COMPLEX FORMATION BETWEEN TRANSFER RNAs WITH COMPLEMENTARY ANTICODONS : USE OF MATRIX BOUND tRNA.

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SUMMARY : It is shown that yeast $tRNA^{Phe}$, chemically coupled by its oxidized 3'CpCpA end behaves exactly as free $tRNA^{Phe}$ in its ability to form a specific complex with E. coli $tRNA_2^{Glu}$ having a complementary anticodon. The results support models of tRNA in which the $3'CpCpA_{OH}$ end and the anticodon are not closely associated in the tertiary structure, and provide a convenient tool of general use to characterize others pairs of tRNA having complementary anticodons, as well as for highly selective purification of certain tRNA species.

INTRODUCTION : Pairing of different tRNA's by their anticodons was first demonstrated for the $tRNA^{Phe}$: $tRNA_2^{Glu}$ pair (1-2). $tRNA^{Phe}$ from yeast has a highly fluorescent base (the so called Y base (3), adjacent to the 3'-end of the anticodon (4). This provides a sensitive natural probe to monitor the pairing reaction (1,5,6); more recently, the same couple of tRNAs has been characterized by gel electrophoresis (2).

The pecularity of the Y base as well as the requirement that the two tRNA's be in highly purified form set obvious limits to this approach; the use of an insolubilized pure tRNA species should provide a convenient tool of general use to characterize tRNA complexes and study their properties; the cognate species of aminoacyl-tRNA need not be isolated from the crude tRNA mixture.

tRNA bound by its 3' end to a solid matrix has been used already for selectively binding the cognate aminoacyl:tRNA ligase (7-9) indicating that a tRNA bound in that manner is accessible to a big molecule. In the present work, it is shown that yeast $tRNA^{Phe}$ chemically coupled by its oxidized 3' end

to derivatized polyacrylamide gel beads (10) retains its full capacity to form a complex with E. coli $tRNA_2^{Glu}$. Implications of this observation as to the structure of tRNA and also as the basis for highly selective method for isolating certain species of tRNA will be discussed.

MATERIAL AND METHODS.

Chemical coupling of oxidized yeast $tRNA^{Phe}$ to derivatized polyacrylamide gel beads : The resin used throughout the work was derivatized from Bio-Gel P-200 (Bio-Rad Lab.Richmond, USA) by treatment with hydrazine hydrate according to Inman and Dintzis (10). The degree of substitution ranges from 0.07 to 0.09 millimoles/g (wet weight) as determined by titration of the succinyl-hydrazine derivative (10).

Purified yeast $tRNA^{Phe}$, obtained from Boehringer Mannheim GmbH (specific activity of 1180 pmol/A_{260}), was oxidized with sodium metaperiodate 2mM final concentration, as described by Goodman et al. (11). The excess periodate was destroyed by éthylène glycol and the nucleic acid was then precipitated with ethanol, washed once and redissolved in 0.1 M sodium acetate buffer (pH 4.8).

5 to 15 A_{260} of oxidized tRNA was coupled to the derivatized gel at 4° in 1 ml final volume of 0.2M sodium acetate buffer (pH 4.8) containing 0.2 g (wet weight) resin. Binding usually reached 80-90 % within 2 hours, as measured by the decrease in absorbance of the supernatant solution. When the tRNA-resin was used for binding equilibrium experiments, the unreacted hydrazide groups of the resin were acetylated by addition of acetic anhydride (3x5 µl per 0.2 g of resin, at 5 min. intervals) to the reaction medium. This prevents hydrazinolysis of the ester bond which occurs with the glutamyl-

tRNA's, interfering with the exact quantitation of tRNA whenever radioactive glutamyl-group is used as the label. For chromatographic use, the resin was contained in columns of 0.5 cm in diameter and 4 cm length; it was not necessary to acetylate the remaining hydrazide groups of the resin since excessive glutamyl-cleavage during separation did not occur (less than 5 % of the original glutamyl-tRNA). With other aminoacyl-tRNA species (e.g. phenylalanyl-tRNA) no significant cleavage occurs.

The chemical stability of the tRNA derivative was checked under various conditions; it was neither impaired by incubation at 30°C or 37°C nor by exposure to high ionic strength buffers within a wide pH range (unpublished results of P. Nokin). Prolonged incubation did not release amounts exceeding 1 % of the bound tRNA (20 hours at 4°C, in 10 mM sodium acetate buffer pH 4.8, containing 10 mM Mg sulfate and 1 M NaCl). The columns described in this work have been run for more than 10 cycles without any noticeable loss of efficiency.

