DIFFERENCES IN BINDING OF OLIGO C TO CHARGED AND UNCHARGED tRNA

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1. Introduction

Preliminary studies by different methods (chromatography [1], magnetic resonance [2], circular dichroism [3]) have suggested that the charged and uncharged forms of tRNA have a different tertiary structure. In order to find the regions involved in such changes, we have studied binding of oligonucleotides to tRNA during the aminoacylation reaction.

Such an investigation is based on the ability of exposed regions of tRNA to bind readily to complementary oligonucleotides [4]. The binding of labelled nucleotides to tRNA can be followed either by equilibrium dialysis, by filtration on Sephadex G-50, or by trichloroacetic (TCA) precipitation of tRNA and filtration on millipores: the bound oligonucleotides, when precipitated with TCA and washed with ethanol, coprecipitate with the tRNA, while the unbound oligonucleotides are found in the filtrate. This last method is convenient and rapid (which is an important advantage in studies involving charged tRNA at physiological pH); however, it is more subject to criticism since it involved coprecipitation. In order to check the latter we compared the association constant of oligonucleotides obtained by this method with that found by equilibrium dialysis or by filtration on Sephadex G-50.

Evidence is presented that more oligo C is bound by amino-acylated tRNA than by the uncharged species, at physiological pH.

2. Material and methods

2.1. *tRNAs*

Unfractionated *E.coli* tRNA was purchased from General Biochemicals (Chagrin Falls, Ohio) and purified on a Sephadex G-50 column. Yeast purified phenylalanine-tRNA was purchased from Boehringer (Mannheim, Germany); its phenylalanyl acceptor activity was calculated as 95% (when 0.02 A_{260} of $tRNA^{Phe}$ is acylated with ^{14}C-phenylalanine, 95% of the theoretical amino acid incorporation is found, according to an absorption of 8.3 A_{260} per mmole in (PO_4^-)). *E.coli* purified valine-tRNA was a gift from Dr. M.Yaniv; its valyl acceptor activity was calculated as 98% (same conditions as for $tRNA^{Phe}$). *E.coli* purified formylmethionine-tRNA was a gift from Dr. B.F.C.Clark; its methionyl acceptor activity was calculated as 90% (same conditions as for $tRNA^{Phe}$).

The acetylating enzymes were the DEAE fraction of *E.coli* supernatant for *E.coli* tRNAs, and the fraction after streptomycin 1% precipitation of baker's yeast crude extract for yeast $tRNA^{Phe}$.

2.2. *TCA coprecipitation*

The tRNAs were acetylated either in the presence of ^{14}C-labelled amino acids or cold ^{12}C-amino acids (see legends for figures). The charging mixture was deproteinized by addition of 0.1 ml isoamylic alcohol and 0.25 ml chloroform, followed by centrifugation. The supernatant was incubated for 5 min with the oligonucleotides and precipitated by 2 ml of cold trichloroacetic acid 10% and 0.2% amino acids, followed by filtration over Millipore filters (or glass filters Whatmann GF/C), and washed by 25 ml of cold ethanol (or isopropanol); the radioactivity remaining on the filters was counted in a Packard scintillation counter with 5 ml of toluene, 5% PPO, 0.3% POPOP. The binding constant is estimated from K = bound oligonucleotides/tRNA × free oligonucleotides.

2.3. *Equilibrium dialysis*

The dialysis is performed in plexiglass cells of 100 μl volume, separated by a collodion membrane, 24 or 48 hr at 20°, in the following mixtures: *Cell 1:* tRNA, 10 A_{260}; tris-HCl (pH 7.5) 50 mM; $MgCl_2$, 15 mM; and oligo C, 50,000 cpm (25,000 cpm/μmole in CMP); *Cell 2:* same mixture minus tRNA.

