POLY U-DEPENDENT RIBOSOMAL BINDING OF Lys-tRNA^{Lys}

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1. Introduction

There is some evidence that U-U base pairs can be formed at the polynucleotide level. Thus, enhanced hypochromicity of poly U at 0° [1] suggests that this polynucleotide acquires some ordered structure at this temperature and infrared studies [2] indicate that hydrogen bonding is involved in this transition. Moreover, polyamines were found to stabilize poly U ordered structure for, in the presence of one spermine equivalent, poly U exhibits a cooperative melting profile with 50% hypochromicity and T_m equal 29° [3]. A similar though much more stable complex is formed with the 5-methyl homolog of poly U [4]. The random coil-spermine complex transition is accompanied by abrupt changes in ORD spectrum, e.g., a four-fold increase of the long wavelength Cotton effect and a violet shift of the first trough [5]. The compactness of the resulting structure is evidenced by the marked increase (from 4.5 S to 9.5 S) of the sedimention coefficient; the $S_{20,w}$ value becomes relatively independent of ionic strength in sharp contrast to the behavior of the random coil poly U [6]. Ordered poly U does not bind to ribosomes unless "melted out" [7]. The kinetics of formation of the poly U ordered state is independent of poly U concentration within a fairly wide range (10^{-5} to 10^{-3} M [3]) suggesting that it is an intramolecular phenomenon. Since poly U as well as oligo U's do not appear to show non-cooperative stacking interactions [6, 8, 9] it may tentatively be assumed that the ordered state of poly U corresponds to a hairpin-like helical molecule with hydrogen-bonded U-U base pairs. This is supported by the observation that poly *N*-methyl U does not form any kind of ordered structure [18].

The above considerations and the fact that the stability of the trinucleotide codon-ribosomes-mRNA complex is higher than that of the corresponding trinucleotide-complementary polynucleotide complex [10] suggested the possibility of detecting U-U base in codon-anticodon interaction, such as occurs in the mRNA-directed ribosomal binding of aminoacyl-tRNAs. In looking for U-U interaction the possibility of competition from A-U base pairing should clearly be avoided. The experimental approach was to look for a poly U-dependent ribosomal binding of Lys-tRNA^{Lys} (lysine codons, AA$\frac{A}{G}$) in the absence of tRNA^{Phe}. Two lysine-accepting tRNA species are known in *E. coli* [11]; they would be expected [12] to have the anticodons (3′–5′) UU$\frac{U}{C}$ or UUU (UUI would appear to be excluded as anticodon for it would recognize asparagine codons AA$\frac{U}{C}$). Evidence for the occurrence of poly U-directed ribosomal binding of Lys-tRNA^{Lys} is presented in this communication.

2. Materials and methods

Pseudomonas sp. 412 was grown and ribosomes and ribosomal subunits prepared as previously described [13]. Ribosomes from this strain, a wild type psychrophile, are particularly suitable for assays near 0°. The ribosomes were washed twice with 1.0 M ammonium chloride [13]. Three samples of *E. coli* tRNA were used. Sample A was commercial (Schwarz Bioresearch) unfractionated *E. coli* tRNA. When charged with ^{14}C-

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lysine it accepted 37 pmoles/A_{260} unit. Sample B was a $tRNA^{Lys}$-enriched fraction from *E. coli* obtained by chromatography on DEAE-Sephadex [14]. It accepted 270 pmoles of lysine and 3.6 pmoles of phenylalanine/A_{260} unit (Lys: Phe = 75). Sample C was a highly purified preparation of $tRNA^{Lys}$ from *E. coli* B, obtained by rechromatography of sample B on hydroxyapatite [15]. It accepted 1115 pmoles of lysine and 0.8 pmole of phenylalanine (Lys:Phe = 1400). $tRNA^{Phe}$ (90% phenylalanine accepting capacity) was provided by the Oak Ridge National Laboratory.

