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Structural investigation of Phe-tRNA^{Phe} from *E. coli* bound to the ribosomal A-site

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SUMMARY

Kethoxal modification of guanosines within Phe-tRNA^{Phe} from E. coli was studied for tRNA in the free state and specifically bound to the ribosomal A-site. Complex formation with the ribosome results in a protection from chemical modification of two distant sites in the tRNA molecule. The guanosines affected are G-18 and G-19, located in the D-loop, and G-34 in the anticodon loop.

Modification of Phe-tRNA^{Phe} in the absence of ribosomes leads to a destabilisation of the tRNA structure. Our data are consistent with the conclusion that modification of G-34 at the anticodon loop triggers a conformational instability in distant parts of the tRNA molecule.

INTRODUCTION

The principal mechanism of one of the main events in protein biosynthesis, namely the coded binding of a tRNA to the ribosome, is still not understood. It is known that codon-anticodon interaction is not sufficient to explain the high stability of a tRNA-ribosome complex and the high accuracy with which it is formed. Additional interactions between the tRNA and the ribosome have been proposed, and it will be of great importance to find out which sites of the tRNA may be involved in such an interaction.

Chemical modification has been shown to be of high potential for the localisation of interacting sites between tRNAs and ribosomes (1-3). We have used the chemical reagent kethoxal to study the accessible guanosines within Phe-tRNA^{Phe} from E. coli, both in the free state and bound to the ribosomal A-site.

Specific binding of Phe-tRNA^{Phe} was achieved enzymatically

via the ternary complex with elongation factor EF-Tu and GTP, while the P-site was effectively blocked with deacylated tRNA. The location of the Phe-$tRNA^{Phe}$ in the A-site was demonstrated by the puromycin reaction before and after modification with kethoxal. The binding conditions were selected to keep non-enzymatic binding of Phe-$tRNA^{Phe}$ at a level of below 5%. Intact tRNA∿ribosome∿mRNA complexes were isolated after modification. The tRNA was then extracted and analyzed. The deacylated tRNA did not appear in the analysis because it was selectively oxidised at its 3'-terminus and could not, therefore, be radioactively labeled at its 3'-end. Thus, only A-site bound tRNA that was still associated with the ribosome after the reaction with kethoxal was analysed in the later sequencing step.

Modified guanosines were identified by a partial RNase T_1 digestion and separation of the tRNA fragments on sequencing gels. The modification sites could readily be assigned since RNase T_1 does not hydrolyse kethoxylated guanosines.

MATERIALS AND METHODS

$tRNA^{Phe}_{E.coli}$, $tRNA^{Phe}_{yeast}$, $tRNA^{Val\ I}_{E.coli}$, $tRNA^{Lys}_{E.coli}$, ribonucleotide 5'-diphosphates and 5'-triphosphates were from Boehringer, Mannheim, [^{3}H]-phenylalanine (specific activity 36.7 Ci/mmole) and [^{32}p]-pCp (specific activity 2000 - 3000 Ci/mmol) were bought from Amersham/Buchler, Braunschweig. Kethoxal was from Nutritional Biochemical Corporation, Cleveland, Ohio. Acrylamide, N^1,N^1--methylene-bisacrylamide and N,N,N^1,N^1-tetramethylethylenediamine were obtained from Bio Rad, Richmond. Polynucleotide ligase (E.C.6.5.1.3) was a product of PL Biochemicals, Milwaukee. RNase T_1 (E.C.3.1.4.8) was supplied from Sankyo Inc., Tokyo. Ribosomes were prepared in the form of 70S tight couples according to ref. (4), and $tRNA^{Phe}$ was charged with [^{3}H]-phenylalanine according to ref. (5). Elongation factor EF-Tu∿GDP was prepared following the procedure of Arai et al. (6).

3'-end oxidation of uncharged tRNA molecules

Uncharged tRNA molecules in the aminoacyl tRNA preparation (about 60% charged) were oxidized at their free 3'-terminal hydroxyl groups in 100 mM Na acetate, pH 5.5, with a 200-fold molar excess of Na periodate for 30 min in the dark at room

temperature. The excess of Na periodate was inactivated by adding sucrose, and the RNA was precipitated with ethanol. The oxidation of the 3'-ends completely inhibited the ligation reaction with radioactive pCp. Deacylated tRNA could therefore not be seen on the sequencing gels and could consequently not affect the results.