Formation of tRNA : tRNA complexes : Unless otherwise stated, tRNA:tRNA complexes were formed at 0-2°C in 10 mM sodium acetate (pH 4.8) containing 10 mM Mg sulfate and 1 M NaCl (buffer 1). After washing the resin with buffer 1, the solution containing unfractionated tRNA labelled with (^{14}C) or (^{3}H) on a particular aminoacyl moiety was added to the resin mixture with constant stirring of the suspension, or was applied on a column at a flow rate of 2.5 ml/hr. In the latter case, washing was continued with 10-20 ml of buffer 1 at 6-8 ml/hr. High saline concentration in the buffer stabilizes the tRNA:tRNA complex and reduces the ionic interactions during the chromatographic process. The retained material was quantitatively

eluted at 30°C with buffer 2 (sodium acetate 10 mM pH 4.8, 1 M NaCl, 10 mM EDTA). Fractions (0.5 ml) were collected and their absorbance at 260 nm was measured; portions were taken for scintillation counting in Triton X-100 based fluid according to Patterson and Greene (12). Fractions were pooled as indicated, and the nucleic acid was recovered by ethanol precipitation or by lyophilization after dialysis against water. When the specific activity of tRNA had to be determined, the material eluted with buffer 2 was reactivated by heating at 65°C for 5 min. in the presence of 20 mM Mg^{2+} before the aminoacylation assays.

Aminoacylation assays and preparation of labelled aminoacyl-tRNA : Aminoacylation of tRNA was carried out routinely at 37°C in a medium containing 50 mM HEPES-KOH buffer (pH 7.6), 3 mM $MgCl_2$, 1 mM adenosine triphosphate, 1 mM dithioerythrol, 0,05 mM (^{14}C) or (^{3}H) labelled aminoacid and a final concentration of 0.1 mg/ml of partially purified E. coli extract; (DEAE fractions referred in 13). After incubation for 30 or 45 min., aliquots were precipitated with 10 % TCA on Whatman 3 MM paper discs, washed with 6 % TCA, dried and counted by liquid scintillation. The rest of the incubation mixture was applied on small DEAE-cellulose columns and labelled aminoacyl-tRNA was recovered as described by Reeves et al. (14).

Chemicals : Unfractionated tRNA from E. coli (strain B) and labelled aminoacids were purchased from Schwarz BioResearch (Orangeburg, USA). Adenosine triphosphate (disodium salt), dithioerythrol and HEPES (N-2 hydroxymethylpiperazine-N'-2-ethane sulfonic acid) were purchased from Sigma (St-Louis, USA). Hydrazine hydrate (80 % w/w) and sodium metaperiodate were from Merck A.G. (Darmstadt, Germany).

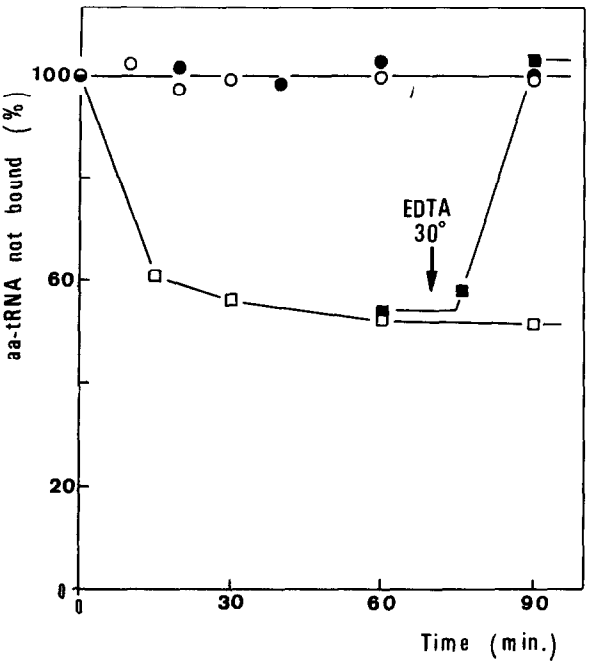

FIGURE 1.

INTERACTION OF Glu-tRNA^{Glu} WITH RESIN BOUND tRNA^{Phe}.

