EFFECT OF AMINO ACID STARVATION ON CONSTITUTIVE SYNTHESIS OF HISTIDINE mRNA

L. PATTHY and G. DÉNES

Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Received 15 July 1971

1. Introduction

In stringent bacteria, amino acid starvation is known to arrest gross RNA synthesis. There is accumulating evidence suggesting that this amino acid control of RNA synthesis is not coordinate: *rel* gene regulates rRNA and tRNA but not mRNA synthesis [1].

It was shown for the tryptophan, histidine and arginine systems that during starvation for the respective amino acid the corresponding mRNA is synthesized preferentially [2–6]. Moreover, the possibility of inducing *lac* mRNA synthesis during amino acid starvation suggests that mRNA synthesis is not restricted to the operon controlling the synthesis of the amino acid which is withdrawn [9–12].

There are two ways for the determination of mRNA synthesized during amino acid starvation. The first utilizes the hybridization technique and employs appropriate genetic tools [2–4, 7–8]. The second assesses mRNA by its capacity to direct enzyme synthesis [6, 9].

Using the second method we have found that in a histidine constitutive mutant histidine mRNA is accumulated during starvation for leucine or threonine.

2. Methods

The regulatory mutant Y10-6 111 was derived from strain Y10 (F^-, *thr*$^-$, *leu*$^-$, B_1^-) of *Escherichia coli* K12 [13]. Growth conditions and assay of histidinol dehydrogenase (EC 1.1.1.23) were described in a previous article [13]. Y10-6 111 was always grown in the presence of exogenous histidine (20 μg/ml). Histidinol dehydrogenase specific activity is expressed relative to that in wild type Y10, corresponding to 78 nmole NADH formed per hr per mg protein. When plotting histidinol dehydrogenase activity (nmole NADH formed per hr) per ml against absorbance at 490 nm in a 10 mm cuvette, the enzyme content of the growth cultures was calculated by normalizing the enzyme activity to the protein content per ml culture.

3. Results and discussion

The Y10-6 111 mutant derived from *Escherichia coli* K12 synthesizes histidine biosynthetic enzymes constitutively irrespective of a high excess of exogenous histidine [13]. The constitutive enzyme level increases with temperature of growth. It was observed with this mutant that after deprival and readdition of leucine there is a burst of histidinol dehydrogenase synthesis. The significance of constitutivity in the appearance of this burst could be verified by demonstrating that this phenomenon depends on constitutivity (varied by temperature of growth). The duration of leucine deprival was also found to affect the size of the burst, the best response being obtained after 36 min starvation [13].

In subsequent experiments starvation was effected by deprival of threonine and similar results were obtained (result not shown). This suggests that the observed phenomenon is not peculiar to leucine starvation.

In the interpretation of this phenomenon it is of significance that: (a) it is elicited by amino acid (leucine or threonine) starvation; (b) the duration of amino acid deprival determines the size of the burst; (c) the size of the burst increases with constitutivity.

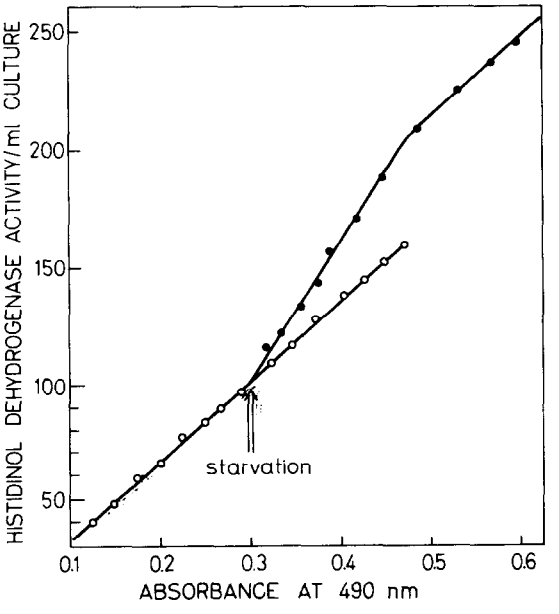

Fig. 1. Amount of histidinol dehydrogenase synthesized during the burst. Y10-6 111 cultures were grown at 41°. Histidinol dehydrogenase activity per ml culture was plotted against absorbance of culture at 490 nm in a 10 mm cuvette. One unit of histidinol dehydrogenase produces 1 nmole NADH per hour. One of the cultures was starved for leucine for 36 min at A_{490} of 0.300 (no change in absorbance) then 40 μg/ml L-leucine was added, -●-●-. The parallel culture was grown in excess leucine, -o-o-.

