STUDIES ON PHENOLASE ENZYME IN PRAWNS
II. THE INHIBITORY ACTION ON THE ENZYME BY CERTAIN CHEMICAL AGENTS

P. D. ANTONY & M. R. NAIR
Central Institute of Fisheries Technology. Cochin-682011

The effect of certain chemical agents on dopa oxidation by phenolases has been examined. Sulphur containing amino carboxylic acids are inhibitory agents for dopa oxidation. Tyrosine, a substrate for the enzyme also acts as an inhibitor for dopa oxidation by the enzyme. The possible mode of action has been discussed. The function of diethyl dithiocarbamate in suppressing the display of enzyme activity has been detailed and its behaviour has been compared to the other chemical agents studied.

INTRODUCTION

The distribution of phenolase enzyme in a few species of prawns has been reported (Bailey *et al.* 1960 and Antony and Nair, 1968). The extent of enzyme activity in different anatomical regions of Indian prawns has also been a subject of study by the latter group of workers(unpublished). The probable factors which contribute to the incidence of black spots have been investigated and it was brought out that the most important of them are pH of the tissue, holding temperature, accessibility to oxygen and copper ions. Preliminary investigations have shown that sulphur containing amino acids such as methionine, cystine, cysteine, as well as tyrosine and diethyl dithiocarbamate are inhibitory agents on the enzyme. The present paper summarises the results of experiments carried out to study the nature and extent of inhibition by some of the above chemical agents on the enzyme.

MATERIAL AND METHODS

The material used for the study was prawn of species *Metapenaeus monoceros* obtained from the Cochin brackish waters. The prawns were immediately beheaded after landing and the head portions collected. Weighed amounts of head were blended with 2.5 vols. of ice cold water for 2 minutes. The homogenate was centrifuged at 7500 rpm at 0°C for 45 minutes in an IEC refrigerated centrifuge. The centrifugate was treated with Alumina C γ gel prepared according to Bauer (1955) in the ratio 10:3 and allowed to stand for 2 hours at 4°C over a magnetic stirrer.

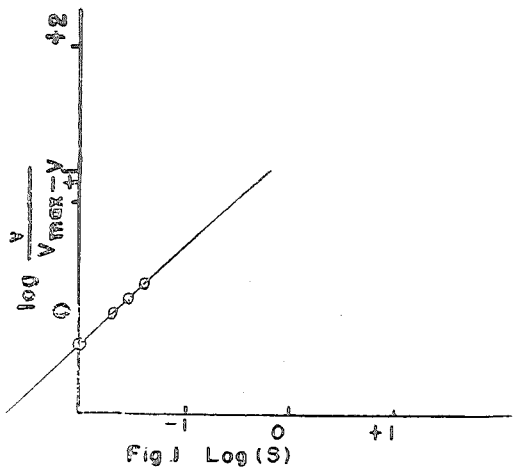

Fig. I

The suspension was centrifuged at 5000 rpm at 0°C for 10 minutes. The supernatant which had practically no enzyme activity was discarded. The residue was thoroughly washed with 0.05M potassium phosphate buffer (pH 7) by stirring for 1 hour over a magnetic stirrer in a cold room at 4°C and the residue collected by centrifugation at 0°C at 3000 rpm for 10 minutes. The centrifugate showed only very feeble enzyme activity. The enzyme was extracted from the residue by repeating the above step with 1 M. potassium phosphate buffer (pH 7) and at 7500 rpm. The centrifugate was dialysed at 4°C for 24 hours against several changes of distilled water. The pale blue dialysate obtained was used for the study after proper dilution. Unless otherwise stated the assay mixture contained 0.2g. equivalent of head tissue.

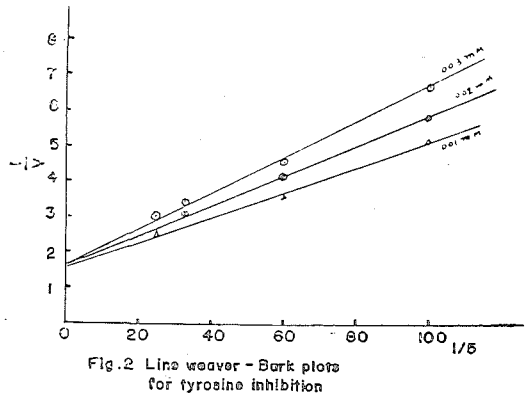

Fig. 2

The chemicals used for enzyme assay and inhibition studies were 3:4 dihydroxy phenyl alanine (dopa) and diethyl dithiocarbamate sodium (salt) of BDH, England, and L. methionine, L. tyrosine and L. cysteine of E. Merck.

