CHEMICAL EVIDENCE FOR A CODON-INDUCED CHANGE OF tRNA CONFORMATION

R. WAGNER and R. A. GARRETT
Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin-Dahlem, Germany

Received 26 September 1977
Revised version received 2 November 1977

1. Introduction

A conformational change occurs within the tRNA structure following the codon–anticodon complex formation, such that the TψCG sequence becomes exposed [1,2]. The accessibility of the latter was demonstrated by binding the complementary tritium-labelled oligonucleotide CGAA. Additional evidence for the structural change came from fluorescent measurements [3].

Kethoxal has been successfully used in investigating the secondary structures of various tRNAs [4–6]. For example, it was shown that in the native tRNA structure the guanosine in the TψCG region was not modified in any of the individual tRNAs tested. If codon–anticodon interaction triggers the exposure of the TψCG loop this should result in an increased reactivity of at least the guanosine in the TψCG and dihydrouridine loops. Therefore, we investigated the accessibilities of G residues within the tRNA structure in the presence and absence of a cognate codon. We used the radioactive kethoxal-like reagent *p*-carboxyphenylglyoxalmethylester to follow the reactivity of $tRNA^{Phe}$ and $tRNA^{Lys}$ with and without their corresponding codons. In both tRNAs an enhanced modification of guanosines was observed when the correct oligonucleotide was used as a codon. For the $tRNA^{Lys}$ an additional 5 mol guanosines were modified per mol tRNA in the presence of the codon after 3 h reaction.

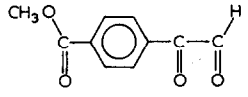
In a second approach, [^{32}P]tRNA and kethoxal were used to characterise the additionally modified guanosines within the tRNA structure. Evidence was obtained for the enhanced modification of at least 4 guanosines, in the presence of the codon, and one of these was tentatively assigned as the G residue of $T\psi CG_p$. Therefore, these results demonstrate that a codon induced structural change occurs in tRNA and that the TψCG sequence and other unidentified sequence regions are involved in this structural change.

Address correspondence to: R. Wagner

2. Materials and methods

2.1. *Materials*

$tRNA^{Lys}_{E.\ coli}$ and $tRNA^{Phe}_{E.\ coli}$ were from Boehringer, Mannheim. Kethoxal was from Nutritonal Biochemicals Corporation, Cleveland, Ohio. The radioactive methylester of *p*-carboxyphenylglyoxal:



was prepared by reacting the acid chloride of *p*-carboxyphenylglyoxal with [^{3}H]methanol [7]. The specific activity was adjusted to 0.3 mCi/mmol.

$(A)_4$, i.e. ApApApA, and $(U)_4$, i.e. UpUpUpU, were prepared enzymatically with polynucleotide phosphorylase as described [8]. Tetranucleotides were used as codons instead of trinucleotides because they have higher binding constants to the tRNA anticodons [9,10].

2.2. *Preparation of [^{32}P]tRNA from* E. coli *cells*

Cells from *E. coli* MRE 600 were grown in the presence of 20 mCi [^{32}P]orthophosphate as described [11]. Extraction of RNA and separation of tRNA

were performed according to the electrophoretic procedure described [12].

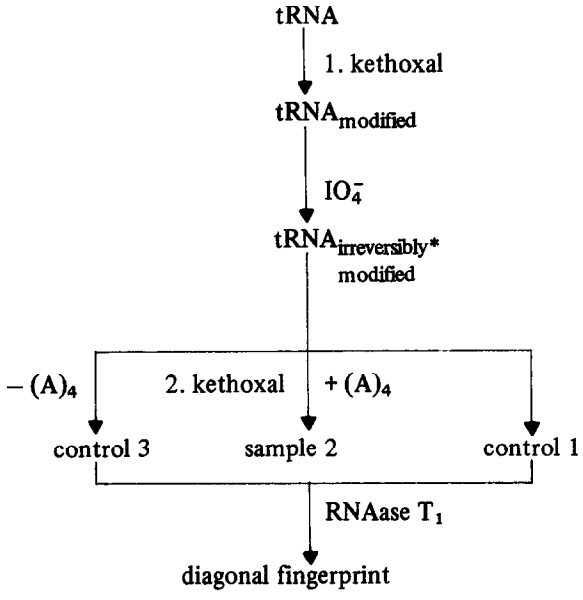
2.3. *Reaction of tRNA with [^{3}H]methylester of p-carboxyphenylglyoxal*

