Genetically determined differences in concentrations of isoaccepting tRNAs in *Escherichia coli*

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ABSTRACT

Two examples of genetically determined altered concentrations of isoaccepting tRNAs are presented:

The concentrations of isoaccepting tRNAs^{Thr} are selectively changed by a mutation causing a fourfold overproduction of the cognate aminoacyl-tRNA-synthetase, the threonyl-tRNA synthetase, whereas the distribution of isoaccepting tRNAs of four control tRNA-species in these *E. coli* mutants was not affected by that mutation.

Secondly evidence is presented for a correlation between mutations in structural genes of aminoacid biosynthetic enzymes and alterations in concentrations of cognate isoaccepting tRNAs in two different *E. coli* strains, auxotrophic for threonine, isoleucine/valine and leucine, and arginine, respectively.

INTRODUCTION

Isoaccepting tRNAs nowadays can well be separated by use of various column techniques, predominantly the RPC-5 technique. A variation in concentrations of isoaccepting tRNAs determined by the latter procedure has been reported during unbalanced growth after aminoacid withdrawal in relaxed *E. coli* strains¹⁻³ or after antibiotal treatment^{2,4,5} and recently also during balanced, exponential growth under amino acid limitation⁶. In these cases by all environmental devices used, the concentration of isoaccepting tRNAs of all tRNA species investigated were affected.

Earlier we reported changes in concentrations of isoaccepting tRNAs^{Thr} in a mutant of *E. coli* exhibiting constitutively a fourfold increased level of wild-type threonyl-tRNA synthetase⁷. Here now we show that this genetically determined alteration in the pattern of isoaccepting tRNAs^{Thr} is restricted to this tRNA species, since the concentrations of isoaccepting tRNAs of four control tRNA species are not affected by this mutation. In addition we demonstrate that our threonyl-tRNA synthetase overproducing *E. coli* mutants can be classified into two groups, only one of them exhibiting altered isoaccepting tRNAs^{Thr} profiles.

Secondly we present evidence that mutations in structural genes of amino acid biosynthetic enzymes might lead to characteristic changes in concentrations of cognate, isoaccepting tRNAs.

MATERIAL AND METHODS

Bacterial strains and growth conditions. The *E. coli* strains used are: the wildtype *E. coli* K12B^{8,9} and the thereof derived threonyl-tRNA synthetase overproducing strains BorRes3, BorRes5, BorRes10 and BorRes15¹⁰. The amino acid auxotrophic strains AB2271 (*thr-1 leu-6 ilvD32 thi-1 lacY1*)¹¹ and III/18 (*argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5*) derived by P1-transduction from MC104¹², kindly provided by Dr. A. Böck.

Growth of the cells in minimal medium was performed as described¹¹, the minimal medium in case of culturing AB2271 being supplemented with 40µg/ml each of threonine, leucine, isoleucine and valine and 5 µg/ml of thiamine, and in case of III/18 with 40 µg/ml each of proline, histidine and arginine.

Isolation of bulk tRNA. The total tRNA of 30 g wet weight of cells was isolated according to the method of Zubay¹³ modified by Kelmers et al.¹⁴ as described⁷.

Determination of the total acceptor activity of a tRNA species.

The total acceptor activity of a tRNA species in bulk tRNA was determined according to Kelmers et al.¹⁴: for this the aminoacylation of the tRNA was performed as described⁶ by using limiting amounts of total tRNA (20 to 80 µg) and as aminoacyl-tRNA synthetase source 40 µg of RNA-free aminoacyl-tRNA synthetase mixture. The total concentration of a tRNA species is expressed in picomole specific tRNA per 100 µg total tRNA.

Separation of isoaccepting tRNAs. Isoaccepting tRNAs^{Thr} were resolved by the benzoylated DEAE-cellulose urea method developed by Fittler et al.¹⁵ as described⁷. For separating isoaccepting tRNAs^{Leu}, tRNAs^{Ile} and tRNAs^{Arg} the RPC-5-technique^{16,17} was applied as described⁶. However when indicated homogeneous RPC-5-material, prefractionated with the device developed by McMartin and Vinster¹⁸ was used in a column of 0.9 x 50 cm, which resulted in an improved resolution of the isoaccepting tRNAs. All elution profiles presented were obtained by cochromatography of double labelled (¹⁴C/³H) aminoacyl-tRNA from the two strains⁶.