Binding of Phe-tRNA^{Phe} to the ribosomal A-site

The ternary complex with EF-Tu∿GTP∿Phe-tRNA^{Phe} was prepared as described in (2) using 50 pmoles of Phe-tRNA^{Phe} in a total volume of 8 µl. The complex was added to 100 pmoles of 70S ribosome tight couples and incubated in 38 µl of 50 mM Na cacodylate, pH 7.2, 7 mM $MgCl_2$, 150 mM KCl and 3 mM dithiothreitol in the presence of 50 µg poly(U) at 37°C for 15 min.

In the control sample, the ternary complex was replaced by Phe-tRNA^{Phe} and a corresponding amount of EF-Tu∿GDP, and ribosomes were omitted.

Modification reaction

2.5 volumes of saturated kethoxal solution in 50 mM Na cacodylate, 150 mM KCl, 5 mM $MgCl_2$, 10% ethanol was added to both the sample and control, and incubation was carried out at 37°C. 25 µl aliquots were withdrawn at time intervals of 10, 30, 45, 60 and 75 min and filtered through nitrocellulose filters (1 cm diameter). They were each washed twice with 100 µl of 50 mM Na cacodylate pH 7.2, 7 mM $MgCl_2$, 100 mM NH_4 acetate, 20 mM Na borate. This procedure removes unbound tRNA and tRNA∿EF-Tu∿GTP, while ribosomes and ribosome-tRNA complexes are retarded on the filter. The filters were then washed with 200 µl 50 mM Na cacodylate, pH 8.2, 100 mM NH_4 acetate, 20 mM Na borate 1 mM EDTA and 0.1% SDS. The control samples were directly dissolved in the latter buffer. All samples were extracted three times with an equal volume of phenol, saturated with 100 mM NH_4 acetate, 20 mM Na borate, and after reextraction with diethylether the samples were precipitated with 2.5 volumes of ethanol.

Deacylation of Phe-tRNA^{Phe}

Whereas uncharged tRNA's had been 3'-end oxidized and in consequence could not be labeled, charged molecules had to be deacylated prior to 3'-end labeling of the tRNA. To deacylate

the tRNA, the samples were dissolved in 10 µl 160 mM Na acetate pH 5.3, 20 mM Cu SO_4 and 20 mM Na borate. They were incubated for 15 min at 37°C, after which 2 µl of 100 mM EDTA was added and the tRNA's were precipitated with ethanol.

3'-end labeling of $tRNA^{Phe}$

The tRNA samples were 3'-end labeled with 1µCi 5'-[^{32}P]pCp and polynucleotide ligase according to ref. (7), except that the Mg^{2+}-concentration was raised to 30 mM and the reaction was performed in the presence of 20 mM Na borate. After precipitation with ethanol, the tRNA was separated from excess pCp and tRNA fragments by gel electrophoresis, using 12% polyacrylamide, 8 M urea gels. The pH during gel electrophoresis was maintained at 7.5. Bands containing the tRNA were cut out according to the autoradiogram of the gel and were eluted with 250 mM Na acetate, 20 mM Na borate, 2 mM EDTA, followed by ethanol precipitation.

Analysis of modification sites

The modified tRNA samples were subjected to a partial RNase T_1 hydrolysis. The enzyme does not cleave at kethoxal modified guanosines (8). The hydrolysis was performed with 0.65 mU of RNase T_1 per sample for 20 min at 0°C in the presence of 3 µg carrier tRNA. Samples were quickly frozen in dry ice and lyophilized. They were separated on 12% acrylamide gels (0.4 x 200 x 400 mm) in the presence of 8 M urea. The running buffer was 50 mM Tris borate pH 8.3, 1 mM EDTA. Separation was performed at 1800 V for 2 or 5 hours, depending on the size of the fragments to be resolved. The gel was exposed to an X-ray film at -80°C and the autoradiograms were scanned using a Vitatron TLD 100 densitometer.