In final vol. 0.5 ml, 5 nmol tRNA were incubated at 37°C in the following reaction mixture: 50 mM Tris-borate, pH 7.2, 16 mM $MgCl_2$, 2.5 μmol radioactive reagent and codon concentrations as given in the legends to fig.1–3. Aliquots were taken at time intervals, precipitated with trichloracetic acid and analysed for [^{3}H]reagent incorporation.

The results from the different experiments were averaged. The variation in the amount of radioactivity incorporated was generally within ± 5%.

2.4. *Reaction of kethoxal with [^{32}P]tRNA*

The [^{32}P]tRNA was reacted as outlined in the following scheme:



Three A_{260} units crude [^{32}P]tRNA (6×10^7 dpm) in 0.5 ml reaction buffer (100 mM Na cacodylate, pH 7.2, 15 mM $MgCl_2$) were incubated for 40 min at 37°C with 1.85 mg kethoxal. The tRNA was twice precipitated with ethanol and washed to remove excess kethoxal. The final pellet was resuspended in 0.3 ml reaction buffer. The modified tRNA was reacted with IO_4^- leading to the more stable N^2-α-ethoxypropionyl guanosine derivatives [13]. Freshly prepared 0.2 M $NaIO_4$ solution, 100 μl, was added and the sample kept in the dark at 20°C for 15 min. A further aliquot of $NaIO_4$ was added and the mixture was incubated for another 15 min. Excess IO_4 was destroyed by the addition of sucrose. The tRNA was precipitated with ethanol, washed and redissolved in 0.5 ml reaction buffer. The sample was divided into three equal parts. Sample 1 was precipitated with ethanol without further reaction and used as a control. Samples 2 and 3 were incubated again with 1 mg kethoxal for 60 min at 37°C; sample 2 in the presence of 2 mM $(A)_4$, and sample 3 without any codon.

Both samples were twice precipitated with ethanol and washed. The tRNA samples were hydrolysed with RNAase T_1 in the presence of phosphatase and the analysis was carried out according to the diagonal fingerprint method described [14].

**Irreversibly modified means that no hydrolysis of modified G residues occurred prior to the second dimension of the diagonal fingerprint [14]. This irreversibility was demonstrated in control 1.*

3. Results

3.1. *Kinetics of chemical modification of tRNA in the presence and absence of the codon*

The time dependence of the reaction of the radioactive guanosine specific reagent with $tRNA^{Phe}$ in the presence and absence of the codon $(U)_4$ is shown in fig.1. As can be clearly seen the reaction in the

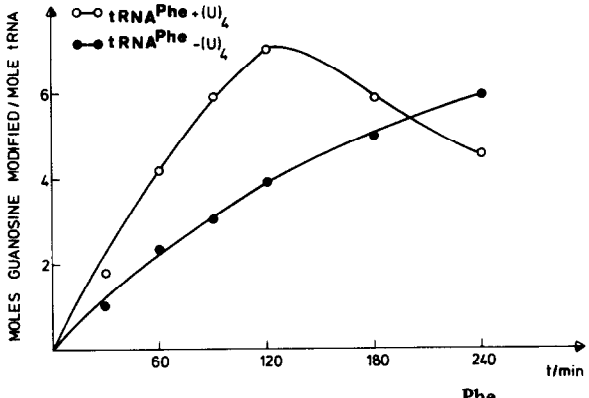


Fig.1. Time dependence of the reaction of $tRNA^{Phe}$ with the [^{3}H]methylester of *p*-carboxyphenylglyoxal in the presence and absence of the codon $(U)_4$. The codon concentration was 1 mM.

presence of $(U)_4$ proceeds faster leading to a difference of the equivalent of 3 additional G residues being modified within 2 h reaction. After that time, however, the number of modified guanosine decreases in the sample with the codon. We believe this decrease is caused by two factors:

(1) Guanosine 34 in the anticodon is modified leading to a decrease in the codon-anticodon complex concentration.