Binding assay samples (0.05 ml) contained, tris-acetate buffer, pH 7.2, 2.5 μmoles; NH_4Cl, 5 μmoles; magnesium acetate, 0.5 μmoles; B-mercaptoethanol, 1 μmole; NH_4Cl-washed *Pseudomonas sp.* 412 ribosomes, 2.5 A_{260} units; poly U (Miles), 10 μg; Lys (^{14}C)-tRNA, 19–26 pmoles (^{14}C-Lysine specific radioactivity, 271 μCi/μmole; 1 pmole = 535 cpm in the Packard Tricarb Scintillation Counter). Samples with 30 S ribosomes contained in addition 0.2 μmole of spermidine. The samples were incubated for 5 min at 30° (to form the poly U-ribosomal complex) followed by chilling to 0° prior to the addition of Lys-tRNA and further incubated for 60 min at 0°. Binding was measured by the procedure of Nirenberg and Leder [16].

3. Results and discussion

As seen in table 1, there was poly U-directed binding of ^{14}C-Lys-tRNA both with highly purified $tRNA^{Lys}$ and with partially purified preparations. Binding was observed with either 30 S or 70 S ribosomes although net binding values were, as usual higher (about threefold) with the latter. In contrast, no poly U-directed binding was observed when unfractionated tRNA was the source of Lys-tRNA. This was true irrespective of the experimental conditions (0° to 37°; 5 to 30 mM Mg^{2+}). In this case, non-specific binding in the absence of poly U was always 25–35% higher than in its presence, and observation made earlier by Nirenberg and Leder [6] in their original study of the binding system.

The effects of Mg^{2+} concentration, temperature, and $tRNA^{Phe}$ on the binding of highly purified Lys-$tRNA^{Lys}$ are shown in fig. 1. Increasing the Mg^{2+} concentration from 5 to 20 mM increased non-specific binding but augmented the poly U-dependent binding only moderately (fig. 1A), whereas increasing temperature (fig. 1B) above 10° markedly decreased poly U-directed binding without affecting the non-specific reaction. This shows that this binding is relatively weak. Fig. 1C shows that binding is prevented by $tRNA^{Phe}$ (ratio $tRNA^{Phe}$: Lys-$tRNA^{Lys}$, 1:6). On the other hand, when $tRNA^{Phe}$ is added after binding of Lys-$tRNA^{Lys}$ has taken place, addition of $tRNA^{Phe}$ has virtually no effect between 0° and 10° whereas at higher temperatures the more stable complex with $tRNA^{Phe}$ is apparently formed at the expense of the less stable one with Lys-$tRNA^{Lys}$.

Table 1
Poly U-dependent binding of Lys-tRNA to ribosomes.
(pmoles/assay, each assay in duplicate).

	Lys-tRNA preparations					
	A		B		C	
	minus poly U	plus poly U	minus poly U	plus poly U	minus poly U	plus poly U
	Binding to 70 S ribosomes					
Expt. 1	0.72	0.51	0.63	0.94	0.55	0.97
Expt. 2	0.78	0.48	0.68	1.04	0.47	0.95
	Binding to 30 S ribosomes					
Expt. 1	0.28	0.18	0.26	0.38	0.32	0.47
Expt. 2	–	–	0.22	0.35	0.33	0.51