RESULTS

Specificity of tRNA binding

Binding of $Phe\text{-}tRNA^{Phe}$ was made via ternary complex formation with EF-Tu∿GTP. The ternary complex is known to bind specifically to the ribosomal A-site (9). Blocking of the P-site was achieved by binding of the deacylated tRNA present in the aminoacyl-tRNA preparation (60% charged). No preincubation with additional deacylated tRNA was necessary under our conditions. The location of the $Phe\text{-}tRNA^{Phe}$ in the A-site has been shown by

Table I Location and stability of enzymatically bound Phe-$-tRNA^{Phe}$

	Puromycin reactivity [%] 1)		tRNA bound 2) [%]
	-EF-G	+EF-G	
enzymatically bound Phe-$tRNA^{Phe}$ in the presence of 40% deacylated tRNA	0	83	94
enzymatically bound Phe-$tRNA^{Phe}$ after preincubation with a molar excess of deacylated tRNA	n.d.	n.d.	96
enzymatically bound Phe-$tRNA^{Phe}$ after 75 min of kethoxal reaction 3)	0	n.d.	94

1) Puromycin reaction before and after translocation with EF-G and GTP was performed as described in (10).
2) Numbers indicate % of total Phe-$tRNA^{Phe}$ input. 100% corresponds to 50 pmoles.
3) Kethoxal reaction was performed as described in Methods.

n.d. - not determined

the puromycin reaction (see Table I). Table I demonstrates that enzymatically bound Phe-$tRNA^{Phe}$ was essentially at the ribosomal A-site and exchange from A- to P-site did not occur during our reaction conditions. Furthermore it is evident from Table I that the Phe-$tRNA^{Phe}$∿ribosome∿poly(U) complex is remarkably stable during the reaction with kethoxal and no complex dissociation can be observed after 75 min of kethoxal reaction.

Identification of modified tRNA sites

After the reaction tRNA samples that had been isolated, deacylated and 3'-end labeled with radioactive pCp were subjected to a partial RNase T_1 hydrolysis. The sites of modification are protected from the partial RNase T_1 degradation and appear therefore as bands of reduced intensity on the sequencing gel. Fig. 1 shows the autoradiogram of such a sequencing gel. The autoradiogram was obtained from a kinetic experiment, in which tRNA-ribosome-complexes were modified with kethoxal for increasing times and were separated alongside tRNA samples that

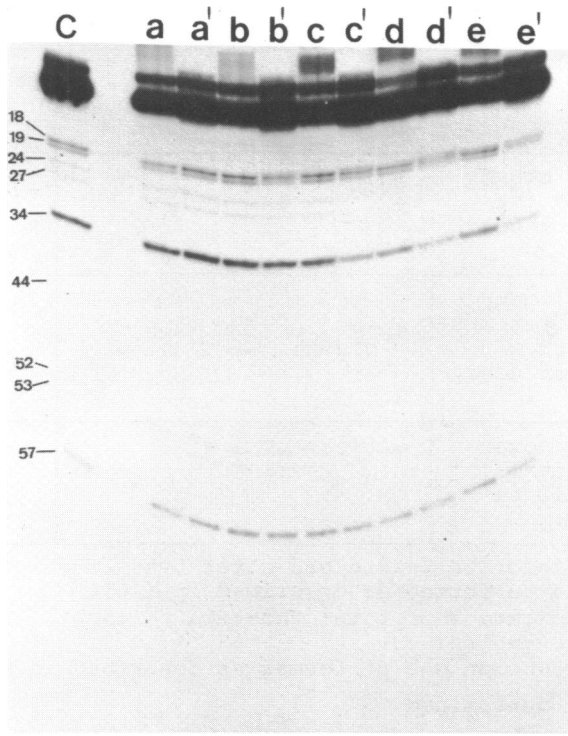

Fig. 1: Autoradiogram of the gel separation of a partial RNase T_1 digest of $tRNA^{Phe}$ samples after kethoxal modification
In C a control sample that was not treated with kethoxal was partially hydrolysed with RNase T_1. Tracks a, b, c, d and e show partial RNase T_1 digests of tRNA's that had been bound to the ribosomal A-site and reacted for 10, 30, 45, 60 and 75 minutes, respectively, Tracks a', b', c', d', and e' show tRNA samples reacted in the absence of ribosomes under the same conditions. The numbers in the left margin indicate guanosine positions. Autoradiograms were exposed for 3 days at -80°C.

had been modified in the absence of ribosomes.

