AMINO ACIDS : MODIFIERS OF XANTHINE OXIDASE ACTIVITY PART II : INTERACTION OF L-LYSINE AND L-GLUTAMIC ACID

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Summary : The interaction of L-lysine and L-glutamic acid with Xanthine Oxidase at pH 7.4 revealed that L-glutamic acid, carrying a net negative charge, could protect as well as reverse the inhibition of the enzyme by L-lysine. The charges carried by them seem to play an important role in the observed effect.

Key words : Xanthine oxidase L-glutamic acid L-lysine interaction

INTRODUCTION

The function of molybdenum, iron and FAD at the active site of xanthine oxidase (xanthine-oxygen oxidoreductase, EC 1.2.3.2.) is well described in its catalytic process(1). In addition, the presence of two groups with pK of 4.4 and 10.7 have also been reported at the active site of the enzyme (2, 3), indicating the role of additional charged groups in the mechanism of its action. The possibility of amino acids with their charged groups and corresponding pK values appeared worth considering for this function. In our previous study (4), therefore, their effect was investigated on the activity of xanthine oxidase at the optimum pH, which revealed on activating and an inhibitory action of L-glutamic acid and L-lysine, respectively. Although the two amino acids carry opposite charges at pH 7.4, we could not assess their exact role in the preliminary studies. It was, therefore, considered of importance to extend the work by examining the interaction of L-lysine and L-glutamic acid on the xanthine oxidase activity in order to gain further insight in the mechanism of action of the enzyme. The present paper incorporates the observations of the above experiments.

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MATERIALS AND METHODS

Milk xanthine oxidase was prepared and purified by the method of Massy *et al.* (5). The enzyme activity was measured by the colorimetric method of Owen (6) with the following modification : The final assay conditions were adjusted to maintain the enzyme activity linear for more than 5 min. The assay mixture, in a final volume of 5 *ml*, contained in the tube of the Thunburg apparatus : Phosphate buffer (pH 7.4), 500 *μmoles*; 2, 3, 5 – triphenyl tetrazolium chloride, 5 *μmoles*; 0.05 *ml* of the diluted enzyme and different concentration of the amino acids, adjusted to pH 7.4, as indicated. Into the lid was placed xanthine, 1 *μmole*. The tubes were evacuated, filled with nitrogen and a preincubation period of 10 min was followed prior to the addition of the substrate. The contents of the tube and the lid were mixed to initiate the reaction which was terminated by adding glacial acetic acid after 5 min incubation at 37°C. The formazan produced was extracted with toluene and the absorbance measured at 495 nm. The change in the absorbance of 0.1/min corresponded to one unit of the enzyme activity.

RESULTS AND DISCUSSION

The effect of the simultaneous addition of L-glutamic acid (5mM) and L-lysine (10mM), presented in Table I, reveals a definite protection of the enzyme activity by

TABLE I : Effect of L-glutamic acid and L-aspartic acid against L-lysine inhibition of xanthine oxidase.

L-Gluamic acid and L-aspartic acid (at two different concentrations) and L-lysine (10 mM) were added to the reaction mixture at pH 7.4. Each value is an average of three separate experiments.

Additions	*Xanthine oxidase activity (percentage)*
None (control)	100
L-Glutamic acid (5mM)	136
L-Glutamic acid (10mM)	142
L-Aspartic acid (5mM)	102.2
L-Aspartic acid (10mM)	104
L-Lysine (10mM)	62.4
L-Lysine + L-Glutamic acid (5mM)	92.2
L-Lysine + L-Glutamic acid (10mM)	118.5
L-Lysine + L-Aspartic acid (5mM)	64.5
L-Lysine + L-Aspartic acid (10mM)	61.3

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L-glutamic acid against L-lysine inhibition. Augmenting the concentration of L-glutamic acid to 10 mM provided complete protection of the enzyme activity.

