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STUDIES ON METHIONYL-tRNA SYNTHETASE

III. ENZYME DEPENDENCE FOR MAXIMUM METHIONYL-tRNA FORMATION

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SUMMARY

1. In the heterologous reaction between methionyl-tRNA synthetase (L-methionine:tRNA ligase (AMP), EC 6.1.1.10) from *Saccharomyces cerevisiae* and tRNA from *Escherichia coli*, the final yield of methionyl-tRNA varies with the enzyme concentration.

2. The yield is also influenced by the presence of monovalent cations.

INTRODUCTION

During the work on activation of methionyl-tRNA synthetase (L-methionine: tRNA ligase (AMP), EC 6.1.1.10) from yeast[1,2] it was frequently observed that the enzyme does not show the expected kinetic behaviour in the heterologous reaction with tRNA from *Escherichia coli*. The reaction rate decreases slightly with time and the maximum incorporation of methionine into tRNA depends on the amount of enzyme present in the reaction volume. Some experiments to characterize this phenomenon are presented in this paper.

MATERIALS AND METHODS

Yeast methionyl-tRNA synthetase was prepared from *Saccharomyces cerevisiae*, strain C836, as described by BERG[3]. Fraction AS-I, dialyzed against 0.01 M Tris–HCl, (pH 7.3), was used throughout this study.

tRNA was prepared from *E. coli* B as previously described[1,2]. The assay for methionyl-tRNA formation was performed at 37°. The reaction mixture contained 0.1 M Tris–HCl (pH 7.3), 2 mM ATP (disodium), 0.1 mM EDTA (dipotassium), 0.02 M $MgSO_4$, 12 μM L-[*Me*-^{14}C]methionine with a specific activity of 15.6 mC/mmole, and different amounts of enzyme and tRNA as indicated in the figures. Aliquots of 0.2 ml were taken out at different times and processed as described earlier[1,2].

The amount of methionine incorporated into tRNA is expressed as uncorrected

counts/min unless otherwise stated. Other methods and materials have been described in the preceding papers[1,2].

RESULTS

In the heterologous, and hence unphysiologic, reaction between yeast methionyl-tRNA synthetase and *E. coli* tRNA, the maximum amount of methionyl-tRNA formed varies with the amount of enzyme present. Fig. 1 shows 4 time curves for the reaction with a 10-fold variation in enzyme concentration. In all 4 cases plateaus are obtained within 50 min reaction time. The apparent magnitude of the plateau increases with increasing enzyme concentration. Part of this effect is due to an increase in blank values as more enzyme is added to the reaction mixture. These blank values

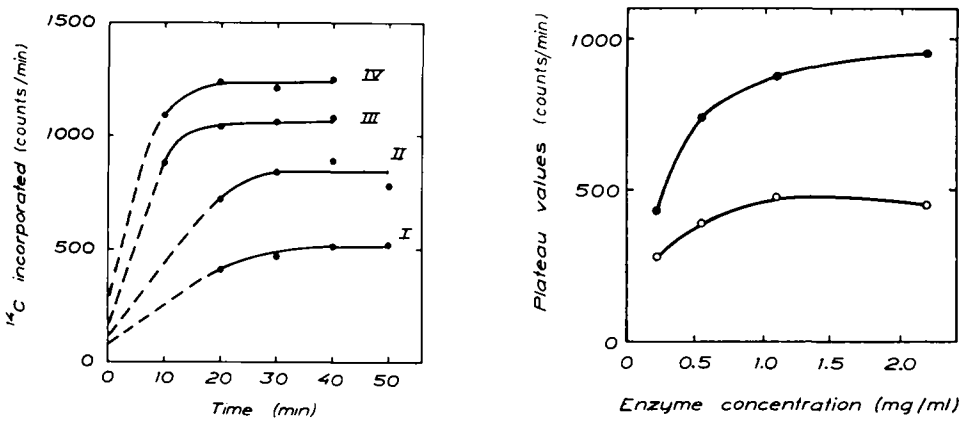

Fig. 1. Time curves for the formation of *E. coli* methionyl-tRNA with yeast methionyl-tRNA synthetase. The reaction mixture contained 1.6 mg of tRNA per ml and varying amounts of enzyme: I, 0.22; II, 0.55; III, 1.1; and IV, 2.2 mg of protein per ml. Aliquots of 0.2 ml were taken out at different times. The curves are drawn to zero-time blank values determined in other experiments.