3. Results and discussion

Fig. 1a,b shows the kinetics of aminoacylation of unfractionated *E.coli* tRNA and yeast $tRNA^{Phe}$ correlated to the binding of oligonucleotides ($(Cp)_n$, n = 1 to 10) to a mixture of charged and uncharged tRNA, at pH 7.5. The amount of oligo C bound parallels the observed amount of aminoacylation.

In fig. 1b, the binding of oligo U to yeast $tRNA^{Phe}$ is also shown; it is likely that this binding occurs with a much smaller affinity, at the anticodon region, since a comparison with unfractionated tRNA does not show similar binding of oligo U. The results of the binding of oligo U to charged and uncharged $tRNA^{Phe}$ are quite different from those found with oligo C: it is not dependent on the charging of tRNA by phenylalanine, and the binding constant is very weak (about 100 M^{-1}).

In the case of uncharged tRNA, two peaks of labelled $(Cp)_4$ are observed, one migrating with the tRNA and the other at the normal oligonucleotide position, with a broad intermediate region showing the exchange between the bound and unbound species. In the case of charged tRNA, not only is the first peak (migrating with the tRNA) higher, but the maximum of the second peak is broader and is shifted towards the tRNA value, thus showing a much bigger affinity of $(Cp)_4$ for the charged tRNA than for the uncharged species. The differences in the binding can be estimated from the curve: the charged tRNA binds oligo C 5 to 10 times more strongly than the uncharged tRNA (cf. legend to fig. 2).

Table 1 shows that the association constants of oligo C with tRNA, obtained by TCA coprecipitation, is a function of the length of the oligonucleotide. The best binding occurs with oligonucleotides having a chain length of 4.

In order to compare the binding constants obtained by TCA coprecipitation with a standard method,

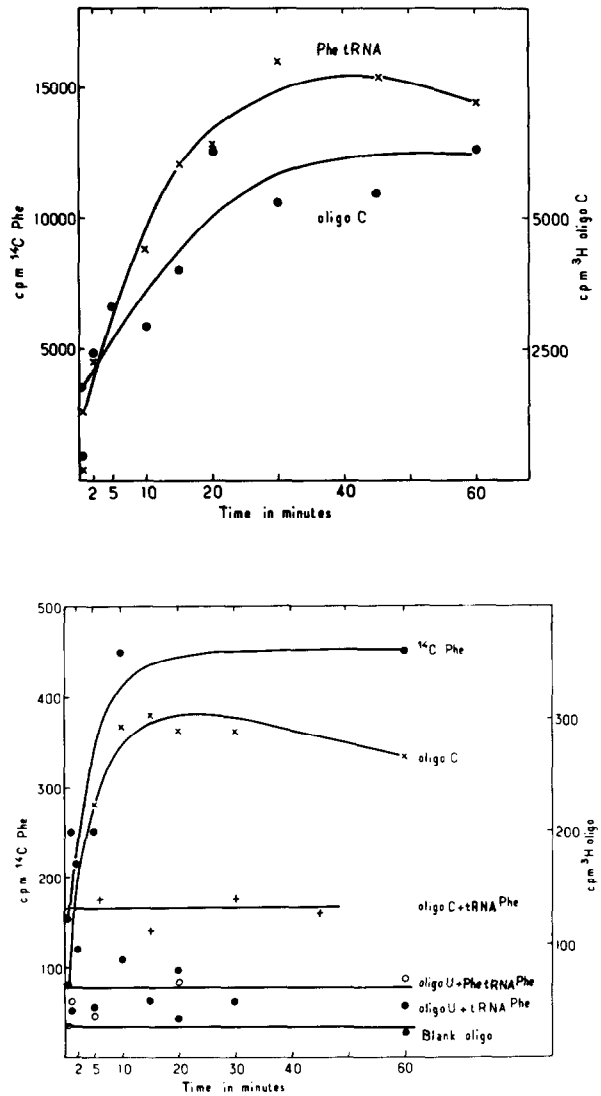

Fig. 1. Binding of oligo C to charged and uncharged tRNA.