The enzyme assay

The assay mixture contained 1 ml. of enzyme at appropriate dilution, 5ml. of potassium phosphate buffer (pH 6.8) and varying amounts of dopa in a final volume of 10ml. The mixture was incubated at 37°C for varying periods and the colour developed measured at 470mμ.

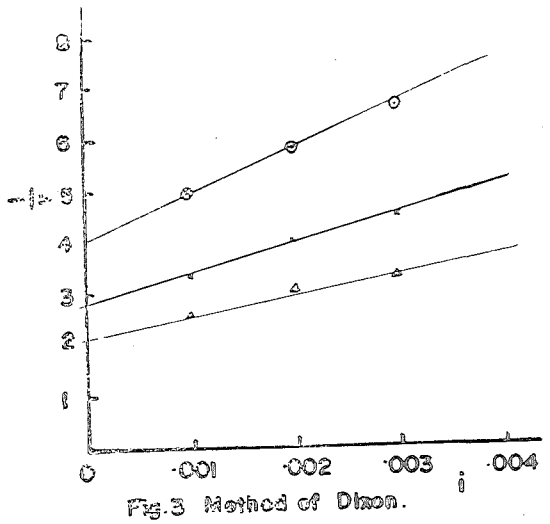

Fig. 3

L. cysteine as inhibitor

Though L. methionine, and L. cysteine showed inhibitory action on the enzyme, only L. cysteine showed a pronounced

inhibition on dopa oxidation. The assay mixture for the study of cysteine inhibition contained, in addition to appropriate amount of dopa, 0.2 to 0.8μ mole of L. cysteine.

L. tyrosine as inhibitor

The assay was carried out as before but the reaction mixture contained 1 to 3μ moles of L. tyrosine in place of cysteine.

Sodium diethyl dithiocarbamate as inhibitor.

The concentration of diethyl dithiocarbamate in the reaction mixture varied from 0.25 to 1μ mole.

Results And Discussion

The effect of L. cysteine on dopa oxidation is shown in Fig. 5. The inhibition increases with increasing concentration of L. cysteine. However, it fails to give straight line with Lineweaver - Burk (1934) plots pointing out a mixed type of inhibition (Keleti, 1966). The behaviour of the enzyme in presence of L. tyrosine in its dopa oxidation property is presented in Fig. 2 & 3. The nature of inhibition by L. tyrosine is competitive type; the ki value by Dixon's plot is presented in Fig. 2 which gives a value of 3.75×10^{-6} Moles. Sodium diethyl dithiocarbamate behaves in an identical way to that of cysteine in its general nature and extent. The percentage of inhibition effected by these agents are presented in Fig. 4, 5 and 6. The fall in velocity in presence of cysteine is presented in Fig. 7.

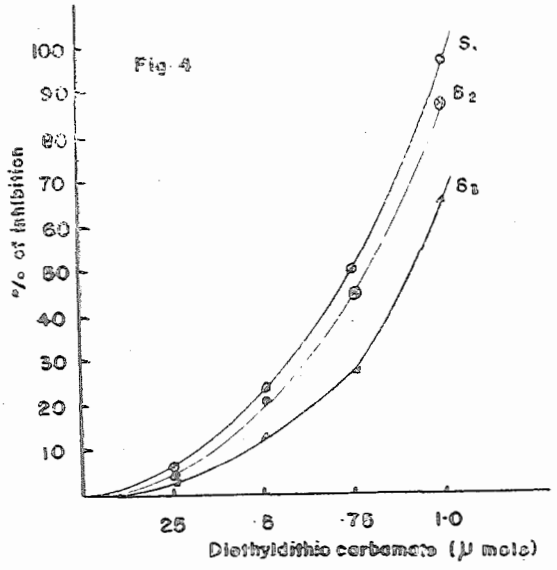

Fig. 4

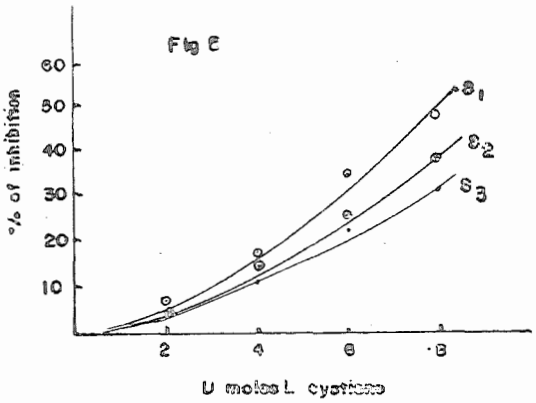

Fig. 5

All the inhibitors which have been investigated have a pronounced effect on the dopa oxidation by the enzyme under study. The behaviour of tyrosine in the system in inhibiting the dopa oxidation can be explained by the fact that tyrosine itself can act as a substrate for the enzyme