(2) The modification is reversible such that the reagent can dissociate and allow the tRNA structure to refold.

In order to avoid the complication of the reagent reacting with either the codon, or the anticodon, we tested the system: $tRNA^{Lys}$ with $(A)_4$ as the codon.

The kinetics of the modification of the guanosine residues in $tRNA^{Lys}$, in the presence and absence of the codon $(A)_4$, are shown in fig.2. In the presence of the codon the modification proceeds faster and ends on a plateau. The equivalent of about 5 guanosines/mol tRNA are modified additionally in the presence of the codon within 4 h.

Association constants between tetranucleotides and tRNAs are as high as $10^3 - 10^4$ M^{-1} only for the anticodon [15,16]. Strong interactions of oligonucleotides with other parts of the tRNA that alter the tRNA structure are therefore very unlikely. It is also evident from the primary structure [17] that with the exception of the anticodon there are no

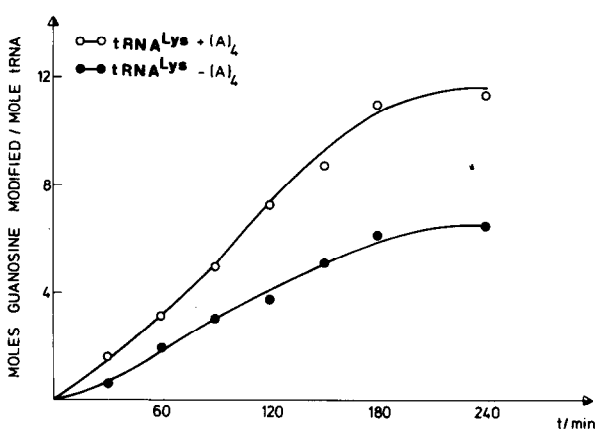


Fig.2. Time dependence of the reaction of $tRNA^{Lys}$ with the [^{3}H]methylester of *p*-carboxyphenylglyoxal in the presence and absence of the codon $(A)_4$. The codon concentration was 1 mM.

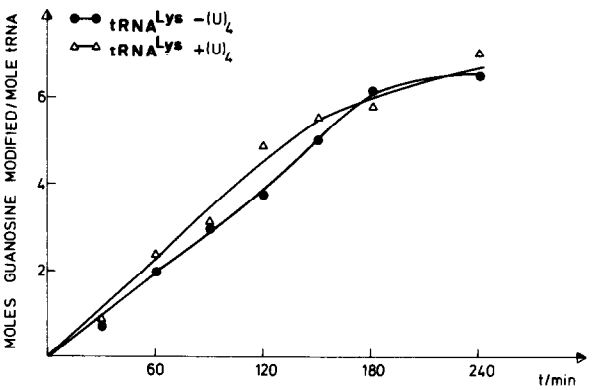


Fig.3. Time dependence of the reaction of $tRNA^{Lys}$ with the [^{3}H]methylester of *p*-carboxyphenylglyoxal in the presence and absence of the non-coding oligonucleotide $(U)_4$. The oligonucleotide concentration was 1 mM.

complementary sequences to $(A)_4$ longer than two bases. In order to ensure that no stacking, or H bonding interactions other than base pairing to the anticodon, occur at the rather high oligonucleotide concentrations in the reaction mixture (2 mM), the system was tested with $tRNA^{Lys}$ and $(U)_4$ that is not recognized as a codon. The result is shown in fig.3. Only a very small increase in reactivity was detected relative to those in fig.1 and 2. We conclude, therefore, that the above observed increase in reactivity of the guanosine residues is triggered by the codon–anticodon interaction.