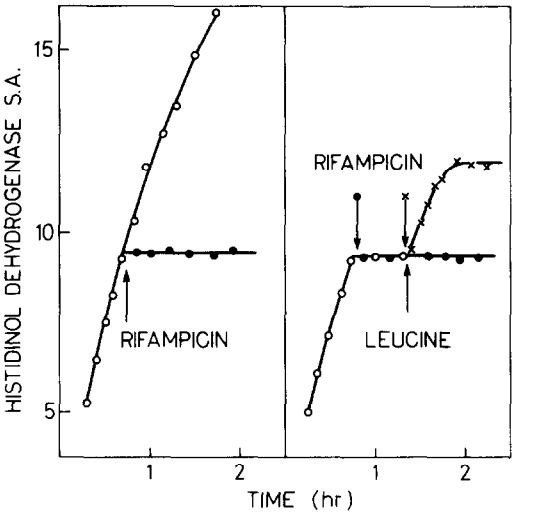

Fig. 2. Effect of rifampicin (60 μg/ml) on histidinol dehydrogenase synthesis. Cultures of Y10-6 111 were pregrown at 31° to decrease the level of histidinol dehydrogenase and at 0 min the temperature was shifted from 31° to 41°. Histidinol dehydrogenase specific activity is expressed relative to wild-type level, corresponding to 78 nmole NADH formed per hour per mg protein. (a) Due to the temperature-shift the specific activity of histidinol dehydrogenase increases to the steady-state level of 41° as shown by -o-o-. Addition of rifampicin stops histidinol dehydrogenase synthesis, -●-●-. (b) Cultures were starved for 36 min at an A_{490} of 0.300 for leucine and leucine (40 μg/ml) was readded. To one of the cultures rifampicin was added at the beginning of starvation, -●-●-, to the other 2 min prior to leucine addition, -x-x-.

An explanation compatible with these data is that during amino acid starvartion the constitutive synthesis of histidine mRNA continues and the accumulated message is translated after readdition of the amino acid. Thus the size of the burst should reflect the amount of message accumulated.

In this respect it is significant that the amount of histidinol dehydrogenase synthesized during the burst is of the same magnitude as would have been synthesized during the starvation period had no starvation occurred (fig. 1).

This explanation could be tested by the application of the RNA polymerase inhibitor rifampicin [14]. Rifampicin (CIBA) was applied at a concentration of 60 μg/ml. The efficiency of rifampicin was shown by instantaneous and complete blockage of histidinol dehydrogenase synthesis in exponentially growing cultures (fig. 2a). Following a period of amino acid starvation rifampicin was added and allowed to act for 2 min before readdition of the amino acid. As can be seen in fig. 2b, in this case there is histidinol dehydrogenase synthesis. On the other hand if the mutant is starved for the same time but rifampicin is added at the beginning of starvation no histidinol dehydrogenase synthesis is observed. These experiments indicate the histidinol dehydrogenase synthesized in the presence of rifampicin was translated from preexisting mRNA accumulated during amino acid starvation and suggest that transcription of the constitutive histidine operon continues during amino acid starvation.

It is known that transcription of operons released physiologically from their specific control continues during amino acid starvation [2–6, 9–12]. The results presented in this article suggest that the same may hold for constitutive systems.

Experiments with tryptophan constitutive mutants starved for amino acids other than tryptophan, however, found decreased levels of *trp* messenger [7–8], in both RC^{str} and RC^{rel} strains. The basis and generality of this depression in *trp* mRNA synthesis is not known but it is not mediated by the *rel* gene.

It seems probable, that during amino acid starvation synthesis of the different mRNA species is not controlled by the *rel* gene. Changes in pattern of mRNA synthesis might be due to repression by biosynthetic intermediates accumulated during starvation.

References

[1] G. Edlin and P. Broda, Bacteriol. Rev. 32 (1968) 206.
[2] G. Edlin, G.S. Stent, R.F. Baker and C. Yanofsky, J. Mol. Biol. 37 (1968) 257.
[3] R. Lavallé and G. De Hauwer, J. Mol. Biol. 37 (1968) 269.
[4] P. Venetianer, J. Mol. Biol. 45 (1969) 375.
[5] R. Faanes and P. Rogers, J. Bacteriol. 96 (1968) 409.
[6] W.L. McLellan and H.J. Vogel, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1703.
[7] J.D. Stubbs and B.D. Hall, J. Mol. Biol. 37 (1968) 303.
[8] J.D. Stubbs and E.A. Stubbs, J. Mol. Biol. 51 (1970) 717.
[9] A. Kepes, Biochim. Biophys. Acta 76 (1963) 293.
[10] D. Nakada and B. Magasanik, J. Mol. Biol. 8 (1964) 105.
[11] A. Kepes and S. Beguin, Biochim. Biophys. Acta 123 (1966) 546.
[12] D.W. Morris and N.O. Kjeldgaard, J. Mol. Biol. 31 (1968) 145.
[13] L. Patthy and G. Dénes, Biochem. Biophys. Res. Commun. 43 (1971) 1246.
[14] G. Hartmann, K.O. Honikel, F. Knüsel and J. Nüesch, Biochim. Biophys. Acta 145 (1967) 834.