The reduction in the intensities of the bands corresponding to guanosines G-34, G-18, G-19, G-24, G-27 (and weakly G-52, G-53 with longer reaction times) is an indication of their kethoxal reactivity. The corresponding band intensities are reduced to a much greater extent, however, when the tRNA was modified in the absence of ribosomes. One has to conclude, therefore, that formation of the tRNA complex with the ribosomal A-site does indeed protect G-18, G-19, G-34 and weakly G-52, G-53

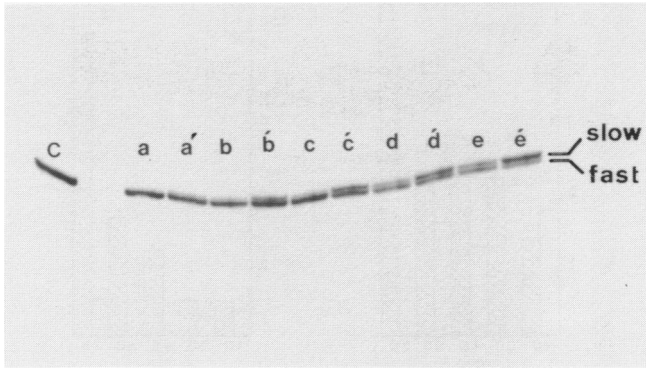

Fig. 2: Autoradiogram of the sequencing gel from Fig. 1 showing the unhydrolysed tRNA bands
The numbering of the samples is the same as in Fig. 1. The slower and the faster tRNA species are indicated. The autoradiogram was exposed for 4 hours at -80°C.

from modification with kethoxal.

Modification results in the formation of a slower migrating tRNA isomer

When autoradiograms were taken at shorter exposure times, an additional effect was observed. Fig. 2 shows an autoradiogram of the same sequencing gel as in Fig. 1 after 4 hours exposure time. Under this condition only the unhydrolysed tRNA, representing about 90% of the total material, is visible. During the time course of the reaction a slower migrating tRNA band becomes apparent. The formation of this double band is much faster in samples that had been modified in the absence of ribosomes (Fig. 2a', b', c', d' and e').

Quantitation of the protection effects caused by ribosomal A-site binding

The protecting effect mediated by ribosomal binding can be assessed more precisely by quantitation of the band intensities for the corresponding guanosines. The autoradiograms were therefore monitored by densitometry, and the relative intensities of the guanosine bands were calculated as a ratio of the intensities of the non-hydrolysed tRNA material and the intensity of the corresponding guanosine band (Fig. 3A and B). In Fig. 3C the relative intensities of the slower migrating tRNA isomers from

