Cox, R. A. (1966b), *Biochem. J. 98*, 841.
Cox, R. A. (1969), *Biochem. J. 114*, 743.
Cox, R. A., Gould, H. J., and Kanagalingam, K. (1968), *Biochem. J. 106*, 733.
Cox, R. A., and Kanagalingam, K. (1967), *Biochem. J. 103*, 431.
Dahlquist, F. W., and Raftery, M. A. (1969), *Biochemistry 8*, 713.
Delihas, N. (1967), *Biochemistry 6*, 3356.
Delihas, N., and Bertman, J. (1966), *J. Mol. Biol. 21*, 393.
Egami, F., Takahashi, K., and Uchida, T. (1964), *Prog. Nucleic Acid Res. 3*, 59.
Fasman, G. D., Lindblow, C., and Seaman, E. (1965), *J. Mol. Biol. 12*, 630.
Fellner, P. (1969), *Eur. J. Biochem. 11*, 12.
Fellner, P., Ehresmann, C., and Ebel, J. P. (1970), *Nature (London) 225*, 26.
Gould, H. J. (1966a), *Biochemistry 5*, 1103.
Gould, H. J. (1966b), *Biochim. Biophys. Acta 123*, 441.
Gould, H. J. (1967), *J. Mol. Biol. 29*, 307.
Gould, H. J., Bonanou, S., and Kanagalingam, K. (1966), *J. Mol. Biol. 22*, 397.
Gould, H. J., Pinder, J. C., Matthews, H. R., and Gordon, A. H. (1969), *Anal. Biochem. 29*, 1.
Gould, H. J., and Simpkins, H. (1969), *Biopolymers 7*, 223.
Huppert, J., and Pelmont, J. (1962), *Arch. Biochem. 98*, 214.
Kasai, H., Takahashi, K., and Ando, T. (1969), *J. Biochem. (Tokyo) 66*, 591.
Leng, M., Rosilio, C., and Boudet, J. (1969), *Biochim. Biophys. Acta 174*, 574.
Markham, R., and Smith, J. D. (1951), *Biochem. J. 49*, 401.
McPhie, P., Hounsell, J., and Gratzer, W. B. (1966), *Biochemistry 5*, 988.
Moore, P. B., and Asano, K. (1966), *J. Mol. Biol. 18*, 21.
Morihara, K., Oka, T., and Tsuzuki, H. (1969), *Arch. Biochem. 132*, 498.
Nichols, J. L. (1970), *Nature (London) 225*, 147.
Nirenberg, M. W., and Matthaei, H. (1961), *Proc. Nat. Acad. Sci. U. S. 47*, 1588.
Penniston, J. P., and Doty, P. (1963), *Biopolymers 1*, 145.
Pinder, J. C., Gould, H. J., and Smith, I. (1969), *J. Mol. Biol. 40*, 289.
Schechter, I., and Berger, A. (1967), *Biochem. Biophys. Res. Commun. 27*, 157.
Shiobara, Y., Takahashi, K., and Egami, F. (1962), *J. Biochem. (Tokyo) 52*, 267.
Spencer, M., and Poole, F. (1965), *J. Mol. Biol. 11*, 314.
Takai, N., Uchida, T., and Egami, F. (1966), *Biochim. Biophys. Acta 128*, 218.
Thach, R. E., and Doty, P. (1965), *Science 148*, 632.
Thach, S., and Boedtker, H. (1969), *J. Mol. Biol. 45*, 451.
Thoma, J. A., Brothers, C., and Spradlin, J. (1970), *Biochemistry 9*, 1768.
Thomas, G. J. (1969), *Biopolymers 7*, 325.
Whitfield, P. R., and Witzel, H. (1963), *Biochim. Biophys. Acta 72*, 338.
Wikman, J., Howard, E., and Busch, H. (1969), *J. Biol. Chem. 244*, 5471.

# 2′-*O*-Methyloligoadenylates as Templates for the Binding of Lysyl Transfer Ribonucleic Acid to Ribosomes*

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ABSTRACT: Certain oligonucleotides containing only 2′-*O*-methyladenosine have been found to stimulate the binding of lysyl-tRNA to ribosomes. The stimulatory activity of these oligonucleotides has been compared to oligonucleotides containing only adenosine and 2′-deoxyadenosine. All adenosine oligomers were highly active. All 2′-deoxyadenosine oligomers were inactive.

The 2′-*O*-methyladenosine triplet without terminal phosphate, AmpAmpAm, was inactive. However, pAmpAmpAm and AmpAmpAmpAm were approximately 30–40% as active as the corresponding adenosine oligomers. Neomycin and streptomycin enhanced the template activity of 2′-*O*-methyloligoadenylates. Thus, free 2′-hydroxyl groups are not essential for codon recognition on the ribosome, and the presence of a 2′-*O*-methyl residue may not block ribonucleic acid codon translation.

