CHANGE OF ISOACCEPTING THREONYL-tRNA AND CONSTITUTIVELY INCREASED LEVEL OF THREONYL-tRNA-SYNTHETASE IN *E. COLI*

Jürgen THOMALE and Gisela NASS

Max-Planck-Institut für experimentelle Medizin, Abt. Molekulare Biologie, 34 Göttingen, Hermann-Rein-Strasse 3, West Germany

Received 14 May 1975

1. Introduction

The level of an aminoacyl-tRNA-synthetase in bacteria can be increased reactively in answer to specific environmental conditions or constitutively due to mutation (cf. [1,2] and [3–6]). In neither case the molecular reactions determining the intracellular level of an aminoacyl-tRNA-synthetase are fully understood.

We have described mutants of *E. coli* K12, in which the level of wildtype threonyl-tRNA-synthetase (ThrRS) is increased constitutively by a factor of three to ten, depending on the mutant [4,7]. Since in many respects aminoacyl-tRNA-synthetases and tRNA can be looked at as a functional unit, we investigated the $tRNA^{Thr}$ in one of our mutants, which has a five-fold increased level of ThrRS. Here we show that this intracellular increase of wildtype ThrRS is accompanied by a specific change in the amount of the three threonine isoaccepting tRNA's.

2. Materials and methods

2.1. *Bacterial organisms and growth conditions*

The *E. coli* wildstrain used was *E. coli* K12B [8,9]. From this strain the Borrelidin resistant mutant Bor Res 3 had been isolated without using any mutagen [4]. The cells were grown in minimal medium at 37°C as previously described [10]. For isolation of tRNA the cells were grown under identical conditions but the volume of the culture was increased to 100 liters and the cells were always harvested in their logphase of growth at an A_{420} nm not higher than 1200.

2.2. *Determination of the activity of aminoacyl-tRNA-synthetase*

Cell-free extracts were prepared as previously described [7]. For aminoacylating the tRNA of K12B or Bor Res 3, a RNA-free aminoacyl-tRNA-synthetase fraction was used, which had been prepared according to Traub et al. [11] as previously described [7], however now the DEAE-step [11] was included into the procedure. The specific activity of the ThrRS and PheRS was determined as described [7]. The experimental conditions for determining the specific activity of the SerRS were the same as for the PheRS except that serine in a concentration of 1.5×10^{-4} M was used. One unit of enzyme activity is defined as the attachment of 1 μmol amino acid to tRNA per hour. The specific activity is expressed as enzyme units per mg protein.

2.3. *Isolation of tRNA*

tRNA of K12B and Bor Res 3 was isolated according to the method of Zubay [12], with the modifications described by Kelmers [13]. However, as reported for $tRNA^{His}$, $tRNA^{Arg}$ and $tRNA^{Val}$ [14], the stripping step was omitted since in control experiments stripped and unstripped tRNA gave the same amount of acceptance for the investigated amino acids Phe, Ser and Thr. The tRNA was prepared in portions of 30 g wet weight of cells, with a yield of 55 mg total tRNA, an absorbance of 23.0 at wavelength of 260 nm being equal to 1 mg tRNA.

2.4. *Separation of isoaccepting tRNA*

Isoaccepting $tRNA^{Thr}$ and isoaccepting $tRNA^{Ser}$ were separated by the BD-cellulose urea method

developed by Fittler et al. [15]. The BD-cellulose was purchased from Serva (Heidelberg, W. Germany) and the urea from Baker (Deventer, Netherlands). A water jacketed column (6 × 30 mm) was used. The column was equilibrated with 0.15 M NaCl containing 7 M urea and the pH had been adjusted to 3.0 with HCl. A total of 1.4 mg tRNA was applied to the column as described in the result section. After placing the tRNA on the column, it was washed with 150 ml of the buffer used for equilibration. Then the column was eluted with a linear gradient (120 ml/120 ml) of 0.22 M NaCl to 0.50 M NaCl containing 7 M urea and pH 3.0 adjusted with HCl. The flow rate of the column was 10 ml/hr and the eluate was collected in 1.5 ml fractions. Throughout the chromatography the temperature of the column was kept constant at 24°C and the ionic strength was monitored continuously by a conductolyzer (LKB 5300 B). The radioactivity in the fractions was measured in aliquots of 1.0 ml which were put in 10 ml of Aquasol (New England, Boston, USA)

2.5. *Protein concentration*

The protein concentration was determined by the standard colorimetric method [16] using human serum albumin as standard protein.

