STIMULATION OF THE BINDING OF AMINOACYL-sRNA TO RIBOSOMES BY TUBERCIDIN (7-DEAZAADENOSINE) AND N^6-DIMETHYLADENOSINE CONTAINING TRINUCLEOSIDE DIPHOSPHATE ANALOGS

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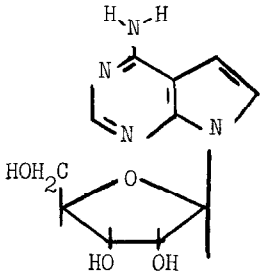
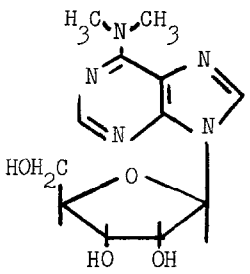
It has recently been shown that ribotrinucleotides serve as templates for the binding of specific aminoacyl-sRNA's to ribosomes (Nirenberg et al., 1964; Nirenberg et al., 1965; Söll et al., 1965). This specificity of binding is presumed to be brought about by hydrogen bond formation between the bases of the trinucleotide and the anticodon bases of the sRNA. It was of interest therefore to investigate the stimulation of aminoacyl-sRNA binding to ribosomes directed by trinucleotides containing base analogs in which this hydrogen bonding may be affected. For this purpose we have taken the trinucleoside diphosphates ApApA* and ApCpC, which serve as codons for lys-sRNA and thr-sRNA respectively, and replaced the 5'-adenosine end of each chain with N^6-dimethyladenosine (Ikehara et al., 1961) and tubercidin (7-deazaadenosine)(S. Suzuki, et al., 1960; Y. Mizuno et al., 1963). As the 6-amino group of adenine is known to be involved in hydrogen bond formation with the 4-O atom of uracil (Rich et al., 1956), conversion of this group to the dimethylamino group should eliminate this bonding. The fact that poly N^6-dimethyladenylic acid fails to form a double stranded helical complex with polyuridylic acid (Griffin et al., 1964) substantiates this idea. Tubercidin on the other hand differs from adenosine only in the substitution of the N^7 of the adenine by CH. Testing the stimulation

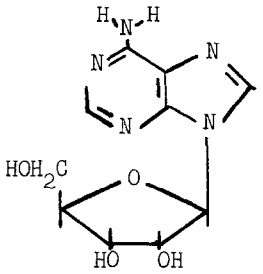
* Abbreviations: ApApA, adenylyl-(3'→5')-adenylyl-(3'→5')-adenosine; A, adenosine; G, guanosine; C, cytidine; U, uridine; DMA, N^6-dimethyladenosine; Tu, tubercidin.



tubercidin (Tu)



N^6-dimethyladenosine (DMA)



adenosine

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of binding of lys-sRNA and thr-sRNA to ribosomes by the triplets TupApA and TupCpC respectively should show whether the N^7 of adenine plays a role in the hydrogen bonding. These studies also are of interest in clarifying the mode of action of the antibiotic tubercidin which is known to be incorporated into RNA and to inhibit protein synthesis in mouse fibroblasts (Acs et al., 1964). It was also possible that DMA or Tu could substitute for G, C or U rather than A in the trinucleotide. Therefore the binding studies with DMApApA and TupApA were compared with those of GpApA, CpApA and UpApA. Similarly the binding studies using DMApCpC and TupCpC were compared with those using GpCpC, CpCpC and UpCpC.

MATERIALS AND METHODS

The ribotrinucleoside diphosphate analogs, TupApA, TupCpC, DMApApA and DMApCpC were obtained by chemical synthesis (Ikehara et al., in preparation). The other trinucleoside diphosphates used in this study were also synthesized chemically (Lohrmann et al., in press). The preparation of all C^{14}-aminoacyl-sRNA's and of E. coli B ribosomes has been described previously (Söll et al., 1965). A partially purified fraction of sRNA which was obtained by counter-current distribution (Nishimura et al., 1965) was used for the preparation of glu-sRNA. The remaining aminoacyl-sRNA's were prepared by charging unfractionated E. coli B sRNA preparation with a given amino acid. Specific activities ($\mu c/\mu mole$) of C^{14}-amino acids used were: C^{14}-ala, 123; C^{14}-gln, 32.2; C^{14}-glu, 205; C^{14}-pro, 165; C^{14}-lys, 222; C^{14}-ser, 123; C^{14}-thr, 164. For the binding

of sRNA to ribosomes, the method of Nirenberg and Leder (1964) was used. The incubation mixture (0.05 ml.) contained 0.1 M buffer, 0.05 M KCl, 0.02 M $Mg(OAc)_2$ unless otherwise indicated, 1.5 - 2.0 O.D. units of ribosomes and 3-12 mμmoles of trinucleotides. The buffer routinely used was sodium cacodylate-HCl, pH 7.2 except where otherwise specified. The concentrations of labeled aminoacyl-sRNA's used are shown for each aminoacyl-sRNA in the Table. The incubations were carried out at 25° for 20 min. unless otherwise specified.