3.2. *Preliminary analyses of the chemically modified G residues*

A partial analysis was made of the regions of the tRNA that were involved in the structural change. The experiment was performed as outlined in methods. The diagonal fingerprints of sample 2 and 3 looked very similar, but showed strong quantitative differences (fig.4, table 1). In both cases, at least 7 new spots migrated away from the diagonal line containing the unmodified oligonucleotides. Approximate quantitation of these 7 spots is shown in table 1. For all except spot 5 (G_p), the radioactivity was much stronger in the codon-containing sample 2. Accurate quantitation of diagonal fingerprints prepared with crude [^{32}P]tRNA was difficult especially for spots moving close to the diagonal. In the diagonal fingerprint of sample 1 no new spots were clearly visible

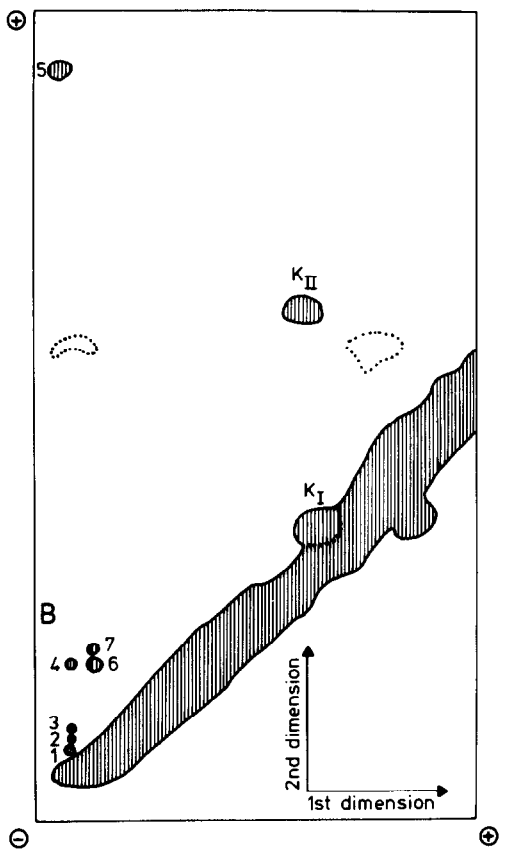


Fig.4. A drawing of the autoradiogram of a diagonal fingerprint from sample 2. The seven off-diagonal spots are indicated. K_I and K_{II} were used as control spots for the standardisation. The position of the blue tracking dye (xylene cyanol FF) is indicated by the letter B.

Table 1
Radioactivity present in the modified oligonucleotides

Diagonal spot no.	Counts per minute		sample 2 / control 3
	sample 2	control 3	
1	15 140	2790	5.4
2	12 550	1480	8.5
3	7280	1120	6.5
4	5370	1100	4.9
5	1550	3540	0.4
6	4740	1490	3.2
7	4110	770	5.3

The cpm values given are averaged from two determinations and are standardized against control spots on and off the diagonal (see fig.4)

off the diagonal which suggests that little or no hydrolysis of modified G-residues had occurred.

A preliminary analysis of the spots from the diagonal fingerprint (fig.4) showed that spot 3 is likely to be identical to $T\psi CG_p$. This was established:

(i) By comigrating the identical marker oligonucleotide $T\psi CG_p$ at pH 1.9.

(ii) By determining the base composition.

For a more refined analysis of this and the other kethoxal modified oligonucleotides it will be necessary to use purified $tRNA^{Lys}$.

4. Discussion

A codon-induced rearrangement of $tRNA^{Phe}_{E.\,coli}$ and $tRNA^{Phe}_{yeast}$ occurs in the presence of mM concentrations of the cognate codon independently of the 30 S ribosome, EF–Tu X GTP and aminoacylation. Thus it was possible to investigate the structural change in a simple reaction system by chemical modification. It was observed, earlier, that the bases in the $T\psi CG$ region are not modified in native tRNA [4–6,18,19]. This result correlates well with the three-dimensional structure of $tRNA^{Phe}$ from yeast that was established from X-ray diffraction analyses of tRNA crystals [20,21], which demonstrated that the $T\psi CG$ sequence interacts with the dihydrouridine loop through base pairs between $G_{18}-\psi_{55}$, $G_{19}-C_{56}$ and a stacking interaction of G_{57} between G_{18} and G_{19}.

