EVIDENCE FOR A NEW STEP BETWEEN ATP AND 5-AMINO 4-IMIDAZOLCARBOXAMIDE RIBOTIDE IN THE CYCLIC PROCESS OF HISTIDINE BIOSYNTHESIS[1]

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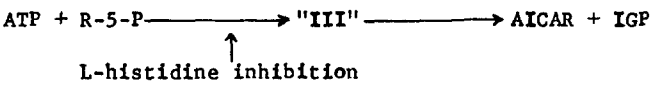
Moyed and Magasanik (1960) have shown that the biosynthesis of imidazole ring of histidine occurs from ATP, ribose-5-phosphate (R-5-P) and glutamine in bacteria. The products of the reaction are imidazole glycerol phosphate (IGP) and 5-amino 4-imidazolcarboxamide ribotide (AICAR). They have suggested that the reaction takes place in two steps, separable by protamine fractionation. The first is the addition of R-5-P to ATP to give "compound III" of a hypothetical structure, and the second is the breakage of the purine ring in the presence of glutamine to give AICAR and IGP. The first step has been shown to be inhibited by histidine providing another example of a feedback control.



We have shown previously that histidine depresses the rate of reversion to prototrophy in a heteroallelic $\underline{ad}_3$ (adenine and histidine requiring) diploid of baker's yeast (Luzzati, Clavilier and Slonimski, 1959). The present paper describes the activity of crude extracts of Saccharomyces cerevisiae in forming AICAR and shows, as a consequence of the study of histidine inhibition, that a new supplementary step in the above mentioned reaction has to be postulated to explain the experimental results.

MATERIALS AND METHODS

Yeast strains LM27 and LM2 (respectively $\frac{ad\ 3/3}{ad\ 3/6}$ diploid and a non-requiring reverted diploid) were cultivated in a synthetic medium (Galzy and Slonimski, 1957) containing 40 mg/l of adenine, 20 mg/l of L-histidine and 20 g/l of glucose. The culture was grown aerobically at 28°C for 20-24 hours, cells harvested in the logarithmic phase (between 250 and 500 mg dry weight/l),

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washed with distilled water and extracts obtained by grinding the cells with Ballotini beads and 0.1 M potassium phosphate buffer pH 7.4 in a Lourdes multi-mixer. The homogenate was centrifuged at 25000 g for 20 min. and the supernatant was dialysed overnight against the same buffer 0.01 M. The extract containing ca 15 mg. protein/ml could be stored for several days at -25°C.

Enzyme activities were measured by a slightly modified method of Love and Boyles (1959). One milliliter of incubation mixture contained : R-5-P, 10 μmoles; K-Na ATP, 2 μmoles; K-phosphoglycerate, 15 μmoles; glutamine, 7.5 μmoles; $MgCl_2$, 100 μmoles; potassium phosphate buffer (pH 7.4), 50 μmoles and 3 mg of protein of the extract. The incubation was made at 37°C, the reaction stopped by TCA and AICAR measured by the Bratton and Marshall (1939) reaction. Two kinds of non-acetylable arylmines were determined : free-AICAR (measured directly) and total-AICAR (measured after a weak acid hydrolysis, 1N HCl, 100°C, 20 min.). The difference between the is denoted as "compound III" (Moyed and Magasanik, 1960).

RESULTS AND DISCUSSION

Extracts produced 200 to 600 mμmoles of total AICAR per ml of incubation mixture per hour. For a given extract the reproductibility of activity measurements was quite satisfactory but different extracts did vary to some extent. The reaction was linear during at least the first 60 minutes and the rate was proportional to the protein content between 1 to 4 mg per ml of the incubation mixture. No major differences were found between extracts from the mutant and reverted strain.

All the components of incubation mixture are indispensable for the reaction except glutamine. In its absence the reaction is slightly diminished. Phosphoribosylpyrophosphate (1 μmole per ml) replaces R-5-P. It is of interest that potassium ions are absolutely required. Their replacement by sodium ions diminishes the reaction by 90%.

L-histidine added at the beginning of the incubation completely supresses the formation of AICAR, its action being highly specific. Other related substances like urocanate, 1-methyl- and 3-methyl-histidine, histamine and thiamine were not inhibitory. Histidyl-histidine showed a much slighter inhibition.

These results suggest that a similar pathway exists in S. cerevisiae and in different bacterial species.

A new feature of the reaction is revealed by the experiment shown in Fig.1. When histidine is added at successive times during the course of the reaction the system becomes progressively more resistant towards histidine, eg., histidine produces 96% inhibition when added at zero time and

only 3% when added at 30 min. Increases in resistance concerns the production of free-AICAR as well as that of "compound III". The latter remains at least constant, or even augments, in spite of the increase of free-AICAR.

