Effect of L-methioninyl adenylate on the level of aminoacylation in vivo of tRNA^{Met} from *Escherichia coli* K12

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ABSTRACT

In cells of *E. coli* K12 grown exponentially in minimal medium, tRNA^{met}, tRNA^{leu} and tRNA^{ile} are aminoacylated at 100 %, 80 % and 64 %, respectively.

On addition of L-methioninyl adenylate to the growth medium, one observes a specific deacylation of tRNA^{met}. When more than 35 % of tRNA^{met} is deacylated, growth rate is reduced and becomes proportional to the amount of methionyl-tRNA formed.

INTRODUCTION

In a previous report¹ we have shown that aminoalkyl adenylates, which are very specific potent inhibitors of aminoacyl-tRNA synthetases in vitro, can operate also in vivo, since they inhibit growth of *E. coli* K12. In the present communication we demonstrate that L-methioninyl adenylate (L-met-ol-AMP) acts in vivo by inhibiting the synthesis of methionyl-tRNA. Furthermore, using this inhibitor, the relation of growth rate to the aminoacylation level of tRNA^{met} has been determined.

MATERIAL AND METHODS

Material

L-methioninyl adenylate prepared as described previously¹ was a gift of Dr M. Robert-Gero. Pure leucyl-tRNA synthetase of *E. coli* K12 was kindly provided by Dr F. Chapeville and partially purified isoleucyl-tRNA synthetase of *E. coli* K12 was a gift of R. Taglang. The methionyl-tRNA synthetase preparation used was purified by the usual procedure².

Bacterial strain and growth conditions

The *E. coli* K12 strain used as reference was Hfr H. This strain was grown at 37°C in minimal medium 63³ with 0.4 % glucose and thiamine at 0.5 mg/litre. Overnight cultures in exponential phase were diluted at approximately 10⁸ cells/ml with

fresh medium containing the antimetabolite at the appropriate concentration or other compounds according to the experiment. Growth was monitored by absorbance at 420 nm.

Determination of the fraction of tRNA aminoacylated in vivo.

The extraction and subsequent treatment for measurement of the levels of aminoacylated tRNA in vivo were performed by the method of Folk and Berg⁴ essentially as described by Lewis and Ames⁵. The procedure involves precipitation of cells (from 300 ml at approximately 10^9 cells/ml) with trichloroacetic acid ; tRNA is extracted from the precipitate by sonication in phenol followed by ethanol precipitation. A portion of the tRNA is treated with periodate to inactivate those tRNA molecules which are not esterified with amino acids and then both the oxidized and unoxidized tRNAs are incubated 2h at 37° in 3.6 M Tris at pH 8.2 to remove esterified amino acids.

Acceptor activity of these tRNA preparations was determined by measuring the amount of (^{14}C) amino acid which could be esterified at 30° in 30 min. with excess of aminoacyl-tRNA synthetase. The assay mixture (0.1 ml) was essentially that used by Fangman and Neidhardt⁶ and contained 0.1 mM (^{14}C) amino acid (specific activity 50-90 mc/mM) and 0.5 to 5.0 A_{260} units of RNA samples as acceptor.

The fraction of a particular tRNA that is aminoacylated in vivo is inferred from the ratio of the specific acceptor activity (picomoles of amino acid per A_{260} unit) of the oxidized RNA to that of the unoxidized RNA. The average total acceptance activity of the RNA preparations examined was 7.9 picomoles of tRNA^{met}, 8.1 picomoles of tRNA^{ile} and 11.5 picomoles of tRNA^{leu}, per A_{260} unit of extract.

RESULTS

To determine if the effect of methioninyl adenylate in vivo is due to its action on methionyl-tRNA synthetase we have measured the aminoacylation level of tRNA^{met} in E.coli cells grown in absence and in presence of L-met-ol-AMP. The concentration of L-met-ol-AMP used was chosen to partially inhibit growth. Moreover the level of aminoacylation of two others tRNAs was determined as control. As shown in Table 1 exponentially growing cells of E.coli K12 have 100 % of their tRNA^{met} aminoacylated.

The presence of 0.11 mM L-met-ol-AMP in the growth medium promotes a 55 % decrease of the aminoacylation level of tRNA^{met}, while those of tRNA^{leu} and tRNA^{ile} are unaffected, as was expected.

Table 1 : In vivo levels of aminoacylated tRNA^{met}, tRNA^{ile} and tRNA^{leu} in strain Hfr H grown under different conditions.

Addition to the culture	Growth rate (%)	Level of aminoacylated tRNA (%)		
		tRNA ^{met}	tRNA ^{ile}	tRNA ^{leu}
none	100*	98	66	-
		98	65	80
		100	63	-
0,11 mM L-met-ol-AMP	69	45	63	79
3 µg/ml chloromycetine	49	98	68	81

*growth rate of 100 % corresponds to a generation time of 70 min.