RESULTS

Concentrations of isoaccepting tRNAs^{Thr} in threonyl-tRNA synthetase overproducing strains.

Fig. 1 shows the known⁷ result, that in BorRes3 the profile of isoaccepting tRNAs^{Thr} has changed insofar as the isoacceptor 1 has decreased and the third isoaccepting tRNA^{Thr} has increased. The specificity of this process is now demonstrated by the result, that the concentrations of three control tRNA-species, of the isoaccepting tRNAs^{Leu}, tRNAs^{Ile} and tRNAs^{Arg} are not affected by the mutation (Fig. 1b-d).

Beside BorRes3 three further mutants had been isolated exhibiting increased level of their wildtype threonyl-tRNA synthetase¹⁰: BorRes5, BorRes10 and BorRes15, the level of threonyl-tRNA synthetase being elevated 6-, 4- and 10-fold, respectively. Fig. 2 shows that in BorRes10 the distribution of the isoaccepting tRNAs^{Thr} is also, though less drastically altered, whereas in BorRes5 and BorRes15 the pattern of the isoaccepting tRNAs^{Thr} are the same as in the parental strain K12B (Fig. 2 B and C).

Fig. 1: Separation of isoaccepting Thr-tRNAs, Ile-tRNAs, Leu-tRNAs and Arg-tRNAs from K12B (●—●) and BorRes3 (○—○).

Thr-tRNAs were separated on BD-cellulose urea and for separating the isoaccepting Leu-tRNAs, Ile-tRNAs and Arg-tRNAs the RPC-5-technique was used. 200 µg total tRNA of each strain was applied to the columns except for the Ile-tRNAs where 400 µg total tRNA each was taken. For further experimental details see MATERIAL AND METHODS.

Fig. 2: Separation of isoaccepting Thr-tRNAs of three threonyl-tRNA synthetase overproducing *E. coli* strains. Part A K12B (●—●) and BorRes10 (○- - -○), Part B K12B (●—●) and BorRes5 (○- - -○), Part C K12B (●—●) and BorRes15 (○- - -○). Experimental details for separating the isoaccepting Thr-tRNAs, cell growth and isolation of bulk tRNA are the same as listed in Fig. 1.

Concentrations of isoaccepting tRNAs in *E. coli* mutants being auxotrophic for certain amino acids. The prototrophic *E. coli* strain K12B and the threonine-, leucine- and isoleucine/valine- auxotrophic AB2271 were grown in minimal glucose medium supplemented with the required amino acids. In Fig. 3 the chromatograms of isoaccepting tRNAs of four tRNA species of these two *E. coli* strains are compared. It can be seen, that there are drastic differences in concentration of isoaccepting tRNAs specific for threonine, isoleucine and leucine, amino acids for which AB2271 is auxotrophic, whereas the profiles of isoaccepting tRNAs^{Arg} are the same in AB2271 and K12B.

Fig. 3 in addition indicates that in case of isoaccepting tRNAs^{Thr} and tRNAs^{Ile} the total amount of chargeable tRNA is higher in AB2271 than in K12B, whereas the total acceptor activity for the tRNA^{Leu} species, a fur-

Fig. 3: Separation of isoaccepting Thr-tRNAs, Ile-tRNAs, Leu-tRNAs and Arg-tRNAs from K12B (●—●) and AB 2271 (○—○). Experimental details are the same as listed in Fig. 1, except that the prefractionated, homogenous RPC-5-material is used throughout, resulting in a refinement of the separation of those tRNAs into isoacceptors, and that throughout 200 μ g total tRNA of each strain was applied to the columns.

