

CONFORMATIONAL CHANGES IN tRNA

Consequences of aminoacylation and codon-anticodon recognition

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By equilibrium dialysis experiments we have shown [1] that the oligonucleotides U-C-C-C and C-G-A-A bind to phenylalanyl-tRNA^{Phe} (yeast) but not to tRNA^{Phe} (yeast), suggesting that the tertiary interactions between the pseudouridine (T ψ C) and dihydrouridine (D) loops are broken on aminoacylation. However, results obtained by the same technique have been employed [2,3] to postulate that the conformational change is not a consequence of aminoacylation but that it is induced by interaction of tRNA (whether aminoacylated or deacylated) with the appropriate codon in the presence of 30 S ribosomal subunits. This interpretation was developed initially to explain results obtained with tRNA^{Phe} (*Escherichia coli*) [2,3], but has been applied subsequently [4] to results obtained with the yeast system. In this communication we reconcile the two equilibrium dialysis studies by showing that the available data indicate a two-step conformational change in tRNA conformation; the first occurs on aminoacylation and the second as the result of codon-anticodon recognition.

Although it was considered [2-4] that neither tRNA^{Phe} nor Phe-tRNA^{Phe} interacts with C-G-A-A unless the tRNA is bound to the codon, closer examination of the data does in fact demonstrate binding of the oligonucleotide to Phe-tRNA^{Phe}. Table 1 summarises results that are taken from

equilibrium dialysis experiments reported in table III of [3]. A mean value of 14 000 M⁻¹ is obtained from these data for the K_a describing the interaction between C-G-A-A and Phe-tRNA^{Phe}; this is in excellent agreement with our estimate [1] of 13 000 M⁻¹.

The low specific activity of [³H](C-G-A-A) used in [3] (0.32 Ci/mmol versus 11 Ci/mmol in our experiments) and the low Phe-tRNA^{Phe} concentration (0.5 μ M versus 5 μ M) may have made it technically more difficult to detect C-G-A-A binding, especially since a much larger K_a ($\sim 10^6$ M⁻¹) was obtained in the presence of the codon and 30 S ribosomal subunits: indeed the conditions were selected to be optimal for study of the stronger interaction. Furthermore, consideration of the existence of the weak interaction with Phe-tRNA^{Phe} was precluded [2-4] on the basis that (i) C-G-A-A did not bind to deacylated tRNA^{Phe} [5] and (ii) that NMR measurements had failed to detect any structural differences between tRNA^{Phe} and Phe-tRNA^{Phe} [6]. However, those NMR measurements, which were carried out at low resolution and in low magnesium concentrations [7], have been supplanted by data at higher resolution [8] which do favor a small change in conformation on aminoacylation of tRNA^{Phe}. Use of laser light scattering [9] has recently provided additional support for the existence of conformational differences between aminoacylated and deacylated tRNA.

From the sum of evidence that has been accrued by different physical methods of measurement, there now appears to be little doubt that tRNA does undergo a conformational change on aminoacylation.

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Table 1
Binding of C-G-A-A to Phe-tRNA^{Phe}

Components		Excess C-G-A-A bound to the complete system (pmol)	C-G-A-A bound to Phe-tRNA ^{Phe} (pmol)	K^b (M ⁻¹)
30 S subunits	(50 pmol)	9.6		
30 S subunits + Phe-tRNA ^{Phe}	(50 pmol) (50 pmol)	9.1	0.5 ^c	4000
30 S subunits + EF-T _u · GTP ^a	(50 pmol) (400 pmol)	10.8		
30 S subunits + EF-T _u · GTP + Phe-tRNA ^{Phe}	(50 pmol) (400 pmol) (50 pmol)	9.6	1.2 ^c	20 000

^a GTP complex of elongation factor T_u

^b Calculated from the expression $K = [\text{Bound C-G-A-A}] / ([\text{Phe-tRNA}^{\text{Phe}}] [\text{C-G-A-A}])$

^c The difference between the last 2 entries in the previous column

The results, taken from table III of [3], refer to equilibrium dialysis experiments in which one compartment contained, in total vol. 0.1 ml, 100 nmol GTP and the components listed. The other compartment (total vol. 0.1 ml) contained the complete system (50 pmol 30 S ribosome subunits + 25 nmol (uridylyl-3',5'),-uridine (U₈) + 50 pmol Phe-tRNA^{Phe} + 400 pmol EF-T_u · GTP) and 250 pmol C-G-A-A (tritiated). Amounts of Phe-tRNA^{Phe} take into account the fact that only 25% of the tRNA^{Phe} preparation was aminoacylated

Furthermore, the equilibrium dialysis studies [2-4] provide definitive evidence of a subsequent conformational change accompanying the codon-anticodon interaction. Clearly this second structural change, which is indicated by a large enhancement (50-100-fold) of the K_a for the interaction between C-G-A-A and Phe-tRNA^{Phe}, would provide a chemical basis for high fidelity translation in protein synthesis [10].

References

- [1] Dvorak, D. J., Kidson, C. and Chin, R. C. (1976) *J. Biol. Chem.* 251, 6730-6734.
- [2] Schwarz, U., Lührmann, R. and Gassen, H. G. (1974) *Biochem. Biophys. Res. Commun.* 56, 807-814.
- [3] Schwarz, U., Menzel, H. M. and Gassen, H. G. (1976) *Biochemistry* 15, 2484-2490.
- [4] Schwarz, U. and Gassen, H. G. (1977) *FEBS Lett.* 78, 267-270.
- [5] Pongs, O., Bald, R. and Reinwald, E. (1973) *Eur. J. Biochem.* 32, 117-125.
- [6] Wong, Y. P., Reid, B. R. and Kearns, D. R. (1973) *Proc. Natl. Acad. Sci. USA* 70, 2193-2195.
- [7] Kearns, D. R. (1976) *Progr. Nucl. Acid Res. Mol. Biol.* 18, 91-149.
- [8] Kan, L. S., Ts'o, P. O. P., Sprinzl, M., Von der Haar, F. and Cramer, F. (1976) *Biophys. J.* 16, 11a.
- [9] Potts, R., Fournier, M. J. and Ford, N. C., jr (1977) *Nature* 268, 563-564.
- [10] Kurland, E. G., Rigler, R., Ehrenberg, M. and Blomberg, C. (1975) *Proc. Natl. Acad. Sci. USA* 72, 248-251.