

## PURIFICATION AND PROPERTIES OF METHIONYL-tRNA SYNTHETASE FROM *E. COLI* K 12 CARRYING THE F32 EPISOME

Doris CASSIO and Jean-Pierre WALLER

*Laboratoire d'Enzymologie du C.N.R.S., 91 - Gif-sur-Yvette, France*

Received 7 December 1970

### 1. Introduction

In a previous report [1], the specific activity of aminoacyl-tRNA synthetases was examined in *E. coli* strains presumed to be diploid for the structural gene of methionyl-tRNA synthetase. Of eighteen different aminoacyl-tRNA synthetases tested, all but one showed similar levels of activity in both the merodiploids strains carrying the F32 episome and the haploid strain of reference Hfr H. The single exception was methionyl-tRNA synthetase which, unexpectedly, was present in the merodiploids at a four fold higher level than in the haploid strain, regardless of growth conditions.

In the present report, the purification and several properties of methionyl-tRNA synthetase from the merodiploid strain EM 20031 carrying the F32 episome are described. By a number of criteria, including specific activity and antigenic properties, this enzyme appears to be undistinguishable from that purified from the haploid Hfr H strain. It is further shown that neutralisation of the methionyl-tRNA synthetase activity in an extract from strain EM 20031, by titration with antibodies directed against the purified enzyme, requires four times as much antibodies as in the case of an extract from strain Hfr H examined at the same protein concentration.

We conclude that the four fold increase in the level of methionyl-tRNA synthetase activity in the strains carrying the F32 episome is due not to the production of an enzyme with higher specific activity, but rather to an increase in net synthesis which may be ascribed to the quadruplicate state of the methionyl-tRNA synthetase structural gene, or to an alteration in the regulatory system of this enzyme.

### 2. Materials and methods

The merodiploid strain EN 20031 was kindly provided by Dr E. McFall. It was grown on minimal medium as described previously [1]. Methionyl-tRNA synthetase from the Hfr H strain was purified according to Lemoine et al. [2]. The methionine-dependent ATP-PP<sub>i</sub> exchange assay was performed as described earlier [2]. One unit of enzyme activity is defined as that amount incorporating 1  $\mu$ mole of <sup>32</sup>P-PP<sub>i</sub> into ATP in 15 min at 37°. The rate of methionyl-tRNA formation was measured in the presence of 2 mg/ml unfractionated *E. coli* B tRNA (Schwarz Bioresearch Inc.). Conditions were those described earlier, except that tris buffer at pH 7.4 was replaced by 100 mM potassium cacodylate buffer at pH 7.0. This resulted in a 1.6 fold increase in the rate of aminoacylation of tRNA. One unit of enzyme activity is defined as that amount insuring aminoacylation of 1  $\mu$ mole tRNA in 10 min at 30°.

For polyacrylamide gel electrophoresis in the absence and in the presence of 8 M urea, the tris buffer systems of Jovin et al. [3] were used. In order to estimate protein subunit molecular weights from electrophoretic runs in gels containing 8 M urea, the procedure of Hedrick and Smith [4] was adapted. Gel concentration was varied as indicated in fig. 1, maintaining a constant bisacrylamide to acrylamide ratio of 0.56 to 21.2 (w/w). The proteins used for calibration were *E. coli* isoleucyl-tRNA synthetase, bovine serum albumin, beef liver catalase, pig liver glutamic dehydrogenase, ovalbumin and yeast alcohol dehydrogenase. Details of this method will appear elsewhere [5].

Table 1  
Purification of methionyl-tRNA synthetase from *E. coli* K12 strain EN 20031 carrying the episome F32.

| Step                                   | Protein<br>(mg) | ATP- <sup>32</sup> PP <sub>i</sub> exchange assay |                                    |                 | Aminoacyl-tRNA formation     |                                    |                 | Ratio of<br>activation<br>vs. charge<br>(arbitrary<br>units) |
|--|-----------------|---|------------------------------------|-----------------|------------------------------|------------------------------------|-----------------|--|
|  |                 | Total<br>activity<br>(units)                      | Specific<br>activity<br>(units/mg) | Recovery<br>(%) | Total<br>activity<br>(units) | Specific<br>activity<br>(units/mg) | Recovery<br>(%) |  |
| Extract*                               | 52,500          | 320,000   | 6.1                                | 100             | 5,120                        | 0.08                               | 100             | 62   |
| Ammonium sulfate<br>fraction 30 to 50% | 16,800          | 249,000   | 14.8                               | 78              | 4,250                        | 0.25                               | 83              | 59   |
| Sephadex G-200<br>eluate               | 5,420           | 252,000   | 46.5                               | 79              | 3,900                        | 0.72                               | 76              | 65   |
| DEAE Sephadex<br>pH 8.1 eluate         | 1,052           | 259,000   | 246                                | 81              | 3,740                        | 3.55                               | 73              | 69   |
| DEAE Sephadex<br>pH 6.8 eluate         | 736             | 247,700   | 336                                | 77              | 3,590                        | 4.88                               | 70              | 68   |
| Hydroxylapatite<br>pH 6.8 eluate       | 215             | 219,000   | 1,018                              | 69              | 3,350                        | 15.6                               | 65              | 65   |
| Hydroxylapatite<br>pH 7.5 eluate       | 158             | 190,500   | 1,210                              | 59              | 3,110                        | 19.6                               | 60              | 62   |