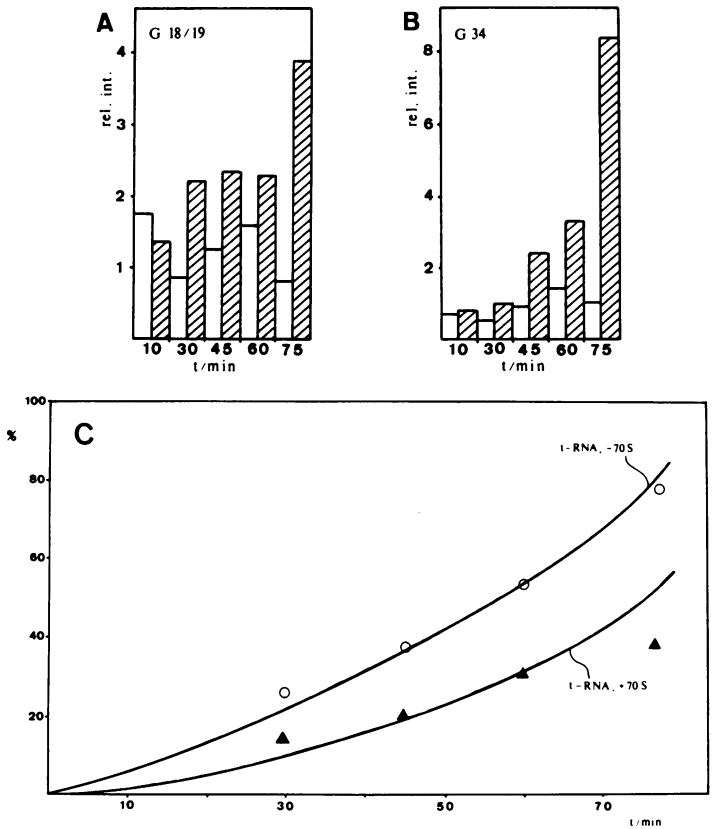

Fig. 3: Quantitation of band intensities of tRNA^{Phe} modified with kethoxal in the free state and bound to the ribosomal A-site. Relative band intensities were calculated from a densitometer scan of autoradiograms from Fig. 1 and 2 as explained in the text. Open bars represent tRNA samples that were modified in the presence of ribosomes, shaded bars are calculated from samples modified in the absence of ribosomes. A shows the relative intensities for guanosine G-18 and G-19 at different time points during the reaction. In B the relative intensities for guanosine G-34 are shown. In C the formation of the slower migrating tRNA isomer is given for: o——o tRNA modified in the absence of ribosomes; ▲——▲ tRNA modified in the presence of ribosomes.

Fig. 2 are shown. In this case, intensities were calculated taking the sum of both the slower and the faster migrating tRNA band as 100%.

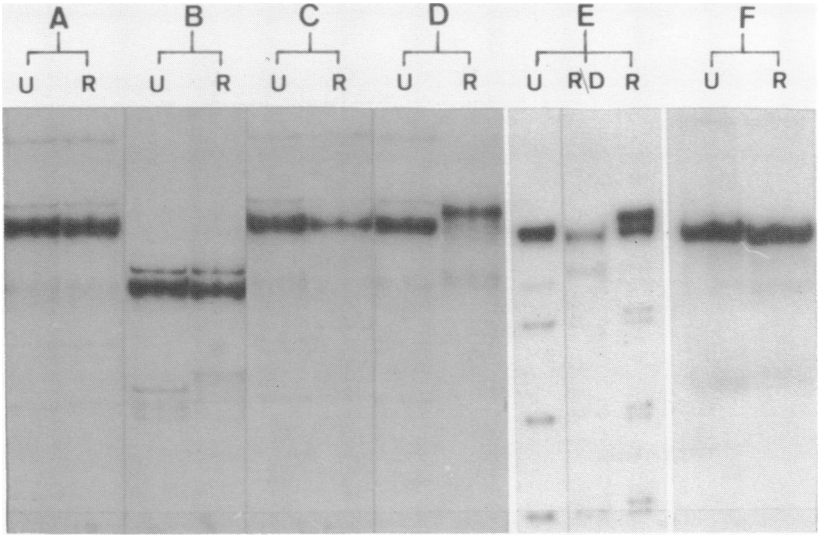

Fig. 4: Gel electrophoretic mobility of tRNA samples. Autoradiograms of six different experiments are shown. Samples A to E are separated on 12% acrylamide gels in the presence of 8 M urea, whereas samples in F are separated in the absence of urea. U stands for unreacted, R for reacted samples. A) $tRNA^{Phe}$ from E. coli modified at acp^3 U-47 with acetic acid succinimide ester. B) $tRNA^{ValI}$ from E. coli modified with kethoxal. C) $tRNA^{Phe}$ from E. coli modified with kethoxal followed by two hours incubation in 15 mM Tris-OH buffer. D) $tRNA^{Phe}$ from E. coli modified with kethoxal. E) $tRNA^{Phe}$ from E. coli modified with kethoxal (not to completion) R and U were additionally digested with RNase T_1 under limiting conditions to show guanosine positions. R/D was a reacted sample denatured by heating to 90°C for 45 minutes prior to gel electrophoresis. F) $tRNA^{Phe}$ from E. coli modified with kethoxal as in D but separated under non-denaturing conditions.

What is the reason for the formation of a slower migrating tRNA band?

The double band of $tRNA^{Phe}$ formed on the sequencing gel (Fig. 2) after kethoxal reaction is of particular interest, because it apparently represents a structural difference between tRNA modified in the free state, as compared to an A-site bound tRNA.

