AMINO ACIDS : MODIFIERS OF XANTHINE OXIDASE ACTIVITY

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(Received on April 1, 1980)

Summary: L–Glutamic acid has been found to be a positive and L–lysine a negative modifier of the xanthine oxidase activity at the optimum pH (7.4) of the enzyme. Increase in pH was observed to be associated with a progressive decrease in the inhibition produced by L–lysine.

Key words: Xanthine oxidase modifiers L–glutamic acid L–lysine

INTRODUCTION

Alteration in the activity of xanthine oxidase (xanthine - oxygen oxidoreductase, EC 1.2.3.2.), the enzyme responsible for the conversion of hypoxanthine and xanthine to uric acid (1), is observed in diseases like gout (2) atherosclerosis (3) and hepatic disorders (4). Recently a decrease in its activity has been reported in leukemia (5) and other neoplastic disorders (6). A large numbers of modifiers of xanthine oxidase are described (7–9) but there seems to have been hardly any work reported on the effect of amino acids on this enzyme other than cysteine (10). The fact that several amino acids are known to act as positive and negative modifiers of various enzyme system (11-13), prompted us to study their interaction with the enzyme xanthine oxidase. Two groups with pK of 4.4 (14) and 10.7 (15) have been identified at the active site of the enzyme. Since the approximate value expected for the intrinsic pk's of the side chain group of aspartic and glutamic acid (β and γ carboxyl) fall in the range of 4-5 and that of lysine (amino) and tyrosine (phenolic) in the range of 10-11 (14), the effect of the above four amino acids on the enzyme activity was therefore examined.

The studies revealed L-glutamic acid to be a positive modifier and L-lysine to be a negative modifier of the enzyme activity at the optimum pH (7.4) of the enzyme.

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MATERIALS AND METHODS

Milk xanthine oxidase was prepared and purified by the alternate method of Massey *et al.* (16). The specific activity was determined as described by the same authors. The final enzyme preparation had an average specific activity of 3.2. The *in vitro* enzyme activity was assayed by the colorimetric method of Owen (17) with the following modifications. The final assay conditions were adjusted to maintain the enzyme activity linear for more than five min. The assay at pH 7.4 were conducted in 0.5 M phosphate buffer; assay at pH 9.6 and pH 10.8 were conducted in 0.1 M carbonate/bicarbonate buffer. Different substrate concentrations were used for the different pH range, since the binding of xanthine is known to decrease with increasing pH (15). At higher pH, the concentration of substrate giving the same activity as that at pH 7.4 was employed. The assay mixture in a final volume of 5 *ml* contained in the tube of Thunberg apparatus: 1.0 *ml* buffer, 0.1 *ml* 2,3,5 – triphenyltetrazolium chloride (1.67 *mg*), 0.05 *ml* diluted enzyme and different concentrations of amino acids, adjusted to the pH of the buffer. Into the lid was placed xanthine (2×10^{-4}M, 3.8×10^{-4}M, 5.6×10^{-4}M at pH 7.4, 9.6 and 10.8 respectively). 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. The reaction was terminated by the addition of glacial acetic acid after 5 min incubation at 37°. The colour of formazan formed was extracted with toluene and the absorbance measured at 495 nm. Velocity was expressed as the rate of change of absorbance per min.

RESULTS AND DISCUSSION

The change in xanthine oxidase activity following the addition of various amounts of L-glutamic acid, recorded in Table I, shows a progressive rise in the enzyme activity (1-10 mM). A definite activating effect of L-glutamic acid was observable at 1 mM concentration whereas maximal activation (42%) was produced by 10 mM L-glutamic acid. Augmenting the concentration of the amino acid did not result in any further activation of the enzyme.

The effect of the addition of varying concentration of L-lysine on xanthine oxidase activity, (Table II) resulted in a progressive decline in the enzyme activity at pH 7.4 and pH 9.6. The inhibition was markedly greater at pH 7.4 compared to the effect observed at pH 9.6. However, at pH 10.8, the inhibitory effects of lysine were completely overcome. The Lineweaver Burk plots (18) of velocity against substrate concentration, plotted at

TABLE I : Effect of L-glutamic acid on milk xanthine oxidase activity. The enzyme activity was assayed as described under "Materials and Methods".