\* 800 g of cells (wet weight) were used.

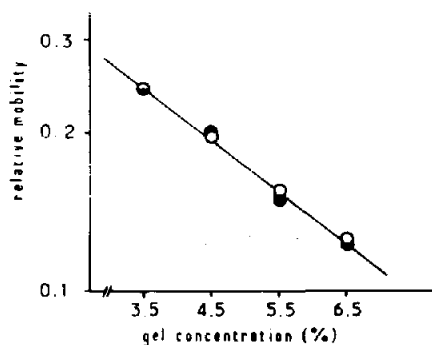


Fig. 1. Comparison by electrophoresis on acrylamide gels in 8 M urea of the subunits of methionyl-tRNA synthetase purified from strains EM 20031 and Hfr H. Each purified enzyme was dialyzed for 24 hr against 8 M urea in 200 mM tris buffer at pH 9.0 containing 150 mM 2-mercaptoethanol. Samples containing 30  $\mu$ g of protein were submitted to electrophoresis in gels of variable concentration as described in Methods. The logarithm of the protein mobility relative to the dye front was plotted versus the gel concentration. Methionyl-tRNA synthetase from EM 20031 ( $\circ$ — $\circ$ ); methionyl-tRNA synthetase from Hfr H ( $\bullet$ — $\bullet$ ).

For the preparation of antiserum, rabbits were immunized by a primary subcutaneous injection of 1 mg of homogeneous methionyl-tRNA synthetase purified from strain EM 20031, followed by two additional injections of 1 mg enzyme, three and six weeks later, respectively. The unfractionated serum was stored at  $-15^{\circ}$  and samples were dialyzed against 20 mM potassium phosphate buffer at pH 7.5 before use.

Crude extracts were prepared as described earlier [1]. Protein concentration was determined by the method of Lowry et al. [6], using bovine serum albumin as the standard.

### 3. Results and discussion

#### 3.1. Purification of methionyl-tRNA synthetase from *E. coli* strain EM 20031

The procedure developed for the purification of the enzyme from the Hfr H strain [2] was followed

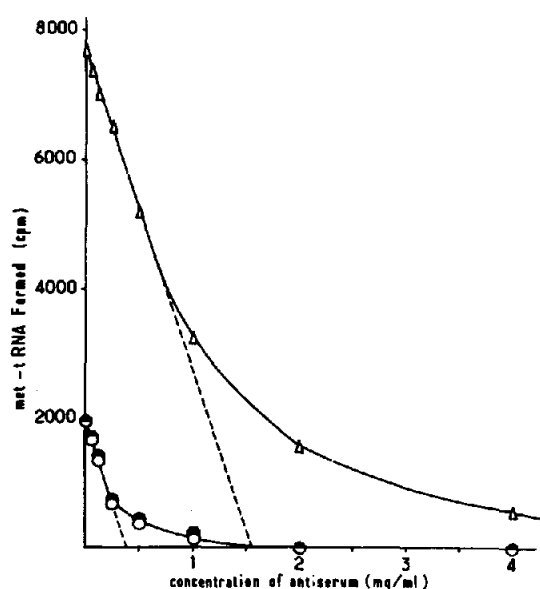


Fig. 2. Neutralisation of the methionyl-tRNA synthetase activity in crude extracts from *E. coli* K12 strains EM 20031 and Hfr H, using antiserum prepared against purified enzyme from strain EM 20031. Purified methionyl-tRNA synthetase from strain EM 20031 (0.5  $\mu$ g/ml) and crude extracts from strains EM 20031 and Hfr H (each at a protein concentration of 0.54 mg/ml in 20 mM potassium phosphate buffer at pH 7.6 containing 200  $\mu$ g/ml bovine serum albumin and 5 mM 2-mercaptoethanol) were incubated at 4° for 2 hr with varying amounts of anti-serum prepared against purified methionyl-tRNA synthetase from EM 20031. Aliquots were removed and after the same appropriate dilution, methionyl-tRNA formation was measured. Methionyl-tRNA synthetase activity of a crude extract from strain EM 20031 ( $\triangle$ - $\triangle$ ) and Hfr H ( $\bullet$ - $\bullet$ ); purified methionyl-tRNA synthetase from EM 20031 ( $\circ$ - $\circ$ ).