Opening up of this structure, as a result of the codon–anticodon interaction, would result in the breakdown of these interactions such that a number of G-residues were accessible for chemical modification including that in the oligonucleotide $T\psi CG$. The results here demonstrate such an increased accessibility of guanosines in the presence of the codon which is compatible with such an opening. However, the slow opening up of the tertiary and secondary structure of the tRNA in the absence of the codon due to chemical modification is also indicated by the steady increase of the reaction with time (fig.1–3). This was also evident from the diagonal fingerprint of control 3, where similar but much weaker modification was observed in the absence of codon.

A determination and comparison of the initial rate constants of the individual nucleotide modification reactions, with and without codon, will give a

more detailed picture of the sequence of events during the rearrangement. Moreover, the identification of the preferred sites of modification in the presence of codon may yield some information about how the structural change is transmitted from the anticodon to the TψCG loop. This remains to be substantiated by analyses with the purified [^{32}P]tRNA^{Lys}.

Acknowledgement

This work was supported by research grants from the Deutsche Forschungsgemeinschaft.

References

[1] Schwarz, U., Menzel, H. M. and Gassen, H. G. (1976) Biochemistry 15, 2484–2490.
[2] Schwarz, U. and Gassen, H. G. (1977) FEBS Lett. 78, 267–270.
[3] Robertson, J. M., Kahan, M., Wintermeyer, W. and Zachau, H. G. (1977) Eur. J. Biochem. 72, 117–125.
[4] Litt, M. (1969) Biochemistry 8, 3249–3253.
[5] Greenspan, C. M. and Litt, M. (1974) FEBS Lett. 41, 297–302.
[6] Hawkins, E. R. and Chang, S. H. (1974) Nucleic Acids Res. 1, 1531–1538.
[7] Wagner, R. and Gassen, H. G. (1975) Biochem. Biophys. Res. Commun. 67, 519–529.
[8] Schetters, H., Gassen, H. G. and Matthaei, H. (1972) Biochim. Biophys. Acta 272, 249–259.
[9] Pongs, O., Wrede, P., Erdmann, V. A. and Sprinzl, M. (1976) Biochem. Biophys. Res. Commun. 71, 1025–1033.
[10] Yoon, K., Turner, D. H., Tinoco, J., Van der Haar, F. and Cramer, F. (1976) Nucleic Acids Res. 3, 2233–2242.
[11] Fellner, P. (1969) Eur. J. Biochem. 11, 12–27.
[12] Monier, R. and Feunteun, J. (1971) in: Methods in Enzymology, Vol. 20, part C, pp. 494–502, Academic Press, New York.
[13] Shapiro, R., Cohen, B. J., Shiuey, S. J. and Maurer, H. (1969) Biochemistry 8, 238–245.
[14] Noller, H. F. (1974) Biochemistry 13, 4694–4703.
[15] Högenauer, G. (1970) Eur. J. Biochem. 12, 527–532.
[16] Uhlenbeck, O. C. (1972) J. Mol. Biol. 65, 25–41.
[17] Chakraburtty, K., Steinschneider, A., Case, R. V. and Mehler, A. H. (1975) Nucleic Acids Res. 2, 2069–2075.
[18] Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C. and Klug, A. (1974) Nucleic Acids Res. 1, 927–932.
[19] Rhodes, D. (1975) J. Mol. Biol. 94, 449–460.
[20] Ladner, J. E., Jack, A., Robertus, J. D., Brown, R. S., Rhodes, D., Clark, B. F. C. and Klug, A. (1975) Proc. Natl. Acad. Sci. USA 72, 4414–4418.
[21] Rich, A. and RajBhandary, U. L. (1976) Ann. Rev. Biochem. 45, 805–860.