Fig. 4: Separation of isoaccepting Leu-tRNAs and Arg-tRNAs from K12B (●—●) and III/18 (▲—▲). Experimental details are the same as listed in Fig. 3.

ther cognate amino acid for which AB2271 is auxotrophic is the same in both strains. The same holds true for the total acceptor activity of the tRNA^{Arg} species, both strains being prototrophic for the cognate amino acid. This notion is verified by a direct determination of the total acceptor activity of these tRNA species in the bulk tRNA of K12B and AB2271 as shown in Table 1.

Table 1: Total acceptor activity of four tRNA species from *E. coli* K12B and AB2271

tRNA species	Total acceptor activity ($\frac{\text{picomole of specific tRNA}}{100 \mu\text{g total tRNA}}$)	
	K12B	AB2271
ile-tRNA	58	91
leu-tRNA	373	361
thr-tRNA	107	168
arg-tRNA	220	231

Experimental details for cell growth, isolation of bulk tRNA and the determination of total acceptor activities are listed in MATERIAL AND METHODS.

To substantiate the result, that in AB2271 the concentrations of isoaccepting tRNAs differ from the wildtype pattern for amino acids for which the strain is auxotrophic, we took the tRNA of another amino acid auxotrophic strain, of III/18, which is auxotrophic for arginine, but not for leucine. As can be seen in Fig. 4 the isoaccepting tRNAs^{Arg} of this arginine-auxotrophic strain differ in their distribution greatly from the one of the prototrophic strain K12B, whereas the concentrations of the isoaccepting tRNAs^{Leu} are identical in both strains.

DISCUSSION

We are reporting two kinds of specific alterations of tRNA profiles which are restricted to certain tRNA species in correlation to two classes of mutations.

One is that two of our four threonyl-tRNA synthetase overproducing strains, BorRes3 and BorRes10, exhibit altered concentrations of tRNAs^{Thr} isoacceptors, i.e. those two mutants, in which the level of threonyl-tRNA synthetase is increased only fourfold, whereas in BorRes5 and BorRes15, showing wildtype patterns of the isoaccepting tRNAs^{Thr} the level of threonyl-tRNA synthetase is increased six- and tenfold, respectively. A further feature placing those

four threonyl-tRNA synthetase overproducing mutants into these two groups comes from recent genetic studies, which revealed, that the mutation responsible for the threonyl-tRNA synthetase overproduction in BorRes3 and BorRes15 is located close, but in different distances to the structural gene of the threonyl-tRNA synthetase (Fröhler, Böck, Thomale and Nass, unpublished), the latter gene mapping at minute 37,7 on the *E. coli* chromosome^{19,20}. These results may have some features in common with those obtained by D. Söll and his coworkers, who found revertants of a structurally altered, temperature sensitive leucyl-tRNA synthetase, which exhibit altered pattern of isoaccepting tRNAs^{Leu} but not of tRNAs^{Ser}, the underlying backmutation mapping near the structural gene of the leucyl-tRNA synthetase (D. Söll, personal communication). Further studies will reveal whether the altered tRNA profiles in those mutants are due to different modifications of those isoaccepting tRNAs or to different activities of corresponding tRNA genes, and by which molecular devices those changes in isoaccepting tRNAs are connected to the overproduction of their cognate aminoacyl-tRNA synthetase.

The second case of altered isoaccepting tRNA concentrations we find in amino acid auxotrophic strains: in two different *E. coli* strains there are characteristic alterations in the chromatograms of those isoaccepting tRNAs for the cognate amino acid of which the cells are auxotrophic, whereas tRNA profiles specific for amino acids for which the cells are prototrophic are like the ones of wildtype cells. And it is of interest to note, that those tRNA-patterns in our amino acid auxotrophic strains are present not only when grown in the here used supplemented minimal glucose medium, but in identical manner when grown in supplemented minimal succinate medium⁶ or in rich medium, as shown for the isoaccepting tRNAs^{Leu} in AB2271 (unpublished results). A similar constancy of isoaccepting tRNAs-pattern we also observed with the amino acid prototrophic strain K12B grown in minimal glucose and in rich medium (unpublished results).