(a) Unfractionated *E.coli* tRNA: In a total volume of 1 ml, aliquots of 100 μl are collected at various times; after precipitating the enzyme with chloroform and isoamylic acid, the supernatant is incubated 5 min at 25°, in the presence of ^{3}H-oligo C (specific activity, 60 μCi/μmole of CMP). The final mixture is precipitated by TCA.

(b) Yeast $tRNA^{Phe}$: Same procedure as above, except that the amount of tRNA is 10 A_{260}/ml, and that one out of two of the aliquots collected contains ^{12}C-phenylalanine instead of ^{14}C-phenylalanine.

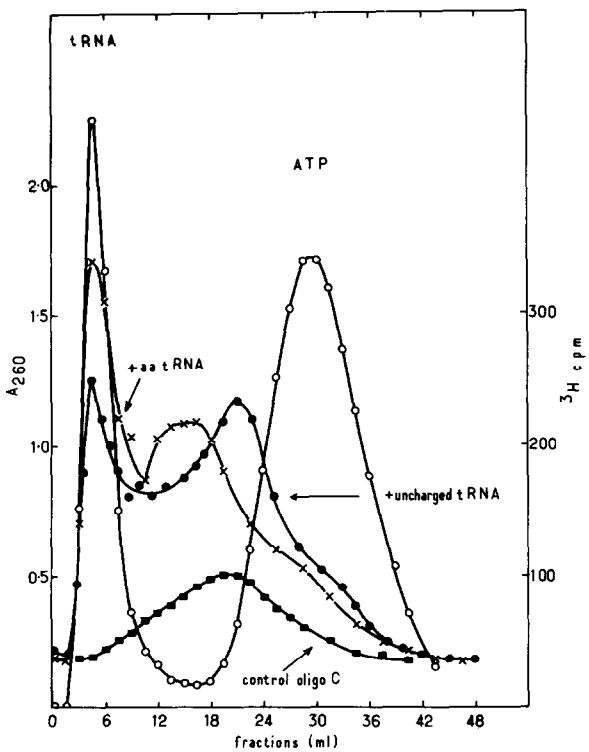

Fig. 2. Binding of tritiated oligo C, $(Cp)_4$, to charged and uncharged tRNA, shown by passage over a Sephadex G-50. (coarse grade) column (1.2 X 75 cm, 60 ml/hr) at 20°. The position of tRNA and ATP used as a marker for uncharged tRNA, are calibrated and may be used to show a change in the oligonucleotide peak. The effluent contains: tris-HCl buffer, pH 7.5, 0.05 M, and $MgCl_2$, 10 mM. The incubation mixture used for the acylation of tRNA contains (for 1 ml): *E.coli* unfractionated tRNA, 10 A_{260}, tris-HCl, pH 7.5, 50 mM; $MgCl_2$, 10 mM; ATP, 10 mM; a mixture of the 20 amino acids (5 mM of each); and 1.5 mg of the DEAE fraction of *E.coli* supernatant. This mixture is incubated 15 min at 37° and then deproteinized by a Sevag method; the supernatant is incubated for 5 min with 10,000 cpm $(Cp)_4$ (25,000 cpm/μmole in CMP) before it is put on the column. The procedure is the same for uncharged tRNA, except that the medium is devoid of amino acids, and contains only 0.5 mM of ATP. o—o : ultraviolet absorbancy; ▪—▪ : ^3H $(Cp)_4$ alone; •—•: ^3H $(Cp)_4$ + uncharged tRNA; x—x: ^3H $(Cp)_4$ + aminoacylated tRNA.

With uncharged tRNA 9000 cpm are recovered, and with charged tRNA, 9400 cpm, the relative intensities of the oligonucleotide peaks migrating at the place of $(Cp)_4$ as a control are in the ratio of 5 to 10, depending on the predicted shape of the peak), thus showing that the binding constants are also in a ratio of 5 to 10.

an equilibrium dialysis was performed with CpCpC and unfractionated *E.coli* tRNA; the values are similar, as shown in the table.