The presence of the slower-migrating tRNA isomer cannot be explained by a difference in molecular weight of the two tRNA species as a result of the kethoxal modification, since i)

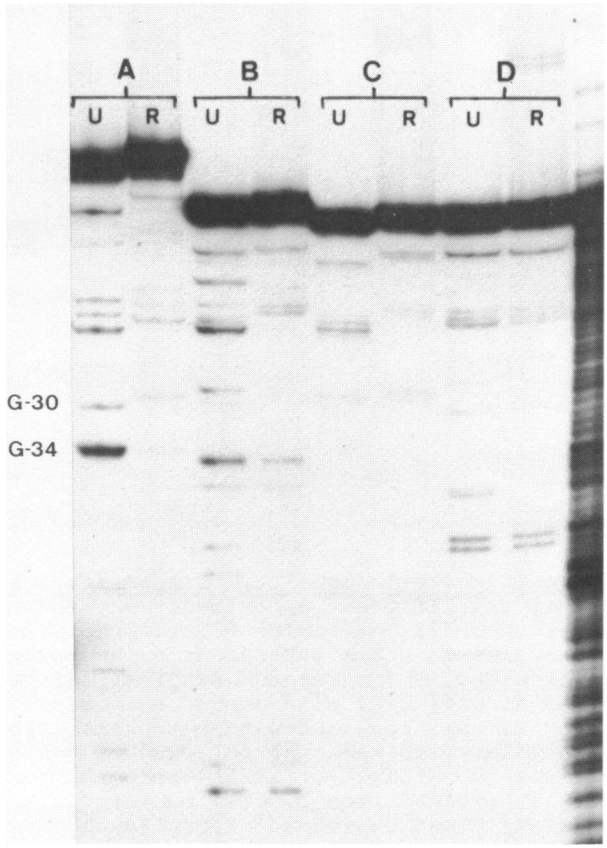

Fig. 5: Autoradiogram of a gel separation of partial RNase T_1 digests of kethoxal reacted tRNAs. U stands for unreacted control samples, R for kethoxal reacted samples. Reaction was performed in 100 mM Na cacodylate pH 7.2, 10 mM $MgCl_2$, with no other components present, by adding 2.5 volumes of saturated kethoxal solution. Reaction was performed for 30 min. RNase T_1 partial digestion was as described in methods. A) $tRNA^{Phe}$ from E. coli; B) $tRNA^{ValI}$ from E. coli; C) $tRNA^{Phe}$ from yeast; D) $tRNA^{Lys}$ from E. coli.

$tRNA^{Val}$ or $tRNA^{Lys}$ molecules, modified to the same extent, do not show such a difference in mobility (Fig. 4B, Fig. 5B, D) and ii) separation of modified $tRNA^{Phe}$ in a gel system without urea does not show a mobility difference compared to the unmodified sample (Fig. 4F).

The slower-migrating band can also not be ascribed to a change in charge due to kethoxal modification of the amino group

of acp^3U at position 47 within the tRNA. When a $tRNA^{Phe}$ sample was selectively modified at the amino group of acp^3U-47 with acetic acid succinimide ester (11) which had been prepared according to (12), no slower migrating band could be detected. This is shown in Fig. 4A.

We were able to show, however, that the formation of the slower migrating $tRNA^{Phe}$ species is a reversible effect. It can be reverted under conditions mild enough to cleave kethoxal-guanosine adducts but no other chemical bonds (Fig. 4C,D). Furthermore, if the slower migrating isomer is denatured by heating, the difference in mobility disappears and the sample migrates on the sequencing gel as an untreated control (Fig. 4E), suggesting that a conformational effect is induced. This idea is further strengthened by the observation that the slower mobility of one of the tRNA isomers can only be observed if the separation is carried out in 8 M urea. If the modified tRNA sample is separated under non-denaturating conditions no difference in mobility was detected (Fig. 4F).

We could not detect a similar effect on the mobility of $tRNA^{ValI}_{E.coli}$ or $tRNA^{Lys}_{E.coli}$ when both tRNA species were reacted with kethoxal (Fig. 4B, Fig. 5B,D). A slower migrating conformer was observed, however, with $tRNA^{Phe}$ from yeast after kethoxal reaction (Fig. 5C). Both, $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{E.coli}$ have in common an accessible and very reactive G-34 at the anticodon loop, while most of the other reactive positions are common to all tRNA species investigated. The difference in mobility of the modified versus the unmodified $tRNA^{Phe}_{E.coli}$ disappears when the 5'-terminal region ranging from nucleotide 1 to guanosine G-30, has been excised by digestion with RNase T_1. This is evident from the partial RNase T_1 digestion pattern shown in Fig. 5A.