Fig. 2. Maximum incorporation of methionine into *E. coli* tRNA as a function of yeast methionyl-tRNA synthetase concentration. The upper curve (●—●) represents the plateau values in Fig. 1 and the lower curve (○– ○) plateau values from a parallel experiment with 0.8 mg per ml of tRNA. All values have been corrected for blank values obtained in parallel experiments identically performed except that tRNA was omitted.

have been determined in separate experiments with the same reaction mixtures except for omission of tRNA, incubated for 30 min. The blank values have been indicated in Fig. 1 by dashed lines to zero time.

The plateau values obtained in the experiment of Fig. 1 and in a similar experiment, all corrected for appropriate blanks, are shown in Fig. 2 as a function of enzyme concentration. It is evident from these curves that maximum incorporation of methionine into *E. coli* tRNA is not achieved unless the enzyme concentration exceeds 1 mg per ml of protein.

One possible explanation for this phenomenon might be that the enzyme is unstable under the conditions of incubation. The low plateau values obtained with low

concentrations of enzyme should then indicate that inactivation of the enzyme was completed before saturation of tRNA had been attained. This possibility was tested by addition of more tRNA after the plateau was reached. As can be seen from Fig. 3, this addition results in increased incorporation of methionine up to a higher level with almost the same rate as in the initial phase of the experiment. The enzyme therefore seems to have retained almost full activity during the time of incubation.

Fig. 3 also shows the effect of other additions to the reaction mixture at 30 min, when the low plateau had formed. More enzyme added at this point caused an increase of the plateau in accordance with the data of Fig. 2, while additional amounts of ATP or methionine had no effect, excluding the possibility that these substances had become limiting during the reaction.

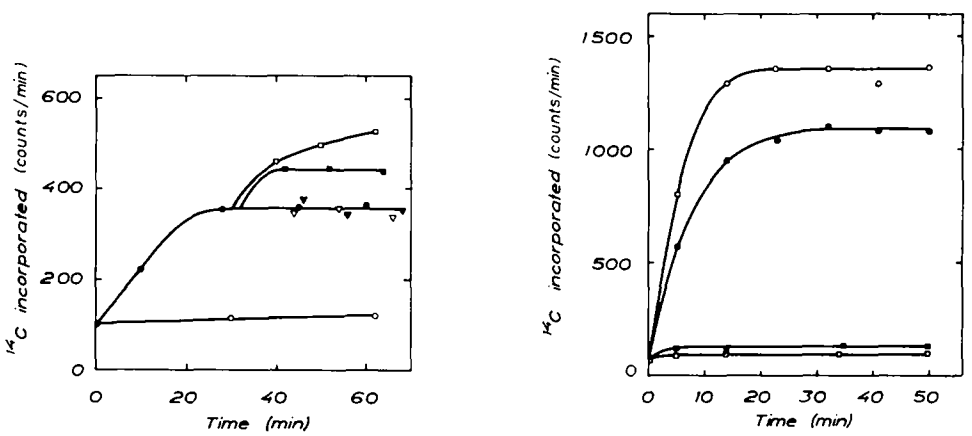

Fig. 3. Effect of added reactants on the maximum formation of *E. coli* methionyl-tRNA with yeast methionyl-tRNA synthetase. The reaction mixture for the control curve (●—●) contained 0.8 mg of tRNA per ml and 0.22 mg of protein per ml. The reaction mixture for the blank curve (○—○) had the same composition except that tRNA was missing. After about 30 min, aliquots of 0.2 ml were taken out for continued incubation with different additions: □ –□, 40 μl of tRNA (0.16 mg); ■—■, 20 μl of enzyme (0.04 mg of protein); ▽ –▽, 40 μl of 0.01 M ATP; ▼—▼, 10 μl of 0.3 mM L-[*Me*-^{14}C]methionine (15.6 mC/mmole).

Fig. 4. Effect of NH_4^+ on the maximum formation of *E. coli* methionyl-tRNA with yeast methionyl-tRNA synthetase. The reaction mixture contained 1.6 mg of tRNA per ml and 0.73 mg of protein per ml. ●—●, no NH_4^+ added; ○—○, 100 mM NH_4^+; ■—■, blank curve without tRNA and NH_4^+; □ –□, blank curve without tRNA but with 100 mM NH_4^+ added.

In a previous paper[1] it was demonstrated that the rate of methionyl-tRNA synthesis with the yeast enzyme was greatly stimulated by NH_4^+ or K^+. It was also found that the final yield of methionyl-tRNA was essentially the same whether or not the monovalent ions were added to the reaction mixture. These experiments were performed with yeast tRNA. If *E. coli* tRNA is used instead, the maximum yield of reaction product with the yeast enzyme at a moderately low concentration is to some extent influenced by monovalent cations. Fig. 4 shows that, in the presence of 0.1 M NH_4^+, the plateau value increases by about 30%, while the blank value is almost unaffected. A similar increase takes place if NH_4^+ is added to the reaction mixture after the low plateau has been reached, analogous to the addition of enzyme at 30 min in Fig. 3.