0.2 g (wet weight) derivatized polyacrylamide beads bearing 4.2 A_{260} units of purified yeast tRNA^{Phe} were dispersed, at 0°C, in 0.1 M Na Acetate buffer (pH 4.8) containing 10 mM Mg^{++}. 2.7 A_{260} units of unfractionated E. coli (^{14}C) Glu-tRNA (□,■) or 3.6 A_{260} units of E. coli (^{14}C) Phe-tRNA (○) were added to the mixture (final volume of 1 ml). Radioactivity of the supernatant solution was estimated on 20 µl aliquots. At the time indicated by the arrow, the sample was heated to 30°C and EDTA was added to a final concentration of 20 mM. As a control the same (^{14}C) Glu-tRNA (●) was incubated in presence of resin without bound tRNA^{Phe}. The absence of aminoacyl-cleavage was ascertained by determination of the TCA precipitable radioactive fraction at 100 min.

RESULTS.

Evidence for strong binding of tRNA^{Glu} to resin bound tRNA^{Phe}.

Fig. 1 shows that insolubilized yeast tRNA^{Phe}, when mixed with unfractionated E. coli tRNA, specifically binds Glu-tRNA for the radioactivity associated with this nucleic acid decreases as a function of time in the supernatant solution. No binding whatsoever was observed on the same resin with unfractionated tRNA containing labelled Phe-tRNA; also, the radioactivity

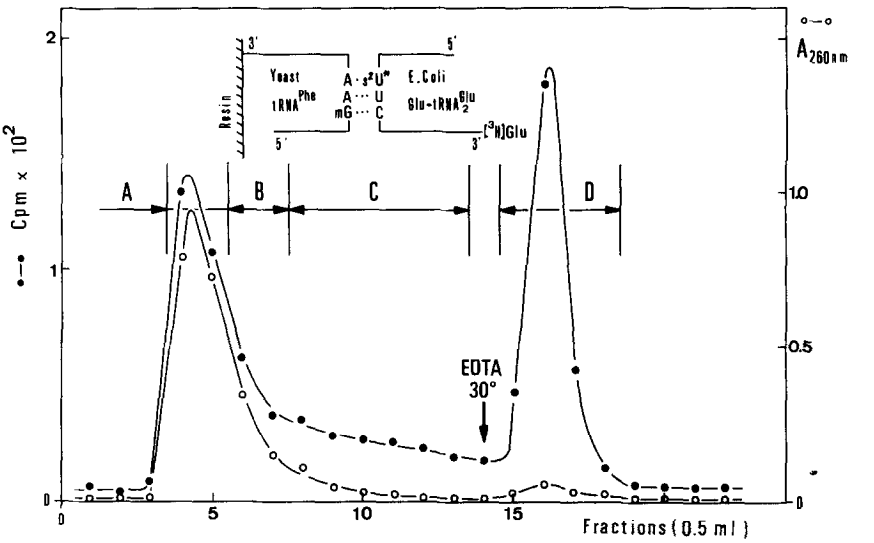

FIGURE 2.

RETENTION OF Glu-tRNA^{Glu} ON A COLUMN PACKED WITH RESIN BOUND tRNA^{Phe}.

The column contained 11 A_{260} units of purified yeast tRNA^{Phe} coupled to 0.2 g (wet weight) resin. 2.7 A_{260} units of E. coli (^{3}H) Glu-tRNA was used. The arrow indicates the change of elution buffer and temperature. The absorbance at 260 nm (O) and the radioactivity (●) were estimated as indicated in Methods. The recovered radioactivity was : 38 % in A + B, 24 % in C and 38 % in D. The anticodon of yeast tRNA^{Phe} is pG*pApA (4) and E. coli tRNA$_2^{Glu}$: ps^{2}U*pUpC (16), G* standing for 2'-o-methyl guanosine and s^{2}U* for 5-methyl-amino-methyl-2- thiouridine.

associated with Glu-tRNA remained almost constant in the supernatant with control resin free of bound tRNA^{Phe}.

Under the condition of the assays, binding equilibrium was reached within 20 to 30 min. at 0°C, and about 50 % of the Glu-tRNA engaged was reversibly bound to the resin-tRNA^{Phe}. The bound Glu-tRNA can be released by the addition of an excess of EDTA and raising the temperature of the mixture to 25-30°C; these treatments presumably alter the conformation of the anticodon loop in tRNA^{Phe} (5).

Assuming that the bound tRNA^{Phe} behaves as if it was free

in solution, and neglecting the small amount of free $tRNA^{Phe}$ introduced as part of the unfractionated tRNA mixture, one finds for the association constant

$$K_a = \frac{(tRNA^{Phe} : Glu\text{-}tRNA^{Glu})}{(tRNA^{Phe})\ (Glu\text{-}tRNA^{Glu})} \simeq 1.5\ 10^5\ mole^{-1}.$$

This value is only slightly lower than 5.10^5 $mole^{-1}$ found by Eisinger at 0°C for the same couple (1) under slightly different ionic conditions. The agreement between the two estimations is good enough to say that the insolubilization technique does not significantly alter pairing and the properties of the complex.