3. Results and discussion

In order to obtain more information about the molecular events involved in the regulation of the level of ThrRS in *E. coli* K12, we investigated the $tRNA^{Thr}$ of our mutant Bor Res 3. The intracellular concentration of ThrRS in this mutant is constitutively increased by a factor of five and its ThrRS has been proven to be the wildtype ThrRS [4,7]. In table 1 it is shown that the total amount of $tRNA^{Thr}$ is the same in the parental strain K12B and in the mutant Bor Res 3. Thus the five-fold increased level of ThrRS in Bor Res 3 is not accompanied by an increase of total threonine accepting tRNA, which means that in Bor Res 3, in comparison to the parental strain, the relation ThrRS to $tRNA^{Thr}$ is increased by a factor of five (table 1).

Next we investigated whether the pattern of isoaccepting $tRNA^{Thr}$ is altered in Bor Res 3. The separation of the threonyl-tRNA of K12B on the BD-cellulose column resulted in three isoaccepting $tRNA^{Thr}$species (fig.1), whereby the first eluting peak contains more than 60% of the total $tRNA^{Thr}$. A resolution of isoaccepting $tRNA^{Thr}$ into three peaks of corresponding sizes of an *E. coli* K12 strain which was also grown in minimal medium has been described by Squires et al. [17]. The apparent difference in the sequence of the elution of the three isoaccepting $tRNA^{Thr}$ species is probably due to the fact that these authors used the technique of reversed phase chromatography in their studies [17]. When separating the isoaccepting $tRNA^{Thr}$ of Bor Res 3, an altered elution profile emerged (fig.1): There are also three isoaccepting $tRNA^{Thr}$ species but their relative amounts differ

Table 1
Specific activity of aminoacyl-tRNA-synthetases and isoaccepting $tRNA^{Thr}$ and $tRNA^{Ser}$ in K12B and Bor Res 3

	K12B	Bor Res 3
Aminoacyl-tRNA-synthetases (spec. act.)		
ThrRS	0.032	0.155
SerRS	0.185	0.165
PheRS	0.076	0.075
Total $tRNA^{Thr}$ (pmol/100 μg tRNA)	114	107
Total $tRNA^{Ser}$ (pmol/100 μg tRNA)	92	100
Isoaccepting $tRNA^{Thr}$ (%)		
peak 1	69	37
peak 2	21	33
peak 3	10	33
Isoaccepting $tRNA^{Ser}$ (%)		
peak 1	56	56
peak 2	44	44

Cell growth, preparation of crude extract, determination of the specific activity of aminoacyl-tRNA-synthetase and the isolation of total tRNA was performed as described in the method section. To determine the content of $tRNA^{Thr}$ and $tRNA^{Ser}$ in the total tRNA, limiting amounts of tRNA were used in several concentrations (100 μg–1000 μg), the threonine concentration was 4×10^{-4} M and as enzyme source 40 μg of the RNA-free aminoacyl-tRNA-synthetase containing fraction of K12B was used, all the other experimental conditions were the same as for the determination of the activity of ThrRS or SerRS, as described in the method section. The percentage of isoaccepting $tRNA^{Thr}$ and isoaccepting $tRNA^{Ser}$ species are calculated from the elution profile in fig.1 and fig.2 respectively.