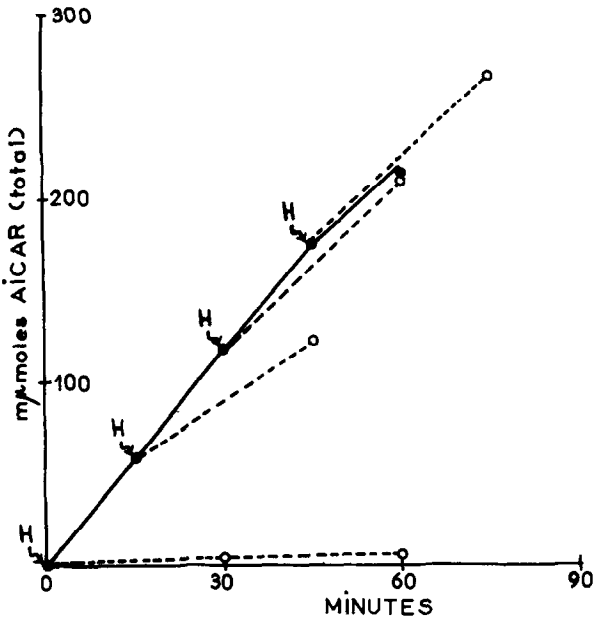


Fig.1 Increases of the resistance towards histidine inhibition during the course of the reaction. L-histidine (1 μmole/ml) added at different times (arrows). Solid line and full circles : no histidine; dashed line and open circles : with histidine. Total-AICAR denotes the sum of free-AICAR and "compound III".

The increase in resistance may be due a priori to two types of phenomena :

1) During the reaction a protectory substance is produced that counteracts or prevents the action of histidine.

2) During the reaction an intermediate metabolite is produced that can be enzymatically transformed into "compound III" and free-AICAR even in the presence of histidine. This new hypothetical intermediate (called X) must be nonhydrolysable into free-AICAR by mild acid hydrolysis which permits it to be differentiated from "compound III".

Studies on inhibition brought about by low concentration of histidine (10^{-4}M) do not support the first hypothesis. If this hypothesis were true the degree of inhibition brought about by a low concentration of histidine (added at zero time) should diminish continually as the reaction

proceeds. In other words in a system partially inhibited by histidine the kinetics of total-AICAR production as a function of time should be exponential. This is not the case; the kinetics being linear.

The experiment shown in Fig. 2 provides evidence for the second one. An extract that forms practically no free-AICAR nor "compound III" (curve A) was obtained from an active one by two cycles of freezing and thawing. Although inactive in the overall reaction, it forms the postulated intermediate "X", when incubated for 30 min. The latter is revealed by subsequent addition of a normal extract which converts it into "compound III" and AICAR even in the presence of histidine (curve F). Control experiments show (curve E) that histidine added at the beginning of the reaction inhibits completely the formation of arylamines in the normal and inactivated extracts either mixed or not. Addition of inactivated extract to the normal one produces a considerable stimulation (curve C to be compared to curve B). The same formation of arylamines is obtained when the active extract is added to the inactivated one which has been pre-incubated for 30 min. (curve D).

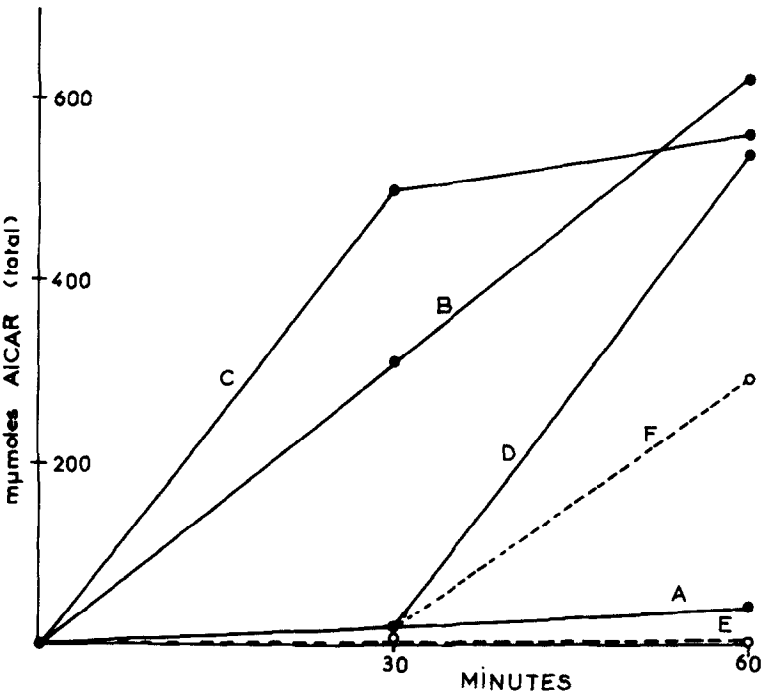
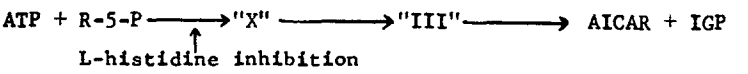


Fig. 2. Inactivated extract alone : A. Active extract alone : B. Both mixed at zero time : C. Addition at 30 min. of active to inactivated extract : D. Conditions corresponding to A, B and C but with histidine added at zero time : E. Conditions corresponding to D but with histidine added at 30 min. : F.

The following scheme summarises our results :



The inactivated extract lacks the second-step activity.

If the results obtained on yeasts are relevant to bacteria, the protamine fraction I of Moyed and Magasanik (1960) should be separable into two activities corresponding to the first two steps of our scheme. The compound absorbing at 290 mμ could correspond to compound "X".

It may be of interest that in spite of an additional step postulated in the early reactions of histidine biosynthesis the feedback control seems to concern the very first step of the pathway in accordance with the general principle of this regulatory mechanism. (Umbarger and Brown, 1958).

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