The possibility was considered that the specific discharge of tRNA^{met}, observed in cells grown in presence of L-met-ol-AMP, was a consequence of the reduction of growth rate. This hypothesis was tested as follows : we have searched conditions where the growth of E.coli K12 was reduced but maintained at a constant rate as with L-met-ol-AMP. This was obtained by use of chloromycetine which inhibits growth of E.coli K12 as do aminoalkyl adenylates. As shown in Table 1 no deacylation of tRNA^{met} nor of tRNA^{ile} or tRNA^{leu}, was observed in cells the growth rate of which was reduced by half with chloromycetine. It appears therefore that L-met-ol-AMP acts directly on the cellular concentration of methionyl-tRNA^{met} by inhibiting methionyl-tRNA synthetase.

The preceeding results indicate that the level of tRNA^{met} aminoacylated in vivo is more sensitive to the action of L-met-ol-AMP than is the growth rate, suggesting that one can partially deacylate tRNA^{met} without affecting growth. This was confirmed by measuring growth rate and level of aminoacylation of tRNA^{met} of cells grown in medium supplemented with different

concentrations of L-met-ol-AMP.

As shown in Fig.1A, increasing the L-met-ol-AMP concentration of the growth medium results in a concomitant decrease of the aminoacylation level of tRNA^{met} and of growth rate. Under these conditions the levels of aminoacylation of tRNA^{leu} and tRNA^{ile} remain unchanged (Fig.1B). In addition, as long as the concentration of L-met-ol-AMP is below 0.05 mM, growth rate is unaffected, while the tRNA^{met} is already partially deacylated. If one represents the variation of growth rate as a function of the level of methionyl- tRNA^{met} (Fig.2), it is clear that when more than 35 % of tRNA^{met} is deacylated, the growth rate is reduced and becomes directly proportional to the fraction of tRNA^{met} aminoacylated in vivo.

These data clearly establish that the site of action of L-met-ol-AMP in vivo is methionyl-tRNA synthetase and the bacteriostatic power of this product is essentially, if not exclusively, a consequence of its action on this enzyme.

DISCUSSION

In E.coli K12 cells grown exponentially in minimal medium tRNA^{met} is completely aminoacylated, while tRNA^{leu} and tRNA^{ile} are only aminoacylated at 80 % and 64 % respectively. These values were obtained with a high accuracy and are in good agreement with those found by Folk and Berg (tRNA^{leu} aminoacylated at 76 %⁴) and by Lewis and Ames (tRNA^{met} aminoacylated at 95 %⁵). The level of aminoacylation of the three tRNAs examined does not change when the growth rate is reduced by half with chloromycetine ; this implies that the limiting factor for the aminoacylation of tRNA^{leu} and tRNA^{ile} is not the utilization rate of aminoacyl-tRNAs in protein biosynthesis.

Furthermore, using L-met-ol-AMP, it becomes possible to modulate specifically and accurately the aminoacylation level of tRNA^{met} in vivo. By this mean the relation of growth rate to the fraction of tRNA^{met} aminoacylated in vivo has been determined: the normal growth rate of the E.coli K12 strain examined is maintained as long as more than 65 % of tRNA^{met} is aminoacylated. Below this level of aminoacylation, growth rate is reduced and becomes directly proportional to the amount of methionyl-tRNA

Fig. 1

Fig. 2

formed. A similar result was obtained by Lewis and Ames⁵ during their studies of histidine regulation in Salmonella typhimurium: in this case the amount of aminoacylated tRNA^{his} in the wild type is at least twice that required to maintain the optimal growth rate in minimal medium.

The participation of some aminoacyl-tRNAs in the regulation of biosynthetic enzymes of the corresponding amino acids has been reported in different organisms. Furthermore aminoacyl-tRNAs also seem to be implicated in the regulation of the corresponding aminoacyl-tRNA synthetases. The best documented example is that of histidyl-tRNA in Salmonella typhimurium. In this case it is clearly demonstrated that there is a direct correlation between the absolute amount of histidyl-tRNA^{his} in vivo and the repression of the his operon⁵; in addition, according to Mc Ginnis and Williams, the histidyl-tRNA is involved in the repression of synthesis of histidyl-tRNA synthetase⁷.

In the case of methionyl-tRNA its participation in the

regulation of methionine biosynthesis has been reported in Saccharomyces cerevisiae⁸ and almost ruled out in Escherichia coli K12⁹, and Salmonella typhimurium¹⁰; however in the three organisms the aminoacylation of tRNA^{met} seems to be a necessary step in repressional control of methionyl-tRNA synthetase^{8,11}.

The study of the role of aminoacyl-tRNAs in these repression mechanisms has proven difficult and laborious since it requires obtention and characterization of mutants (regulatory mutants, mutants of aminoacyl-tRNA synthetases or auxotrophic strains). Considering their specific action in vivo, amino-alkyl adenylates may prove extremely useful in investigations on the possible involvement of aminoacyl-tRNAs in different regulation processes. In addition, L-met-ol-AMP is of particular interest since, as described in this communication, it inhibits the synthesis of methionyl-tRNA in vivo and consequently affects protein biosynthesis, not only at the level of chain elongation, but also at the level of chain initiation.

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