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Histidine Decarboxylation in the Halophile *Dunaliella salina*

by

ROHAN H. WICKRAMASINGHE* AND YVES LE GAL

Laboratoire de Biologie Marine, Concarneau, Finistère, France

Recent investigations made on histidine degradative mechanisms in bacteria have shown that histidine transaminase, L-amino-acid oxidase and histidase may be present in *Escherichia coli*, *Proteus vulgaris* and *Clostridium tetanomorphum* respectively (Wickramasinghe, Hedegaard and Roche, 1967 ; Wickramasinghe, 1970 ; Wickramasinghe and Fry, 1954). The inducible or repressible nature of some of these enzymes is established while micro-organisms are also known to degrade histidine and other imidazole derivatives by enzymes such as decarboxylases and oxygenases (Meister, 1965).

We have recently looked at the histidine degrading enzymes in a unicellular marine alga, *Dunaliella salina*. To the best of our knowledge little work has been done on the metabolism of histidine in members of this type of organism and we have made our cultures in sterile media of (supplemented) sea-water in order to better identify what may be the enzyme composition in the physiological state. To this end, conditions of culture medium which may encourage abnormal production of transaminase (Wickramasinghe, 1969*b*) or decarboxylase (Meister, 1965) were avoided.

MATERIALS AND METHODS

Dunaliella salina strain LB 19/3 of the Cambridge Culture Collection was used in this investigation.

Cultivation of cells of *D. salina* was carried out in the medium adapted by S. Maestrini from the " Gonyaulux medium " of Haxo and Sweeney (1955). The medium consisted essentially of filtered sea-water fortified with sterile soil extract and the requisite trace elements and buffered with Tris (hydroxymethyl)aminomethane (0.5 gm per litre) to pH 7.8-8.0. No exogenous carbon source is added. After sterilisation by autoclaving, inoculation was performed and the culture carried out aerobically at room temperature. (Small one-litre quantities of culture were contained in two-litre flasks and placed on a shaker while for 15-litre batches a sintered glass bubbler connected to a sterile supply of air was used.) The progress of growth of the culture was followed by readings of the optical density of samples withdrawn aseptically.

The cells at the end of the log growth phase were harvested by centrifugation (13,000 g, 20 mins). They were washed in ice-cold potassium phosphate buffer 20 mM, pH 7.2 and then suspended in phosphate buffer 0.2M, pH 7.2 (All operations in the preparation of

*On leave from the Department of Biochemistry, Medical School, Edinburgh University, Scotland,

the enzyme were carried out at 0-4°). Sonication (MSE 20 kc/s, 5 × 1 mins) was performed and the resultant preparation dialysed overnight against 200 volumes of phosphate buffer 0.02M, pH 7.2. The protein was separated into soluble and particulate fractions by centrifugation at 35,000 g for 20 mins. The very dark green particulate preparation was resuspended in phosphate buffer 20 mM, pH 7.2 and the protein concentration of this and the light green coloured "soluble" preparation were measured by the Folin reaction.

Incubations were carried out and the products identified as described previously (Wickramasinghe et al., 1967 ; Wickramasinghe, 1970) using a tracer of C^{14}—ring labelled histidine (Amersham Radiochemicals) added to a neutral solution of "cold" amino-acid. All incubations contained 30μM pyridoxal phosphate which is known to activate the histidine transaminase of *E. coli* (Wickramasinghe, 1969*a*) and decarboxylases (Meister, 1965). Controls were carried out in neutral and acid assays using equivalent quantities of enzyme inactivated by boiling.

Investigations for L-amino-acid oxidase were carried out at pH 7.2, for histidine transaminase also at pH 7.2 but in the presence of neutralised 8 mM oxoglutarate while histidine decarboxylase was looked for by incubations carried out at pH 5.0. In all instances the histidine input was 10^{-3}M. The soluble and particulate protein preparations were investigated separately using from 100-500 mg protein per 10 ml reaction medium and the incubation was carried out for 1 hour at 30°. The reaction was stopped by the addition of glacial acetic acid and boiling. Standards of urocanic acid, imidazolelactic acid, histamine and histidine were used for comparison with the reaction products visualised after paper-chromatography by spraying with diazotised p-chloroaniline. (Imidazolepyruvate, the first product of histidine oxidation or transamination, migrates with imidazolelactate on descending paper-chromatography in n-butanol:acetic acid: water, 50:12:50, upper phase).

RESULTS AND DISCUSSION

Screening of the products of the reaction mixtures showed that neither "soluble" nor particulate preparations had catalysed the conversion of L-histidine to urocanate, imidazolepyruvate or imidazolelactate. Thus the organism does not produce histidase, L-amino-acid oxidase or histidine-oxoglutarate transaminase under the culture conditions described. However, under acid pH conditions of enzyme assay, conversion of histidine to histamine by the particulate preparation (only) was observed. This requirement of an acid pH optimum for the activity of this algal decarboxylase is in contrast to the decarboxylases of animal and higher plant origin and more similar to the nature of the bacterial enzymes.

The location of the enzyme in the particulate preparation is interesting in that it may indicate "compartmentalisation" of some significance (cf. the distinct forms of histidine-pyruvate transaminase in different fractions of rat liver cells—Spolter and Baldridge, 1964). Many bacteria show an increased amino-acid decarboxylase production when cultivated in an acid medium and it has been suggested that the concomitant excretion of amines may be an adaptation by these organisms to "buffer" excess acid (Hanke and Koessler, 1924). However histamine has potent physiological and pharmacological effects and histidine itself is

directly involved in the metabolism of ergothioneine and hercynine in *Neurospora crassa* and probably in several fungi. It is therefore possible that the conversion of histidine to histamine in the marine alga, *Dunaliella salina*, may eventually prove to have considerably more significance than merely for producing a means of counteracting acid conditions.

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