IMIDAZOLE LACTIC ACID: AN INTERMEDIATE IN L-HISTIDINE DEGRADATION IN ESCHERICHIA COLI B.

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Received October 12, 1966

The enzymatic degradation of L-histidine proceeds in most organisms through two main pathways with either imidazole acrylic (urocanic) acid or histamine as intermediates and results respectively in the formation of glutamic or aspartic acid derivatives (Meister, 1965). Recently Sen et al.(1962) reported the in vivo reduction of urocanic acid to imidazole propionic acid. The biological significance of this last reaction step is however unclear and Schlesinger and Magasanik (1965) detect no degradation of imidazole propionic acid in Aerobacter aerogenes.

In early work Hanke and Koessler (1922) mention the presence of imidazole acrylic, imidazole lactic and imidazole acetic acids in the broth of L-histidine grown enterobacteria. No satisfactory explanation was however given as to the reactions leading to the formation of these compounds. More recently Revel and Magasanik (1958) tested 6 strains of Escherichia coli and concluded that this microorganism was unable to metabolize L-histidine. During earlier work we have observed a partial disappearence of "total imidazole" compounds from the histidine-containing growth medium of E. coli B (Hedegaard et al., 1959).

In this paper we present further evidence for L-histidine metabolism in several strains of E. coli B. Our results indicate a new degradation pathway with, as the probable initial reaction, an oxidative deamination of histidine and consecutive formation of imidazole pyruvic acid. The second step is a reduction of the imidazole pyruvic acid by a pyridine nucleotide dependent dehydrogenase to imidazole lactic acid which, in turn, can be further metabolized through a ring-opening step of the imidazole nucleus to one or several acyclic compounds.

Material and Methods.

The following strains of Escherichia coli B were tested: No. 54.125, No. 224, No. 548, No. 5452 and No. HGCYA 151 (collection of Institut Pasteur, Paris). When not otherwise indicated all results given in this paper were obtained with E. coli B 54.125.

L-histidine, imidazole pyruvic acid and the pyridine nucleotides NAD and NADP were purchased from Calbiochem (Calif.). The imidazole lactic acid was synthezised according to the procedure of Fränkel (1903).

E. coli B was cultivated on a shaker at 30° C in 1 liter Erlenmeyer flasks containing 100 ml of a synthetic salt-glucose medium (Hedegaard et al., 1959). Growth was initiated from an 18 hours old adaptation culture and followed by O.D. measurements at 560 mμ. The cells were harvested after 10-12 hours of growth, washed twice with ice cold phosphate buffer, 0.01 M, pH 7.2 and resuspended in the same buffer for incubations.

Histidine metabolism in vivo was followed by chromatography, high voltage electrophoresis and colorimetric analysis of aliquots from incubations of cell suspensions in the phosphate buffer containing L-histidine or imidazole pyruvic acid (final conc. 10^{-3} M) (Hedegaard et al., 1965). Imidazole compounds were visualized on chromatograms after spraying with diazotized para-chloroaniline and measured quantitatively in solutions according to the technic of Jorpes (1932). Histidine degradation in vitro was studied with crude extracts obtained after sonic disruption of the cells followed by high speed centrifugation. The cell-free extracts were incubated at 30° C with histidine or imidazole pyruvic acid (final conc. 10^{-3} M) in phosphate buffer, pH 7.2 and aliquots were analyzed as described above after precipitation of the proteins with glacial acetic acid. Histidine-adapted E. coli B cells were obtained by addition of the amino acid (final conc. 10^{-3} M) to both the adaptation and growth medium. In certain cases histidine-adapted cells were grown under nitrogen starvation conditions in a medium where the normal free ammonia ion concentration was lowered from 2.66×10^{-3} M to 0.76×10^{-3} M. The purity of the E. coli B strains was controlled after growth and extended resting-cell incubations by the indol, methyl-red, Voges-Proskauer, and citrate tests (Gastinel, 1949).

Results and Discussion.

When normal E. coli B cells are incubated in the presence of L-histidine, high voltage electrophoreses show (Fig. 1 a) that histidine is degraded to imidazole lactic acid (Hedegaard et al., 1965). Analyses of incubations of similar cells carried out in the presence of imidazole pyruvic acid show that the latter compound is also rapidly transformed to imidazole lactic acid (Fig. 1 a). In vitro evidence for this last reaction is given in Fig. 1 b. Imidazole pyruvic acid, stable under the