The binding of oligo C to purified *E.coli* $tRNA^{Val}$ and yeast $tRNA^{Phe}$ shows the same phenomenon, i.e. that charged tRNA binds more oligo C than the uncharged species. However, neither with *E.coli* $tRNA_F^{Met}$ nor with Met-$tRNA_F^{Met}$ could we detect any binding of oligo C. Whether this reflects a real phenomenon or whether TCA coprecipitation is not an optimum method in the case of Met-$tRNA_F$ cannot be decided at this point since the binding probably depends markedly on experimental conditions.

We must emphasize here that all the experiments reported above were done immediately after the charging of tRNA and at physiological pH (7.5). When the incubation of charged tRNA with oligo C was done at pH 5.4, we could not detect any difference in the binding of oligo C, either by TCA coprecipitation or by Sephadex filtration; but the Sephadex filtration revealed that under such conditions the oligo C are markedly self-associated, especially in the presence of an RNA carrier, so that neither method is suitable at acid pH. However, such a self-association at acidic pH may explain the efficiency of the TCA coprecipitation since an oligo C bound to tRNA might act as a primer to self-association, amplifying the binding phenomenon so that it is really visible. Thus, the binding constant might be overestimated. However, this does not change the relative differences in binding to charged and uncharged tRNA.

In conclusion, the mixture of *E.coli* tRNA, purified yeast $tRNA^{Phe}$, and *E.coli* $tRNA^{Val}$, when charged, bind more oligo C than when uncharged, while the binding of $tRNA^{Phe}$ to oligo U, which presumably occurs at the anticodon region, is independent of the attachment of the amino acid. It is therefore likely that during the attachment of amino acid, a region containing a sequence of at least two Gs is exposed, whereas this region is usually not exposed when the tRNA is uncharged. (The association constant for two consecutive G.C pairs was calculated from equilibrium dialysis by Uhlenbeck et al. [4] and is of the same order of magnitude as the one reported here, i.e. 10^4). These region containing a sequence of G should be common to most of the tRNAs since mixed tRNA behave similarly to purified yeast or *E.coli* tRNA.

Table 1
Binding constants of oligo C to charged and uncharged tRNA obtained by TCA coprecipitation.

	$(Cp)_n$ *	CpCpC	$(Cp)_4$	$(Cp)_5$	$(Cp)_6$	$(Cp)_7$
Unfractionated $tRNA^{coli}$						
charged	1300	10,000	25,000	20,000	10,000	10,000
uncharged: 1) TCA	**	2,000	3,000	2,000	1,500	1,500
2) Equil. dialysis ***		2,600				
$tRNA^{coli}_{Val}$						
charged		5,000	5,000			
uncharged		1,500	1,500			
$tRNA^{yeast}_{Phe}$						
charged	1000	5,000	5,000			
uncharged	**	1,000	1,000			
$tRNA^{coli}_{F}$						
charged		**	**			
uncharged		**	**			

* $(Cp)_n$ is a mixture of oligo Cs of length 1 to 8.
** Not detected.
*** The equilibrium dialysis is given as comparison.

It is tempting to speculate that the two Gs in the dihydro U loop, common to all tRNAs, are the ones which become exposed when tRNA is charged. It should be noted at this point that the dihydro U loop has to be in suitable configuration in order to allow the aminoacylation reaction to occur [5].

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References

[1] U.Z.Littauer and R.Stern, FEBS Oslo (1968) 93.
[2] M.Cohn, A.Danchin and M.Grunberg-Manago, J. Mol. Biol. 39 (1969) 199.
[3] P.S.Serin and P.C.Zamecnik, Biochem. Biophys. Res. Commun. 20 (1965) 400.
[4] O.C.Uhlenbeck, J.Boller and P.Doty, Nature 225 (1970) 308.
[5] A.Danchin and M.Grunberg-Manago, Biochem. Biophys. Res. Commun. 39 (1970) 683.