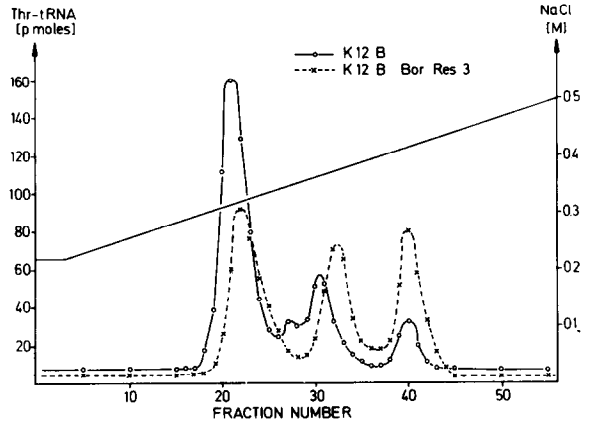

Fig.1. Separation of isoaccepting $tRNA^{Thr}$ of K12B and Bor Res 3. Growth of K12B and Bor Res 3, the isolation of the total tRNA of these cells and the operation of the BD-cellulose column was performed as described in the method section. A total of 1.4 mg tRNA was applied to the column, 0.7 mg of it being tRNA of K12B charged with [^{14}C] threonine and 0.7 mg being tRNA of Bor Res 3 charged with [^{3}H] threonine. The tRNA of K12B and Bor Res 3 were aminoacylated in separate incubation mixtures, the composition of the incubation mixtures was the same as described in the method section for determining the activity of the ThrRS, however the total volume was 2.0 ml, the threonine concentration was 4×10^{-4} M and as enzyme source, 160 μg of the RNA-free aminoacyl-tRNA-synthetase fraction of K12B were used. The incubation time was 10 min and the reaction was stopped by adding 8.0 ml of the buffer used for equilibrating the column plus 0.1 ml of 0.5 N HCl. Immediately thereafter, the two incubation mixtures were mixed and applied to the column. The vice versa charging of the tRNA (tRNA of K12B with [^{3}H] threonine and tRNA of Bor Res 3 with [^{14}C] threonine) resulted in an identical pattern of resolution of isoaccepting $tRNA^{Thr}$, which was also the case when using tRNA isolated from different batches of K12B and Bor Res 3 grown under identical conditions. However, the slight shift of peak 2 of Bor Res 3 in relation to peak 2 of K12B was not always observed.

drastically from the one of the parental strain insofar as only 37% of the total $tRNA^{Thr}$ is present in the first eluting peak and a relative increase of the $tRNA^{Thr}$ in peak 2 and peak 3 has occurred (fig.1, table 1).

Recently the primary structure of one of the three isoaccepting $tRNA^{Thr}$ has been determined and found to correspond to the codon ACU/C [18]. From the comparison of the elution profiles of $tRNA^{Thr}$ by Carbon [17] and the one presented here, we conclude that the sequenced $tRNA^{Thr}$ is the one which elutes as peak 2 from our BD-cellulose column. If this is correct, it would mean that in Bor Res 3 the $tRNA^{Thr}_{ACU/C}$ is increased from 21% to 33% of the total $tRNA^{Thr}$ in comparison to the wildtype K12B. The knowledge of the primary structure of the two other isoaccepting $tRNA^{Thr}$ will reveal whether different genes code for the $tRNA^{Thr}$ species and whether post-transcriptional modification is involved, which might then shed further light upon the biological meaning of the altered distribution of isoaccepting $tRNA^{Thr}$ in Bor Res 3.

As a control the pattern of isoaccepting $tRNA^{Ser}$ was determined in K12B and Bor Res 3 (fig.2); it is identical in both the parental and the mutant strain. A resolution of $tRNA^{Ser}$ into two main peaks had been observed by other authors [19].

There have been a few reports about the involvement of tRNA in the regulation of the level of the corresponding aminoacyl-tRNA-synthetase: In a mutant of *Salmonella typhimurium* in which the acceptor acitivity of total tRNA for histidine was found to be 50% of the one of the wildtype, a derepressed formation rate of histidyl-tRNA-synthetase was observed [20]. Furthermore when the formation rate of structurally altered methionyl-tRNA-synthetase (MetRS) was increased, the in vivo charging of total $tRNA^{Met}$ was reduced to about one-third [21]. The relationship of aminoacylated $tRNA^{Met}$ to the formation rate of