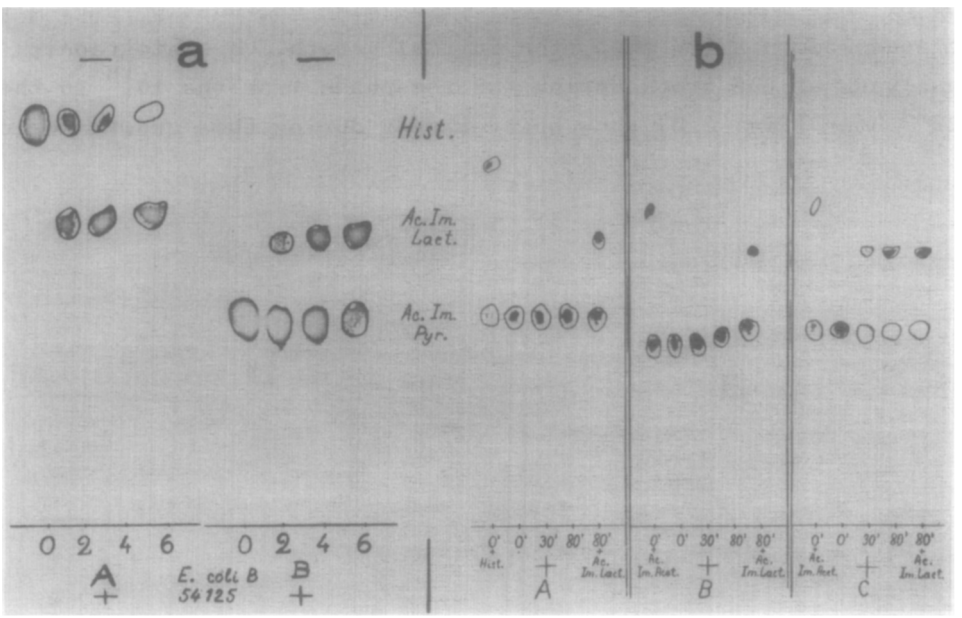

Fig. 1 a: Formation of imidazole lactic acid in vivo by cell suspensions of E. coli B 54.125 containing:
A: L-histidine.
B: Imidazole pyruvic acid.
Aliquots (0, 2, 4, and 6 hours) analyzed by high voltage electrophoresis (6000 volt, pH 1.8).

Fig. 1 b: Formation of imidazole lactic acid in vitro by cell free extracts of E. coli B 54.125 incubated with :
A: Imidazole pyruvic acid alone.
B: as A + cell extract.
C: as A + cell extract + $NADH_2$ + $NADH_2$-regenerating system (G-6-P + G-6-P dehydrogenase).
Aliquots (0', 30', and 80') analyzed by high voltage electrophoresis (6000 volt, pH 1.8).

experimental conditions, is rapidly reduced by a $NADH_2$-dependent dehydrogenase from E. coli B to give imidazole lactic acid.

Normal E. coli B cells grow rapidly in the synthetic salt-glucose medium with free ammonia ions (starting conc. 0.76 x 10^{-3} M) becoming a limiting growth factor after approximately 8 hours of growth(Fig. 2 a). However, when E. coli B is inoculated into the same medium containing L-histidine (starting conc. 10^{-3} M) the growth continues after the 10^{th} hour (Fig. 2 a) and proceeds through a rapid growth period, followed by a new lag-phase from the 22^{th} to the 26^{th} hour and a new log-period to level off after approximately 48 hours of growth. Analyses of "total imidazole" compounds in the medium (Fig. 2 a) indicate no or only insignificant "total imidazole" degradation during the glucose-ammonia ion supported initial growth. Chromatographic analyses of the broth during the log-phase from the 10^{th} to the 22^{th} hour (Fig. 2 b) show however that during this growth period

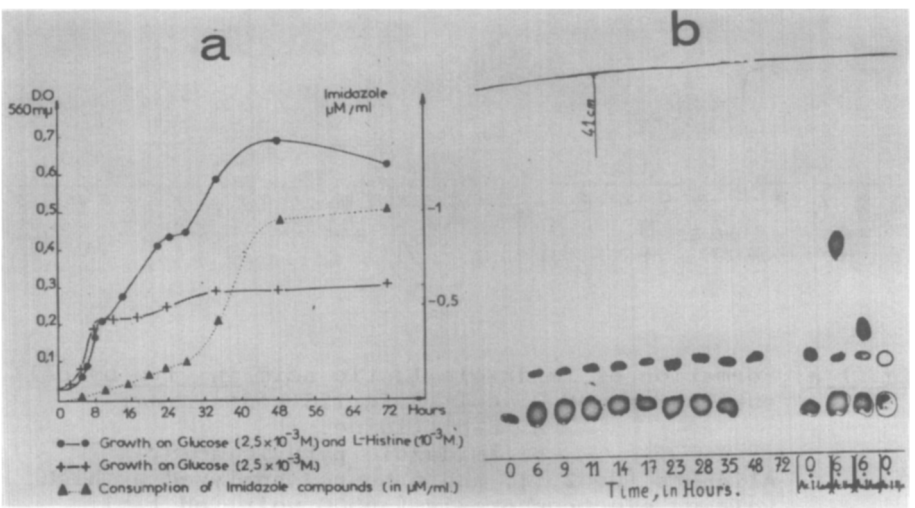

Fig. 2 a: Histidine induced growth of E. coli B 54.125 and degradation of "total imidazole" compounds

Fig. 2 b: Paper chromatographic analyses of aliquots from the experiment Fig. 2 a.
(Solvent: n-Butanol-Ac. acid-Water; 50-12-50).

histidine is actively degraded to imidazole lactic acid. The log-phase from the 26^{th} to the 48^{th} hour coincides with a rapid disappearence of "total imidazole" compounds in the medium (Fig. 2 a). The simultaneous chromatographic analyses of the aliquots (Fig. 2 b) indicate a continuous degradation of histidine to imidazole lactic acid which, in turn, is further metabolized to one or several non-heterocyclic compounds. At the end of the growth (after 48 hours) the medium contains only small amounts of one single imidazole compound, imidazole lactic acid, which is completely metabolized after 72 hours of growth (Fig. 2 b).

It appears from these results that L-histidine is metabolized by Escherichia coli B through imidazole pyruvic acid and imidazole lactic acid to acyclic compounds. This histidine degradation initiates under growth conditions where free ammonia ions in the medium are the growth limiting factor. Cell respiration in the presence of histidine indicates that the first reaction in this new degradative pathway giving rise to imidazole pyruvic acid is due to an oxidative deamination rather than to a transamination (Hedegaard et al., 1965). The enzymatic reduction of imidazole pyruvic acid to imidazole lactic acid exhibits a cofactor requirement which can be fulfilled by both $NADH_2$ and $NADPH_2$. Histidine-adapted E. coli B cells degrade imidazole lactic acid when grown under nitrogen starvation conditions but the metabolic step involved in the ring-opening of the imidazole nucleus is yet unknown.

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