The possibility whether L-glutamic acid treatment could also affect the reactivation of L-lysine inhibited xanthine oxidase was further investigated. The results (Fig. 1) demonstrate that preincubation for 20 min with 0.5–5.0 mM, L-lysine further reduced the enzyme activity. When L-glutamic acid was added to the reaction mixture, a complete reversal of the inhibition produced by L-lysine was observed. The data thus indicate the inhibition of the enzyme by L-lysine to be re/ersible.

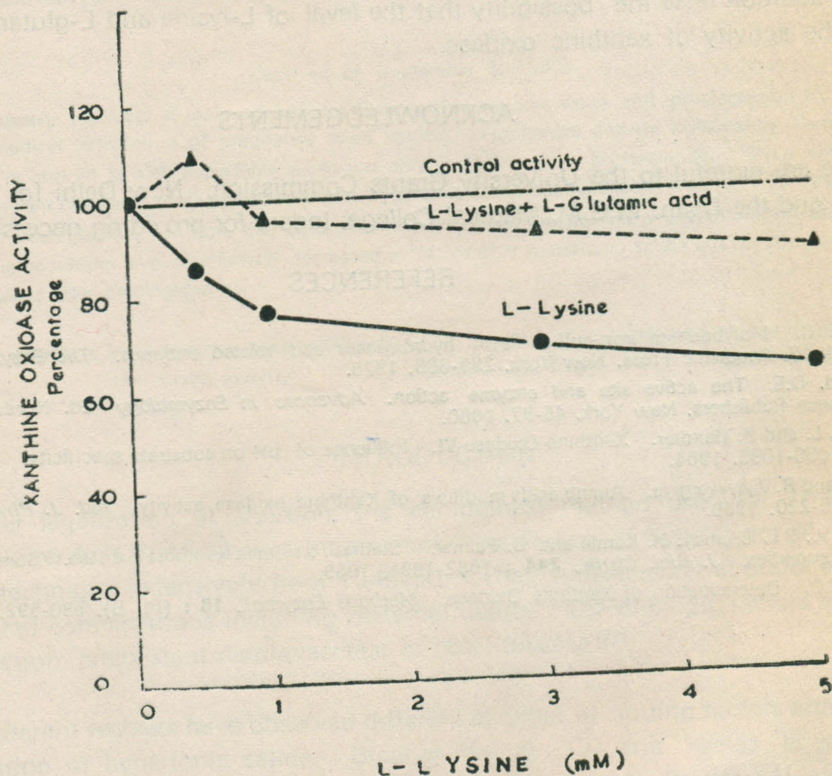

Fig. I : Reversal by L-glutamic acid of Xanthine Oxidase inhibition by L-lysine.

0.4 *ml* of purified diluted enzyme was preincubated on ice for 20 min with 0.4 *ml* of L-lysine (0.5–5.0 mM). A 0.2 *ml* aliquot was withdrawn from the preincubation mixture and mixed with 0.2 *ml* either distilled water or L-glutamic acid (10 mM). This was then used for assaying xanthine oxidase activity.

——●—— Without L-glutamic acid treatment.

——▲——L-glutamic acid treated enzyme.

In our previous study, the inhibition of xanthine oxidase by L-lysine was demonstrated to be a function of the pH, indicating the importance of the positive charge in the inhibitory effect. In the present investigations, L-glutamic acid carrying a net negative charge, could protect as well as reverse the inhibition of the enzyme by L-lysine at pH 7.4. Thus, for the mechanism by which the two amino acids influence the rate of the oxidation of the substrate by the enzyme, the role of the charges carried by them merits consideration. However, since negatively charged L-aspartic acid could not affect the enzyme activity under similar conditions (Table I), the presence of γ-carboxyl group of L-glutamic acid along with the negative charge appears to be of importance in the observed action. The results, in addition raise the possibility that the level of L-lysine and L-glutamic acid may regulate the activity of xanthine oxidase.

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