Fig. 2 shows a chromatographic application of the above system. Unfractionated (^3H) Glu-tRNA was passed at a low constant flow rate-through a column containing the resin-RNA^{Phe} compound. Clearly, only a fraction of the radioactive material elutes in the flow-through; most of the Glu-tRNA is retained by the column. A slight tailing of (^3H) Glu-tRNA occurs within the two hours needed for the complete washing-procedure.It reflects most probably the release of some nucleic acid initially associated with the insolubilized $tRNA^{Phe}$. The tRNA remaining in the column can be recovered quantitatively as a single peak, by eluting at 30°C with buffer containing 10 mM EDTA. The ratio of retained to unretained Glu-tRNA is in good agreement with the result of Fig. 1; as can be judged by the absorbance profile at 260 nm, the bulk of the tRNA engaged flowes through the column, less than 5 % of the nucleic acid being recovered in the bound fraction.

Under the same experimental conditions, but using unfractionated tRNA labelled with phenylalanine, the radioactivity associated with the Phe-tRNA is quantitatively recovered in the flow-through, and less than 2 % of the initial radio-

TABLE I

AMINOACYLATION ASSAYS OF THE NUCLEIC ACIDS RECOVERED IN DIFFERENT FRACTIONS FROM A COLUMN PACKED WITH RESIN BOUND $tRNA^{Phe}$.

ac.am. tested	control : unfractionated tRNA	Specific activity in pooled fractions			
		A	B	C	D
GLU	48	26	185	313	940
LYS	68	54	449	487	87
ASP	53	54	80	45	53
ARG	88	92	172	117	59
TYR	34	31	137	39	68
LEU	118	128	80	35	24
ALA	57	50	57	20	0
CYS	33	29	6	0	0
Others ac.am.	-	-	-	-	0-20
Total	1010	-	-	-	1334

Resin coupled tRNA and conditions used for fractionation were identical to those of Fig. 2, except that 19 A_{260} units of non acylated E. coli tRNA were used. 20 ml of buffer 1 was passed through the column before eluting the complexed tRNA with buffer 2. The nucleic acid contained in pooled fractions A (16 A_{260}) (see Fig. 2) was recovered by precipitation with ethanol. Fractions B (1.5 A_{260}), C (0.7 A_{260}) and D (0.4 A_{260}) were dialyzed overnight against water, then lyophilized and redissolved in water. The aminoacylation assay was performed as described in methods; results are expressed in pMoles of aminoacid incorporation per A_{260} unit. The purity of $tRNA^{Glu}$ in fraction D is 60 %, assuming that the UV absorbing material is made of active molecules exclusively, with an average molecular weight of 25,000.

activity is eluted in the complex fraction (result not shown).

The same results are obtained when uncharged tRNA is used. In this latter case, the accepting activity for several

aminoacids of the tRNA recovered in fractions eluted from the resin-coupled $tRNA^{Phe}$, was determined by the aminoacylation assay.

The eluates were pooled in four fractions containing respectively (Fig. 2) the unretained material (fraction A), the tailing edge of the first peak (fractions B and C) and the material eluted with buffer 2 (fraction D). The results shown in table 1 are expressed as specific aminoacid acceptor activity. They indicate clearly that the activity of $tRNA^{Glu}$ is depleted in fraction A, which passed the column; conversely, fraction D contains almost exclusively $tRNA^{Glu}$ with a 19 fold increase in specific activity, as compared to unfractionated tRNA. No significant acceptor activity for most of the others aminoacids was detected in fraction D, except for Lys, Asp, Arg and Tyr. This observation, as well as the increased activity for these particular tRNAs in the tail of the first peak (fractions B and C), are indicative of a weak interaction with the unsolubilized $tRNA^{Phe}$.

Detailed analysis of these systems will be discussed in another paper (15).

DISCUSSION.

The data presented here clearly show that tRNAs having complementary anticodons form readily a complex even when one of the tRNAs is chemically coupled to a rigid matrix by its 3'-end. The association constant is close to the value found by fluorescence studies (1) and by gel electrophoresis (2) indicating that the insolubilization technique does not introduce major changes in the pairing reaction; the only significantly altered parameter is the time required to approach equilibrium : 20 min with bound tRNA as against a few minutes for the free system (2).

A significant fraction of the bound tRNA remains accessible to macromolecules and the derivatized acrylamide matrix does not create much hindrance around the anticodon region. This observation falls in good agreement with the structures proposed for tRNA, in which the 3'$pCpCpA_{OH}$ end is located at the opposite side of the molecule with respect to the anticodon, or at least some distance from it(for review see at 17,18)(19).