RESULTS AND DISCUSSION

While the trinucleoside diphosphate ApApA stimulated the binding of C^{14}-lys-sRNA to ribosomes, the analog DMApApA gave no stimulation (Table I) showing that for template activity DMA cannot replace A in this trinucleotide. This result may be explained either in terms of the importance of the 6-NH_2 group in the hydrogen bonding of codon to anticodon, or that the bulk of the dimethylamino group interferes with the binding. Of these explanations the former is preferred. Other experiments (Table I) showed that whereas GpApA and CpApA stimulated the binding to ribosomes of C^{14}-glu-sRNA and C^{14}-gln-sRNA respectively, DMApApA had no effect on the binding of these sRNA's. Thus DMA can replace neither G or C in the codons tested. It is not possible to test whether DMA can replace U in the trinucleotide UpApA since this triplet fails to stimulate the binding of any aminoacyl-sRNA. In the same way DMApCpC failed to stimulate the binding to ribosomes of C^{14}-thr-sRNA, C^{14}-ala-sRNA, C^{14}-ser-sRNA and C^{14}-pro-sRNA, for which the codons are ApCpC, GpCpC, UpCpC and CpCpC respectively. Thus for template activity DMA is unable to replace A, G, C or U in these trinucleotides.

The tubercidin analog TupApA stimulated the binding of C^{14}-lys-sRNA to ribosomes, while not stimulating the binding of C^{14}-gln-sRNA or C^{14}-glu-sRNA (Table I), showing that tubercidin can substitute for A but not for C or G in this codon. TupCpC stimulated the binding of C^{14}-thr-sRNA but not the binding of ala-sRNA, ser-sRNA or pro-sRNA, again showing that tubercidin substitutes only for A.

TABLE I

Binding of Aminoacyl-sRNA to Ribosomes Stimulated by Triribonucleotides

	C^{14}-Gln-sRNA* (40.8 μμmoles)		C^{14}-Glu-sRNA (2.96 μμmoles)		C^{14}-Lys-sRNA (17.9 μμmoles)	
	6 mμmole	12 mμmole	6 mμmole	12 mμmole	6 mμmole	12 mμmole
ApApA	0.77	0.73	0.07	0.04	8.32**	8.28**
TupApA	0.75	0.77	0.08	0.13	6.48**	7.71**
DMApApA	0.79	0.83	0.04	0.06	1.37	1.35
GpApA	1.19	1.31	0.97	0.93	1.66	2.63
UpApA	0.75	0.77	0.06	0.05	1.76	1.87
CpApA	2.92	1.31	0.05	0.07	1.44	1.72
Control	0.76		0.04		1.42	

	C^{14}-Thr-sRNA (19.2 μμmoles)		C^{14}-Ala-sRNA (6.5 μμmoles)		C^{14}-Ser-sRNA*** (22.0 μμmoles)		C^{14}-Pro-sRNA (4.8 μμmoles)	
	6 mμmole	12 mμmole	6 mμmole	12 mμmole	6 mμmole	12 mμmole	6 mμmole	12 mμmole
ApCpC	1.91	2.11	0.18	0.15	0.53	0.53	0.30	0.39
TupCpC	1.94	1.77	0.23	0.23	0.52	0.56	0.35	0.36
DMApCpC	0.55	0.78	0.16	0.16	0.55	0.54	0.35	0.44
GpCpC	0.48	0.52	0.55	0.74	0.57	0.56	0.35	0.33
UpCpC	0.46	0.48	0.14	0.16	0.86	0.97	0.29	0.40
CpCpC	0.49	0.63	0.15	0.18	0.59	0.49	0.42	0.40
Control	0.68		0.16		0.51		0.31	

Buffer used was sodium cacodylate-HCl, pH 7.2 for 0.02 M $Mg(OAc)_2$. Incubations were at 25° for 20 min. Other conditions, see text.

* For accuracy these experiments were carried out on twice the scale, as gln-sRNA had a low specific activity.