Looking through literature it is surprising how comparable RPC-5 profiles of isoaccepting tRNAs are from one laboratory to another: so in case of isoaccepting tRNAs^{Leu} RPC-5 separations have been reported by six different research groups^{2,21-25}, out of cells prototrophic for the branched chain amino acids leucine, isoleucine and valine, and they all look like the one we found for K12B (Fig. 1), using unfractionated RPC-5 material. The same holds true for the pattern of isoaccepting tRNAs^{Arg 2,4,16,26,27} and tRNAs^{Ile 4,23,26} though the latter isoacceptors less frequently have been separated. These facts underline the specificity of the altered tRNA-profiles in our amino acid auxotrophic strains AB2271 and III/18, where beside the auxotrophy for

leucine, isoleucine/valine and threonine and arginine we find altered concentrations of isoacceptors of those cognate tRNA-species and the changes being restricted to these four tRNA-species. Furthermore the changes in the profiles of those isoaccepting tRNAs differ drastically from those reported to take place after amino acid withdrawal^{1-3,6} or antibiotal treatment^{2,4,5}, indicating different structural alterations of the isoaccepting tRNAs in both cases. Structural analysis of some isoaccepting tRNAs appearing after amino acid withdrawal or antibiotal treatment showed^{28,29}, that some bases common to most tRNAs are undermodified, explaining easily that after posing those devices upon the cells the pattern of all tRNA species change. In case of our amino acid auxotrophic cells, only related tRNA species are affected, and if this is also due to certain base undermodifications, we expect only bases to be involved which are special for those tRNA species.

In case of the leucine-auxotrophy a good candidate for being altered would be the modified G38 or 39 of tRNA^{Leu}₁, since the modifications of those bases are absent in the tRNA^{Leu}₁ of CP79²⁹, whereas in the leucine prototrophic strain IB11, derived by transduction from CP79, in its tRNA^{Leu}₁ the modified base G38 is shown to be present again³⁰.

The deficiency of the modification of the bases 38 and 39 in tRNA^{Leu}₁, however does not lead to different migration properties of this tRNA on RPC-5 columns²⁹. But in our strain AB2271 auxotrophic for leucine and in addition for isoleucine/valine and threonine, there is a shift of some of the tRNA^{Leu}₁ to the right on that column (Fig. 3). This we take as indication that other structural changes have occurred in this isoaccepting tRNA in AB2271, which we like to correlate to the isoleucine/valine auxotrophy of this strain, amino acids for which CP79 is prototrophic.

The results and thoughts mentioned so far, prompt us to speculate, that mutations in genes coding for amino acid biosynthetic enzymes lead to structurally altered tRNA-molecules, which are involved in the regulation of formation of these enzymes. And it is well documented, that isoaccepting tRNA^{Leu} takes part in the regulation of isoleucine/valine biosynthesis³¹⁻³³. Support for this interpretation of our data are also the results, that due to a structurally altered tRNA^{His}- and tRNA^{Leu}- modifying enzyme, the regulation of the biosynthesis of the amino acids histidine, leucine, isoleucine and valine are disturbed^{23,34,35}. And in addition our hypothesis, that mutations in genes of amino acid biosynthetic enzymes lead to structural alterations of related isoaccepting tRNAs, is favored by the recent report³⁶ of a tRNA-mutant, which does not map in the structural gene of tRNA^{Trp}, but supposingly is due to an altered tRNA^{Trp} modifying enzyme, and which exhibits

altered chromatographic properties of its tRNA^{Trp} and a disturbed regulation of tryptophane biosynthesis. Thus it is tempting to speculate, that also in case of the altered chromatograms of isoaccepting tRNAs^{Thr} and tRNAs^{Ile} in AB2271 and of isoaccepting tRNAs^{Arg} in III/18 there is a correlation to the corresponding amino acid auxotrophy.

Now it will be of great interest on one hand to determine the structure of those altered, isoaccepting tRNAs, especially of the isoaccepting tRNAs^{Leu} of AB2271, and on the other hand to investigate their binding properties to cognate amino acid biosynthetic enzymes.

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