginase activity, at various arginine concentrations, plotted against time.
(———) arginine 4×10^{-3}M; (– – – –) arginine 2.5×10^{-3}M; (·–·–·–·) arginine 2×10^{-3}M.

From the obtained data it is evident that, in the experimental conditions reported in the text, enzyme activity is of zero order kinetics.

In Table I enzyme activity at various substrate concentrations, in presence or not of added inhibitor, is reported.

TABLE I (*)

Arginine μ moles	Urea Formed (μmoles)		% Inhibition
	without inhibitor	with inhibitor added	
4.0	2.3	1.2	47.8
5.0	2.8	1.5	46.4
6.0	2.9	1.7	41.3
7.5	3.6	2.0	44.4
8.0	3.7	2.1	43.2
10	4.0	2.5	37.5

(*) Each value is the mean of many experiments giving comparable results.

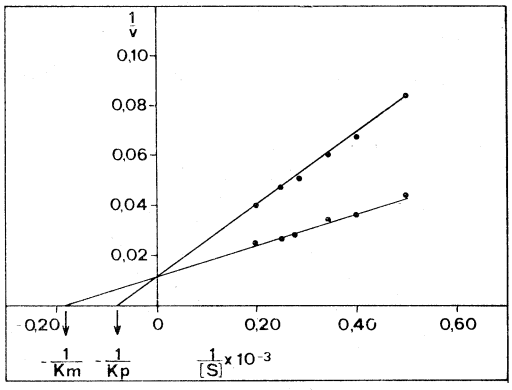

Fig. 2. – Effect of inhibitor from chick liver on the plotting the effect of substrate concentration on enzyme reaction velocity according to Lineweaver and Burk (5). The data of each curve are those reported in table I. $K_m = 5.55 \times 10^{-3}$ M; $K_p = 1.1 \times 10^{-2}$ M.

From the data reported in Table I, inhibition type has been investigated according to Lineweaver and Burk [5] (see fig. 2).

The inhibition type, as shown in fig. 2, is of competitive type.

References.

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