** Control value was 1.04.

*** Since the binding stimulation in cacodylate buffer was too small, tris chloride, pH 7.2 was used.

While the extent of the binding of C^{14}-thr-sRNA stimulated by TupCpC and ApCpC was similar (Fig. 1), TupApA stimulated C^{14}-lys-sRNA binding to a smaller

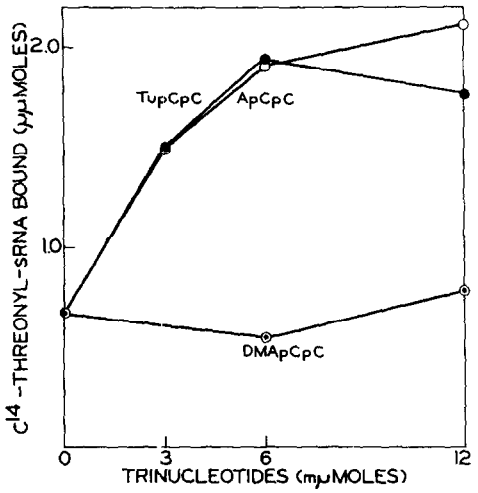

Fig. 1. Stimulation of the binding of thr-sRNA (19.2 μμmoles) to ribosomes by trinucleotides. Conditions are described in Materials and Methods.

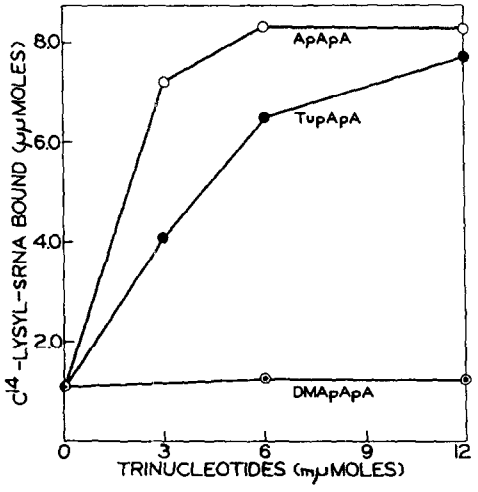

Fig. 2. Stimulation of the binding of lys-sRNA (17.9 μμmoles) to ribosomes by trinucleotides. Conditions are described in Materials and Methods.

extent than did ApApA at 25° and 0.02 M Mg^{++} (Fig. 2). At higher temperature (37°) and lower Mg^{++} concentration, however, TupApA gave better binding of C^{14}-lys-sRNA than did ApApA (Fig. 3). A possible explanation for the difference in binding capacity of the trinucleotides may be an effect of the replacement of the N^7 position of the adenine by CH on Watson-Crick (1953) type hydrogen bond formation. An alternative explanation could be that hydrophobic interactions or non Watson-Crick type hydrogen bonding are involved in this phenomenon to some extent. DMApApA was again inactive at the higher temperature (Fig. 3). At 37° and 0.01 M Mg^{++} concentration DMApCpC, TupCpC and ApCpC all failed to stimulate C^{14}-thr-sRNA binding to ribosomes.

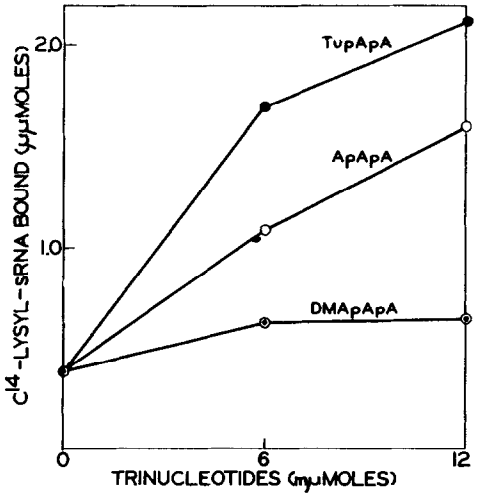

Fig. 3. Stimulation of the binding of lys-sRNA (17.9 μμmoles) to ribosomes by trinucleotides at 37°. Incubations were for 8 min. in Tris-HCl buffer pH 7.5 and 0.01 M Mg^{++}. Other conditions are described in the text.

Triplets containing the antibiotic tubercidin at the 5'-end position have been shown to serve as templates in the binding of sRNA to ribosomes. This may indicate that incorporation of tubercidin into mRNA does not interfere with the translation step of protein synthesis. If this is so then the rapid inhibition of protein synthesis by tubercidin (Acs et al., 1964) must occur at another step

in the protein synthesis. Tubercidin containing ribopolynucleotides are currently being synthesized in order to demonstrate their protein synthesizing ability *in vitro*.

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SUMMARY

The stimulation of the binding of aminoacyl-sRNA to ribosomes by chemically synthesized trinucleoside diphosphate analogs tubercidinyl-(3'→5')-adenylyl-(3'→5')-adenosine (TupApA), N^6-dimethyladenylyl-(3'→5')-adenylyl-(3'→5')-adenosine (DMApApA), TupCpC and DMApCpC was tested by Nirenberg's method and compared with that caused by other natural triplets. The binding of lys- and thr-sRNA to ribosomes was significantly stimulated by TupApA and TupCpC respectively. No stimulation was observed by the triplets containing the dimethyladenine residue. These results may best be interpreted in terms of Watson-Crick type hydrogen bond formation between the codon triplet and the anticodon site of sRNA.

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