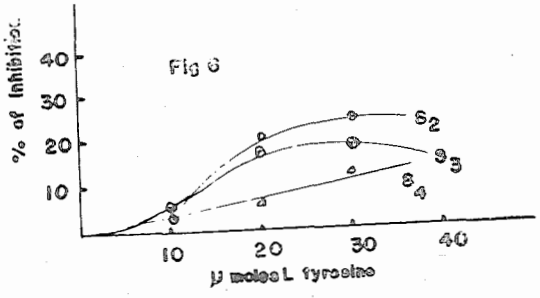

Fig. 6

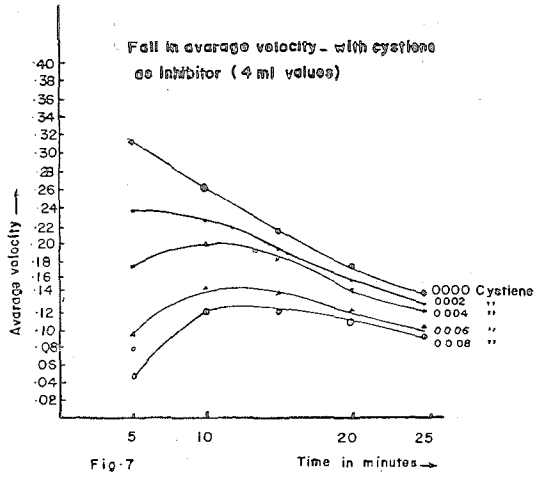

Fig. 7

getting itself converted to dopa (Seymour, 1966). Since the dopa oxidation rate shows a decrease rather than an expected increase due to the increased substrate concentration from tyrosine it is to be assumed that tyrosine hydroxylation as well as dopa oxidation are effected at the same site on the enzyme. Tyrosine and dopa, both having similar functional groups in similar positions, compete for the same site on the enzyme thereby giving rise to the competitive type of inhibition. The enzyme shows either lack of interacting sites or only one active site (Jayaraman, 1970), the latter probability being more or less confirmed from Fig. 1. The fact that Lineweaver Burk plots for cysteine inhibition fail to give straight lines suggests that it is a mixed type inhibition. As cysteine can form addition products with quinones and these addition products are rather not easily removed from the site of action of the enzyme, or may be, the quinone-cysteine adduct is not easily dissociated, it prevents the formation of the measured dopachrome. The inhibition by diethyl dithiocarbamate is mainly due to the metal chelating action on the copper ions present in the enzyme (Masey Ikeda, 1967). It is well known that dialysis of EDTA complex of the enzyme leaves the enzyme devoid of its activity and addition of copper ions to the resulting inactive enzyme restores the activity. However an upward inflexion of the curve of percentage inhibition *v.* concentration of diethyl dithiocarbamate suggests that sulphur atoms present in this agent also exhibits an added inhibition property to the inhibition by chelation ofcopper ions which(Fig. 4) are highly exposed and easily accessible to the complexing agent.

REFERENCES.

Antony, P. D. and M. Rajendranathan Nair. 1968. *Fish. Technol;* **5**, 1:31.

Bailey, M. E; E. A. Fiegar and A. F. Nosak. 1960. *Fd. Res;* **25**, 5:557.

Bauer, E. 1955. *Methods in Enzymology* Vol 1. Ed: S.P. Colowick and N.O. Kaplan, Academic Press, New York.

Jayaraman, H. N; T. Ramakrishnan; M. Sirsi and C. S. Vaidyanathan. 1970. *Indian J. Biochem;* **6** :137.

Keleti, T. & M. Telegdi. 1966. *Enzymologia;* **7**, 30: 1.

Lineweaver, H and D. Busk. 1934. *J. Am. Chem. Soc;* **56** : 658.

Masey uki Ikeda, H. Miston Levi & Sydvey Udenfriend. 1967. *Archives. Biochem. Biophys;* **120**, 2:420.

Seymour, H. Pomerantz. 1963. *J. Biol. Chem.*, **241**, 1: 161.