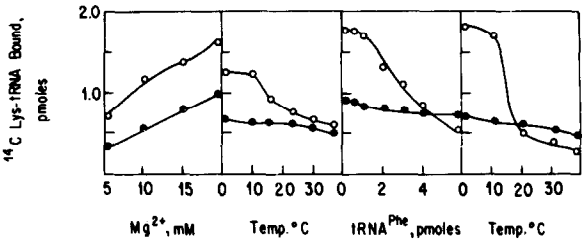

Fig. 1. Effect of Mg^{2+}, temperature and $tRNA^{Phe}$ on poly U-dependent binding of Lys-$tRNA^{Lys}$ "1100" to 70 S ribosomes.
A. Effect of Mg^{2+} concentration, incubation for 60 min at 0°.
B. Effect of temperature, 20 mM Mg^{2+}.
C. Effect of $tRNA^{Phe}$ added at 0 time. Incubation for 60 min at 0°, 20 mM Mg^{2+}.
D. As in C., but $tRNA^{Phe}$ (6 pmoles) was added after 60 min at 0° and samples were incubated for another 30 min at temperatures indicated. ●–●–●, without poly U; ○–○–○, with poly U. Other conditions as in table 1.

Addition of polyamines (spermine, spermidine) to samples with 70 S ribosomes appeared not to increase poly U-dependent binding of Lys-tRNA^{Lys} although it augmented non-specific binding in the absence of the polymer. However, with 30 S ribosomes no binding was detected in the absence of polyamines.

The results described provide further support for the idea that weak U-U base pairs can be formed under appropriate conditions. The codon-anticodon interaction leading to poly U-dependent ribosomal binding of Lys-tRNA^{Lys} would appear to be of the same nature as that responsible for the assumption of ordered structure by poly U. Weakness of the poly U-tRNA^{Lys} interaction is evidenced by the easy dissociation of the complex by tRNA^{Phe} and by elevated temperature.

Some relaxation of base pairing specificity in the presence of the ribosome at the 3′-terminus of the codon, or in the case of tRNA$_F^{Met}$ at the 5′-terminus, is known but the involvement of the ribosome, if any, is not understood. Uhlenbeck, Baller and Doty [17] have recently shown that in the case of tRNA$_F^{Met}$ "wobble" may be demonstrated in the absence of the ribosome. The formation of a Lys-tRNA-poly U ribosomal complex likewise implies little or no involvement on the part of the ribosome in affecting U-U base pairing specificity.

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References

[1] M.N.Lipsett, Proc. Natl. Acad. Sci. U.S. 46 (1960) 445.
[2] F.H.Howard, J.Frazier, M.F.Singer and H.T.Miles, J. Mol. Biol. 16 (1966) 415.
[3] W.Szer, J. Mol. Biol. 16 (1966) 585.
[4] W.Szer, Acta Biochim. Polon. 13 (1966) 251.
[5] G.T.Rogers, T.L.V.Ulbricht and W.Szer, Biochim. Biophys. Res. Commun. 27 (1967) 372.
[6] E.G.Richards, C.P.Flessel and J.R.Fresco, Biopolymers 1 (1963) 431.
[7] W.Szer and L.Nowak, J. Mol. Biol. 24 (1967) 333.
[8] H.Simpkins and E.G.Richards, J. Mol. Biol. 29 (1967) 349.
[9] M.M.Warshaw and I.Tinoco Jr., J. Mol. Biol. 20 (1966) 29.
[10] C.S.McLaughlin, J.Dondon, M.Grunberg-Manago, A.M. Michelson and G.Saunders, J. Mol. Biol. 32 (1968) 521.
[11] J.Goldstein, T.P.Bennett and L.C.Craig, Proc. Natl. Acad. Sci. U.S. 54 (1964) 119.
[12] F.H.C.Crick, J. Mol. Biol. 19 (1966) 548.
[13] W.Szer and J.Brenowitz, Biochem. Biophys. Res. Commun. 35 (1970) 653.
[14] S.Nishimura, F.Harada, U.Narushima and T.Seno, Biochim. Biophys. Acta 142 (1967) 133.
[15] P.Schofield, Biochim. Biophys. Acta 209 (1970) 253.
[16] M.W.Nirenberg and P.Leder, Science 145 (1964) 1399.
[17] O.E.Uhlenbeck, J.Baller and P.Doty, Nature 225 (1970) 508.
[18] W.Szer and D.Shugar, Acta Biochim. Polon. 8 (1961) 235.