In conclusion, the results with respect to the formation of the slower-migrating tRNA isomer can be summarized as follows:

1. Modification of the rare base acp^3 U-47 does not lead to the formation of the double band.
2. The formation of the double band is a reversible process.
3. The formation of the double band seems to be a conformational effect caused by the partly denaturating conditions of the sequencing gel.

4. The formation of the double band seems to involve the tRNA fragment 1 to 30.
5. The formation of the double band seems to be specific for tRNA molecules showing a rapid kethoxal modification at the exposed G-34 of the anticodon loop.

DISCUSSION

Specificity of the bound tRNA

Binding of Phe-tRNA^{Phe} was specifically directed to the A-site by the ternary complex with EF-Tu and GTP at low Mg^{2+} concentration. The EF-Tu preparation was essentially free of G-factor activity and the P-site was effectively blocked with deacylated tRNA. As shown by puromycin reaction the A-site bound tRNA remained in the A-site, and no translocation or sliding to the P-site did occur. Although the tRNA that was analysed was exclusively bound to the A-site, it must be borne in mind that the P-site was simultaneously filled with deacylated tRNA molecules, and protection effects between the two tRNA's bound to the ribosome cannot be excluded.

Limitations of the method

Due to the specificity of kethoxal, the study we describe is limited to the investigation of guanosines within the tRNA structure. If other bases are to be monitored, different reagents have to be used. It would be of interest to see whether nulceotides other than guanosine modified in the anticodon region result in a similar structural effect on the tRNA molecule.

A second limitation is inherent to all studies using a limited enzymatic digestion. Enzyme cutting positions should only be affected at sites where a kethoxal modification occurs. No long range effects caused by different structures should be observed. There is good evidence to exclude this type of hydrolysis artifact in our study. Changes in the RNase T_1 digestion pattern (Fig. 1) are limited to those guanosines which are known to be the target sites of kethoxal modification (8,13). In case of the slower migrating conformer of tRNA^{Phe} the same situation applies, since, i) under the ionic conditions of the limited RNase T_1 hydrolysis but in the absence of the enzyme both conformers are indistinguishable by gel electrophoresis under non-denaturing

conditions (Fig. 4F) and are, therefore, very likely identical, and ii) we could not detect any change in the ratio of intensities of the slower and the faster migrating band for various guanosine positions of the partial RNase T_1 hydrolysate of modified $tRNA^{Phe}$ samples (Fig. 4E). This proves that RNase T_1 does hydrolyse both conformers at the same speed. It furthermore strengthens the hypothesis that the difference in mobility is caused by a conformational difference of the part of the tRNA involving the fragment 1 to G-30, since only fragments exceeding position 30 in 5'-direction show a slower mobility on the sequencing gels (Fig. 5A). The site of kethoxal modification, however, initiating such a difference must be located 3' to position 30.

The origin of the slower migrating $tRNA^{Phe}$ conformer

The experiments we performed to determine the origin of the double band formation of kethoxal modified $tRNA^{Phe}$ are consistent with the following observations and conclusions i) kethoxal modification of $tRNA^{Phe}$ results in a rapid formation of a slower-migrating tRNA conformer visible on denaturating gels. The formation of this double band is inhibited when the tRNA was bound to the ribosomal A-site. ii) The decrease in mobility of the modified tRNA is very likely the result of an unfolding of the tertiary structure, possibly involving the D- and T-loop interactions. iii) Within the unmodified tRNA these interactions are normally so stable that they are not fully denatured under the electrophoresis conditions. iv) Modifications of guanosines at the D-loop only are not sufficient to create a slower migrating conformer. This is evident from Fig. 5B/D, where guanosines of the D-loop were kethoxylated but no change in mobility occurs. v) It seems as if kethoxal modification of the G-34 at the anticodon loop triggers a conformational instability of the tRNA molecule, which is expressed in a region distant from the site of modification.

Comparison with other tRNA binding studies

Effects on tRNA structure upon binding to the ribosome have been described in several recent studies. In one study (1) the chemical modification of guanosines, cytidines and adenosines within $tRNA^{Phe}$ from E. coli was investigated when the deacylated

molecule was bound to the ribosomal P-site. A protection from modification was observed for the 3'-terminal nucleotides C-74 and C-75, while a strong enhancement of reactivity could be detected for G-24 and G-46 in addition to a weak enhancement of eleven more guanosines. All the effects were observed, however, regardless as to whether a specific message was present or not.