in all details, except for the following improvements: cells were ruptured with a Gaulin 15M-8TA Lab Homogenizer, which resulted in a close to two fold higher yield of protein and enzyme, compared to that obtained by the sonication procedure previously used; to avoid handling of large volumes during dialysis steps, pools of active fractions obtained at various stages of the purification were first concentrated in an Amicon Model 401 Ultrafiltration Cell equipped with a Diaflo PM-10 membrane.

The purification procedure is summarized in table 1. The overall recovery of enzyme activity was close to 60%, corresponding to a yield of 190 mg purified enzyme/kg of wet weight cells. Closely similar yields

Table 2  
Specific activities of purified methionyl-tRNA synthetase preparations from *E. coli* K12 strains Hfr H and EM 20031.

| Enzyme source   | ATP-PP <sub>i</sub> exchange assay (units/mg) | Aminoacyl-tRNA formation (units/mg) |
|-----------------|---|-------------------------------------|
| Strain Hfr H    | 1,080   | 17.6                                |
|                 | 1,170   | 19.1                                |
| Strain EM 20031 | 1,210   | 19.6                                |
|                 | 1,160   | 19.7                                |

Each preparation was performed using approximately 1 kg of cells (wet weight); recovery of enzyme activity was within the range of 50-60%.

were obtained from three independent preparations. As shown in table 1, the activity ratio of the two reaction catalyzed by the enzyme did not vary significantly during the purification. Furthermore, the behaviour of the enzyme at each step of the purification was undistinguishable from that of the enzyme derived from strain Hfr H, and no evidence was obtained for the existence of more than one kind of methionyl-tRNA synthetase.

### 3.2. Comparative properties of purified methionyl-tRNA synthetase isolated from strains EM 20031 and Hfr H.

Methionyl-tRNA synthetase purified from strains EM 20031 and Hfr H had the same apparent molecular weight of 180,000, estimated by gel filtration through a calibrated column of Sephadex G-200. Furthermore, their mobilities were undistinguishable by acrylamide gel electrophoresis performed in the absence and in the presence of 8 M urea. Fig. 1 shows a plot of the log of protein mobility relative to the dye front ( $R_m$ ) versus gel concentration, in the presence of 8 M urea. The purified enzyme from both sources gave rise to only a single band with identical  $R_m$ , corresponding to a molecular weight of 43,000, in agreement with the known molecular weight of the subunits of methionyl-tRNA synthetase [2,7].

As shown in table 2, no differences were found in the specific activities of methionyl-tRNA synthetases purified from strains EM 20031 and Hfr H. Moreover, the two enzymes were undistinguishable in their kinetic parameters ( $K_m$  or  $K_i$  and  $V_{max}$ ) for meth-

ionine, ATP, tRNA<sup>Met</sup> and the competitive inhibitor adenylyate, and both showed the same divalent ion requirement for the two reactions catalyzed by the enzyme.

Additional evidence for the identity of the methionyl-tRNA synthetase from strains EM 20031 and Hfr H was obtained from a comparison of their antigenic properties towards antibodies directed against the purified enzyme from strain EM 20031. As shown in fig. 2, the level of methionyl-tRNA synthetase activity in a crude extract from strain EM 20031 was four fold higher, as expected [1], and required four times as much antibodies for neutralisation, compared to an extract from strain Hfr H at the same protein concentration. Moreover the neutralisation curves of methionyl-tRNA synthetase activity from strain EM 20031 by these antibodies are similar, whether the enzyme is purified or not (fig. 2).

#### 4. Conclusion

Taken together, the present findings clearly demonstrate that the four fold increase in the level of methionyl-tRNA synthetase activity found in the merodiploid strain EM 20031 compared to that in strain Hfr H, results from an increase in the net synthesis of the enzyme, rather than from the production of an enzyme with higher specific activity.

#### Acknowledgements

The excellent technical assistance of Y. Mathien is gratefully acknowledged. We thank Dr M. Kaminsky for the preparation of the antisera used in this work. This investigation was supported by grants from the Délégation à la Recherche Scientifique et Technique (Convention 66 00 140) and from the Commissariat à l'Energie Atomique (France).

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