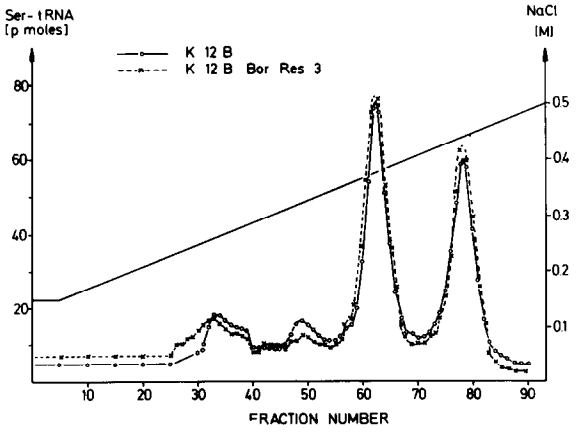

Fig.2. Separation of isoaccepting $tRNA^{Ser}$ of K12B and Bor Res 3. The experimental conditions were exactly those described for fig.1 but instead of threonine, serine was used in a concentration of 1.5×10^{-4} M.

wildtype MetRS has recently been thoroughly investigated and it has been demonstrated that in *E. coli* K12, a derepression of the formation rate of MetRS begins as soon as more than 25% of the $tRNA^{Met}$ is deacylated [6]. However when posing the same Met-tRNA deacylating conditions upon *E. coli* K12 strains which possess various constitutively increased levels of wildtype MetRS, no further increase of the intracellular levels of MetRS is observed, and in some such strains, even a 40% decrease of the level of MetRS occurs [6].

Here for the first time we describe a characteristic change of the pattern of isoaccepting tRNA in a cell in which the level of the corresponding wildtype aminoacyl-tRNA-synthetase is constitutively increased. Future experiments will have to answer the question of whether a common though still unknown regulatory molecule is responsible for both the increase of the level of ThrRS and the change in the profile of isoaccepting $tRNA^{Thr}$ or wether this change of isoaccepting $tRNA^{Thr}$ is a causative event for the five-fold concentration of ThrRS in Bor Res 3.

Acknowledgements

We wish to thank Dr Bernd Hennig for stimulating advice during parts of this work and the Deutsche Forschungsgemeinschaft for generous support.

References

[1] Kisselev, L. L. and Favora, O. O. (1974) Adv. Enzymol. 40, 141–238.
[2] Söll, D. and Schimmel, R. (1974) in: The Enzymes Vol. X, 489–538, (Boyer, ed.), Acad. Press.
[3] Parcker, J., Flashner, M., McKeever, W. G. and Neidhardt, F. C. (1974) J. Biol. Chem. 249, 1044–1053.
[4] Nass, G. and Thomale, J. (1974) FEBS Lett. 39, 182–186.
[5] Cassio, D. and Waller, J. P. (1975) J. Bacteriol., in press.
[6] Cassio, D. (1975) J. Bacteriol., in press.
[7] Paetz, W. and Nass, G. (1973) Europ. J. Biochem. 35, 331–337.
[8] Benzer, S. (1955) Proc. Natl. Acad. Sci. USA 41, 344–351.
[9] Fangman, W. L., Nass, G. and Neidhardt, F. C. (1965) J. Mol. Biol. 13, 202–219.
[10] Nass, G. (1967) Mol. Gen. Genet. 100, 216–224.
[11] Traub, P. and Zillig, W. (1966) Hoppe Seylers Ztsch. physiol. Chemie 343, 246–260.
[12] Zubay, G. (1962) J. Mol. Biol. 4, 347–356.
[13] Kelmers, A. D., Novelli, G. D. and Stulberg, M. P. (1965) J. Bio. Chem. 240, 3979–3983.
[14] Lewis, J. A. and Ames, B. N. (1972) J. Mol. Biol. 66, 131–142.
[15] Fittler, F., Kruppa, J. and Zachau, H. G. (1972) Biochim. Biophys. Acta 277, 513–522.
[16] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[17] Squires, C., Konrad, B., Kirschbaum, J. and Carbon, J. (1973) Proc. Natl. Acad. Sci. U.S. 70, 438–441.
[18] Clarke, L. and Carbon, J. (1974) J. Biol. Chem. 249, 6874–6885.
[19] Söll, D., Cherayil, J. D. and Bock, R. M. (1967) J. Mol. Biol. 29, 97–112.
[20] McGinnis, E. and Williams, L. S. (1972) J. Bacteriol. 109, 505–511.
[21] Archibold, E. R. and Williams, L. S. (1973) J. Bacteriol. 114, 1007–1013.