In other experiments, K^+ has been shown to influence the plateau level to some extent, causing a 20% increase at a concentration of 0.1 M, while Na^+ at the same concentration lowers the plateau with about 30%. All these experiments have been performed in the presence of Tris buffer. The possible effect of $Tris^+$ alone, however, has not been investigated.

DISCUSSION

Reactions between aminoacyl-tRNA synthetases and tRNA ordinarily show the expected kinetic behaviour with a rate phase which is dependent on enzyme concentration and a final yield phase (plateau) independent of enzyme concentration, but related to the amount of tRNA. The results in this paper show that yeast methionyl-tRNA synthetase does not behave in the predicted way when catalyzing the incorporation of methionine into *E. coli* tRNA. The maximum amount of methionyl-tRNA synthesized is clearly a function of the amount of enzyme present, as shown in Fig. 2.

A similar phenomenon has been reported by ZILLIG, SCHACHTSCHABEL AND KRONE[4], who found a stoichiometric proportionality between the maximum amount of *E. coli* leucyl-tRNA formed and the amount of *E. coli* leucyl-tRNA synthetase used. This finding was disputed by BERG *et al.*[5], who studied the attachment of leucine and other amino acids to *E. coli* tRNA, using purified enzymes from the same organism. They observed the final yield of aminoacyl-tRNA to be independent of enzyme concentration. An early finding that the yield of valyl-tRNA varied with enzyme concentration was explained as due to inactivation of the enzyme. This could be prevented with suitable protecting agents.

This trivial explanation does not, however, seem to hold for the yeast methionyl-tRNA synthetase acting on *E. coli* tRNA. Inactivation of the enzyme would be in conflict with the increase in yield obtained after addition of more tRNA (Fig. 3).

Also, the low yield of reaction product is not a result of degradation or inactivation of tRNA, since addition of more enzyme after attaining the plateau raises the net yield of methionyl-tRNA.

One possibility might be the presence of some factor in yeast necessary for complete aminoacylation of heterologous tRNA. MAKMAN AND CANTONI[6] have shown that, in the heterologous reaction between *E. coli* tRNA and a highly purified seryl-tRNA synthetase from yeast, the final yield is lower than with the *E. coli* enzyme but can be increased by addition of an "enhancing factor" isolated from yeast.

Another possibility would be that the enzyme enters into a complex with the heterologous methionyl-tRNA and that this complex plays an important role in establishing an equilibrium between substrates and product. This idea requires that the enzyme and the product at equilibrium are present in molar quantities of the same magnitude. It can be estimated, assuming a purity of 5% and a molecular weight of about 100 000, that in one of the experiments of Fig. 1, 0.06 mμmole of enzyme was present. The final amount of product formed was 0.05 mμmole. The requirement for equimolarity therefore seems to be fulfilled.

It is interesting that the monovalent cations K^+ and NH_4^+ increase the final yield of methionyl-tRNA, while Na^+ has a negative effect. These results are in accordance with the data given by RUBIN, KELMERS AND GOLDSTEIN[7], who have made a careful study of factors influencing the maximum yield of leucyl- and phenylalanyl-

tRNA with a crude enzyme preparation from *E. coli*. They found that the yield of leucyl-tRNA is decreased by 30% when Tris buffer is used and increased by 10% in the presence of 5 mM NH_4^+, while the yield of phenylalanyl-tRNA decreases 7% with Na^+ and increases 7% with K^+ or NH_4^+, all 5 mM.

A similar effect has been reported by PETERKOFSKY, GEE AND JESENSKY[8]. These authors found that yeast leucyl-tRNA synthetase does not charge *E. coli* tRNA to completion if NaCl, KCl, NH_4Cl or Tris–Cl are present in concentrations exceeding 0.05 M. When these salts are added to the reaction mixture, the final yield of leucyl-tRNA becomes dependent on enzyme concentration. The explanation is offered that the enzyme is slowly modified so that it no longer recognizes the heterologous tRNA.

The present study on methionyl-tRNA synthetase and the studies cited from the literature show that deviations from the expected kinetic behaviour are not too infrequent and may occur for many reasons. Since phenomena of this kind may be significant in the interpretation of results from plateau experiments, they deserve continued attention.

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