L-Glutamic acid (mM)	*Percentage activation*
1.0	12.2
2.0	20.8
5.0	36.0
10.0	42.4
50.0	41.2

TABLE II : Effect of L-lysine on the activity of milk xanthine oxidase. The enzyme activity was assayed as described under "Materials and Methods".

Lysine (mM)	*Percentage inhibition*	
	pH 7.4	pH 9.6
1.0	13.4	—
5.0	23.7	9.6
10.0	37.6	17.4
20.0	42.0	22.0
50.0	44.3	26.5

pH 7.4, revealed the inhibition to be non-competitive (Fig 1.) The Ki calculated from the above data is of the order of 1.47×10^{-2}M.

Although with the results of the present observations, it is difficult to assess the role of opposite charges of the two amino acids at pH 7.4, a definite activating effect of L-glutamic acid and an inhibitory effect of L-lysine has been detected on the xanthine oxidase activity.

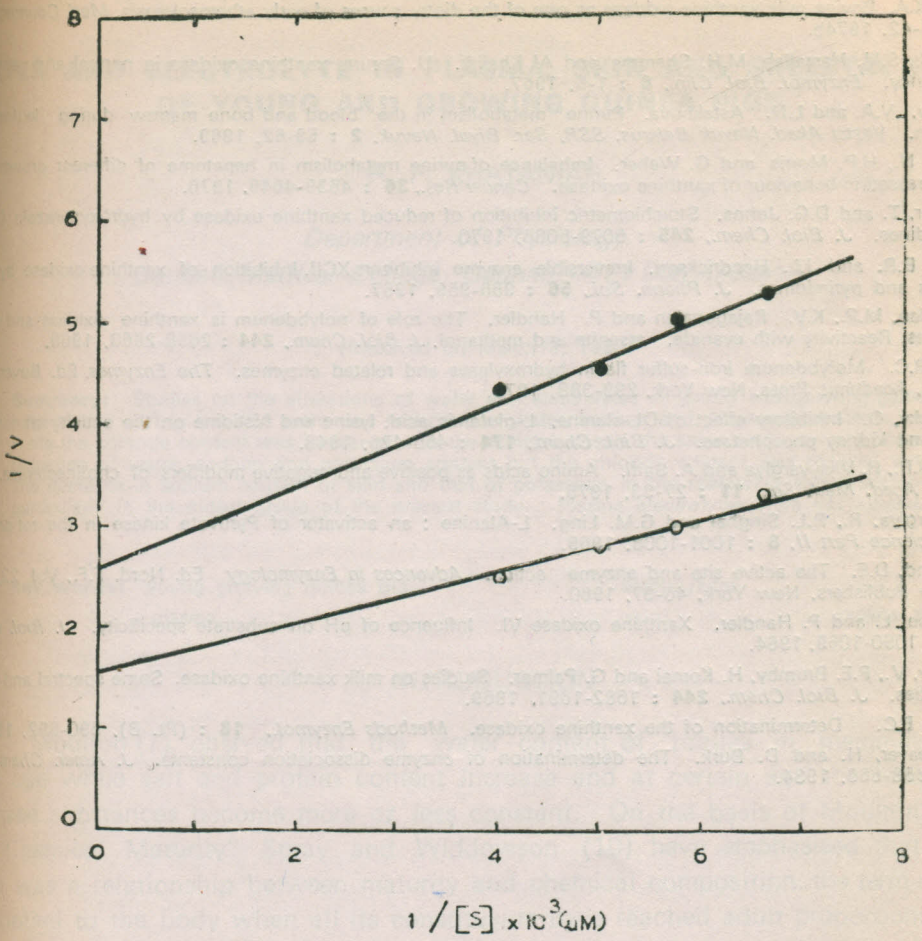

Fig. 1 : Noncompetitive inhibition of milk xanthine oxidase by L-lysine at pH 7.4. Velocity is expressed as the rate of change of absorbance per minute. ——0—— xanthaine (Km = 1.56×10^{-4}M), ——●—— with 1×10^{-2}M L-lysine.

ACKNOWLEDGEMENTS

The authors are grateful to the University Grants Commission, New Delhi, for their financial assistance and to the Dean, M.G.M. Medical College, Indore for providing the necessary facilities.

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