Recent models suggesting that the 3'$pCpCpA_{OH}$ end is stacked with the anticodon (20, 21) cannot be strictly excluded on this basis, because of the possible mobility of this $pCpCpA_{OH}$ end (22) but it would seem that the region of the acrylamide fibre bearing the attachment point would restrict the space available around the anticodon of the bound species.

In a recent model derived from X-rays studies, the 3' $pCpCpA_{OH}$ end of the same tRNA species has been located at right angle to the anticodon and at 82 Å from it (23).

Our method involving resin coupled tRNA provide a remarkable improvement upon earlier methods using resin bound homopolynucleotide which initially led to only slight retardation of certain species as compared to the bulk of tRNA (24-26). Its rapidity and selectivity may provide a simple procedure for separating species which are difficult to separate otherwise (27). It provides also a convenient tool of general use for exploring the formation of other complementary tRNAs pairs, especially those having modified nucleotides in or next the anticodons; works are now in progress in this direction.

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REFERENCES :

(1) EISINGER, J. (1971) Biochim. Biophys. Res. Comm., 43, 854.

(2) EISINGER, J. (1971) Biochim. Biophys. Res. Comm., 44, 1135.

(3) NAKANISHI, K., FURUTACHI, N., FUNAMIZU, M., GRUNBERGER, D. and WEINSTEIN, I.B. (1970) J. Am.Chem.Soc., 92, 7617.

(4) RAJBAANDARY, U.L., CHANG, S.H., STUAR, A., FAULKNER, R.D., HOSKINSON, R.M. and KHORANA, H.G. (1967) Proc.Natl. Acad. Sci., 57, 751.

(5) EISINGER, J., FEUER, B. and YAMANE, T. (1970) Proc. Natl. Acad. Sci., 65, 638.

(6) EISINGER, J., FEUER, B. and YAMANE, T. (1971) Nature, 231, 126.

(7) NELIDOVA, O.D. and KISELEV , L.L. (1968) Molecular Biology USSR, 2, 47.

(8) REMY, P., BIRMELE, C. and EBEL, J.P. (1972) FEBS Letters, 27, 134.

(9) BARTKAWIAK, S. and PAWELKIEWICZ, J. (1972) Biochim. Biophys. Acta, 272, 137.

(10) INMAN, J.K. and DINTZIS, H.M. (1969) Biochemistry, 8, 4074.

(11) GOODMAN, H.M., ABELSON, J.N., LAUDY, A., ZADRAZIL, L.S. and SMITH, J.D. (1970) Eur. J. Biochem., 13, 461.

(12) PATTERSON, M. and GREENE, R. (1965) Anal. Chem., 37, 854.

(13) ZUBAY, G., (1962) J. Mol. Biol., 4, 347.

(14) REEVES, R.H., IMURA, N., SCHWAM, H., WEISS, G.B., SCHULMAN, L.O. and CHAMBERS, R.W. (1968) Proc. Natl. Acad. Sci., 60, 1450.

(15) Paper in preparation.

(16) OHASHI, Z., HARADA, F. and NISHIMURA, S. (1972) FEBS Letters, 20, 239.

(17) ARNOTT, S. (1971) Progr. in Biophys. and Mol. Biol., 22 179.

(18) CRAMER, F. (1971) Progr. in Nucl. Ac.Res. and Mol.Biol., 11, 391.

(19) BEARDSLEY, K. and CANTOR, C. (1970) Proc.Natl.Acad.Sci., 65, 39.

(20) MELCHER, G. (1969) FEBS Letters, 3, 185.

(21) JANG, C.G. (1973) Biochim.Biophys.Res.Comm., 50, 612.

(22) MELCHER, G., (1973) Biophysik, 9, 13.

(23) KIM, S.H., QUIGLEY, G., SUDDATH, F.L., McPHERSON, A., SNEDEN, D., KIM, J.J., WEINZIERL, J., BLATTMANN, P. and RICH, A. (1973) Science, 179, 285.

(24) HUNG, P.P. (1965) Science, 149, 639.

(25) EHRAN, S., NORTHRUP, L.G. and LEACH, F.R. (1965) Proc. Natl.Acad.Sci., 53, 646.

(26) KISELEV, L.L. and AVDONINA, T.A. (1971) Molecular Biology USSR, 3, 88.

(27) TAKADA, C., GROSJEAN, H. and PETRE, J. (1972) Abstract 9th Internat.Congress Biochem. - Stockholm (July 1973).