In a similar study (3), using the same modifying reagents as in (1), protection from modification occurred at C-74, C-75 near the 3'-terminus, G-34-A-A-ms^2i^6A at the anticodon loop and A-21 at the D-stem, whereas A-73 at the acceptor stem was enhanced in its reactivity for $tRNA^{Phe}_{E.coli}$ bound to the ribosomal A-site.

These results are in good agreement with the protection effects at the anticodon loop and the D-loop region presented in this study. Data for the acceptor stem could, unfortunately, not be obtained, because there are no kethoxal reactive guanosines in this part of the molecule.

In a different approach, using [^{3}H] exchange at the purines of the tRNA bases (14), protection from exchange could be observed for the 3'-terminus, the anticodon region, as well as for parts of the T-stem, when the tRNA was bound to the ribosomal P-site. An enhanced exchange was apparent for the sequence T-C-G_{57} at the T-loop and D-D-G_{18} at the D-loop, indicating a structural change in these two loops during ribosomal binding. This result is of particular interest with respect to the structural destabilisation caused by anticodon modification shown in this study.

In summary, there seems to be solid evidence for a close contact between a bound tRNA and the ribosomal A-site not only at the anticodon loop where codon-anticodon recognition takes place, but, in addition, in the central part of the tRNA molecule where the D- and T-loops interact. Furthermore, not observed in this study but demonstrated with other methods (1,3,14), the 3'-terminus of the tRNA is tightly bound to the ribosome.

Kethoxal modification of $tRNA^{Phe}$ leads to a structural destabilisation of the tRNA molecule. Binding of Phe-$tRNA^{Phe}$ to the ribosomal A-site inhibits such a destabilisation.

Although the effect on the $tRNA^{Phe}$ structure, probably

caused by modification of the anticodon loop, is only indirectly shown in this study, it supports the allosteric properties of the tRNA molecule shown by us (8) and others (15) earlier. It furthermore demonstrates the use of denaturing acrylamide gels in detecting stable RNA structures and structural changes (16).

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REFERENCES

1. Peattie, D.A. and Herr, W. (1981) Proc. Natl. Acad. Sci. USA 77, 4679-4682.
2. Bertram, S. and Wagner, R. (1982) Biochemistry International 4, 117-126.
3. Douthwaite, S., Garrett, R.A. and Wagner, R. (1982) Eur. J. Biochem. submitted.
4. Chiam, C.L. and Wagner, R. (1982) Biochemistry, in press.
5. Traub, P., Mizushima, S., Lammy, C.V. and Nomura, M. (1971) in Methods in Enzymology, Moldave, K. and Grossmann, L. eds., Vol. 20 pp. 391-407, Academic Press New York.
6. Arai, K.J., Kamakita, M. and Kaziro, Y. (1972) J. Biol. Chem. 247, 7029-7037.
7. Bruce, A.G. and Uhlenbeck, O.G. (1978) Nucleic Acids Res. 5, 3665-3677.
8. Wagner, R. and Garrett, R.A. (1979) Eur. J. Biochem. 97, 615-621.
9. Miller, D. and Weissbach, H. (1977) in Molecular Mechanisms of Protein Biosynthesis, Weissbach, H. and Pestka, S. eds., pp. 324-374, Academic Press New York.
10. Leder, P. and Burzstyn, H. (1966) Biochem. Biophys. Res. Commun. 25, 233-237.
11. Schwartz, I. and Ofengand, J. (1982) Biochim. Biophys. Acta 697, 330-335.
12. de Groot, N., Lapidot, Y., Panet, A. and Wolman, Y. (1966) Biochem. Biophys. Res. Commun. 25, 17-22.
13. Wagner, R. and Garrett, R.A. FEBS Lett. (1978) 85, 291-295.
14. Faber, N. and Cantor, C.R. (1980) Proc. Natl. Acad. Sci. USA 77, 5135-5139.
15. Gassen, H.G. (1980) Progr. Nucleic Acid Res. Mol. Biol. 24, 57-86.
16. Van Charldorp, R., Heus, H.A., Von Knippenberg, P.H. (1981) Nucleic Acids Res. 9, 267-275.