The template activity of oligonucleotides in directing the binding of aminoacyl-tRNA to ribosomes is sensitive to modifications in the codon structure. For example, the template activity of ApApA for the ribosomal binding of lysyl-tRNA is enhanced by adding a 5′-phosphate and is diminished by adding a 2′- or a 3′-phosphate. A trinucleotide

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containing riboadenylate residues linked by 2′,5′-phosphodiester bonds is inactive as a template and does not inhibit the activity of the corresponding 3′,5′-linked trinucleotide (Rottman and Nirenberg, 1966). Oligomers of 2′-deoxyadenylic acid are essentially devoid of template activity (Nirenberg and Leder, 1964). In addition, some mixed trinucleotides containing both adenylate and 2′-deoxyadenylate residues are from 0 to 70% as active as ApApA depending on the number and location of 2′-deoxyadenylate residues (Moon *et al.*, 1966). Furthermore, oligonucleotides containing uracil arabinoside, in which the 2′-hydroxyl group is inverted relative to that in uridine, have been reported both to have low activity (Schramm and Ulmer-Schürnbrand, 1967) or to be essentially inactive (Nagyvary *et al.*, 1968) as templates for phenylalanyl-tRNA binding. All of these experiments suggested that free ribose 2′-hydroxyl groups may be essential for oligonucleotides to serve as templates for the binding of aminoacyl-tRNA to ribosomes.

A naturally occurring modification of the 2′-hydroxyl group of polynucleotides is the 2′-*O*-methyl group, known to occur in bacterial and animal tRNA and rRNA in amounts ranging from 0.1 to 1.9% of the component nucleotides (Starr and Sells, 1969). Since these RNA species are presumably not translated *in vivo*, it was of interest to determine whether the presence of a 2′-*O*-methyl group could prevent the translation of certain RNA species. We have recently discovered (B. E. Dunlap and F. Rottman, manuscript in preparation) that a polymer containing only 2′-*O*-methyladenylic acid is inactive as a template for the *in vitro* incorporation of lysine into polypeptides under conditions in which poly(A) is active. However, poly(Am)[1] is known to have a more stable secondary structure than poly(A) (Bobst *et al.*, 1969a–c). Thus, the lack of template activity may reflect an inability of the highly ordered poly(Am) to bind to ribosomes as well as a possible requirement for a free 2′-hydroxyl group. We have investigated here the effect of the 2′-*O*-methyl group on the interaction of oligonucleotide template with the aminoacyl-tRNA and the ribosome. This report describes experiments on the relative ability of an homologous series of ribo-, 2′-deoxyribo-, and 2′-*O*-methylribooligoadenylates to serve as templates for the binding of lysyl-tRNA to ribosomes.

## Material and Methods

*Oligoadenylates without Terminal Phosphates.* Poly(A) (Miles Chemical Co.) and poly(Am) (Rottman and Heinlein, 1968) were partially degraded with micrococcal nuclease (Rottman and Nirenberg, 1966). After exhaustive treatment with *Escherichia coli* alkaline phosphatase to remove terminal phosphate, the oligonucleotides were separated according to chain length on DEAE-cellulose or DEAE-Sephadex columns with a triethylammonium bicarbonate gradient. Oligodeoxyadenylates were chemically synthesized and separated by the method of Ralph and Khorana (1961) and were subsequently treated with *E. coli* alkaline phosphatase. All oligonucleotides were further purified by descending chromatography on Whatman No. 40 paper in 95% ethanol–1 M ammonium acetate (pH 7.5) (7:3, v/v), followed by paper electrophoresis in 0.1 M triethylammonium bicarbonate (pH 7.8). Oligonucleotides were characterized by digestion with Russell's viper venom phosphodiesterase free of phosphomonoesterase activity; the nucleoside and nucleoside 5′-monophosphate products were separated and quantitated as described previously (Rottman and Johnson, 1969). The results are shown in Table I. All assigned chain lengths of oligonucleotides were consistent with their elution pattern from DEAE-cellulose and their electrophoretic mobility during paper electrophoresis.

TABLE I: Characterization of Adenine Oligonucleotides by Venom Phosphodiesterase Products.

| | Oligonucleotide | Nucleotide: Nucleoside | |
|---|---|---|---|
| | | Calcd | Found |
| Di-A | ApA | 1.0 | 0.9 |
| Tri-A | ApApA | 2.0 | 2.0 |
| Tetra-A | ApApApA | 3.0 | 3.0 |
| Penta-A | ApApApApA | 4.0 | 4.2 |
| Di-Am | AmpAm | 1.0 | 0.9[a] |
| Tri-Am | AmpAmpAm | 2.0 | 1.8[a] |
| Tetra-Am | AmpAmpAmpAm | 3.0 | 3.0[a] |
| Penta-Am | AmpAmpAmpAmpAm | 4.0 | 4.6[a] |
| Di-dA | dApdA | 1.0 | 1.1 |
| Tri-dA | dApdApdA | 2.0 | 2.0 |
| Tetra-dA | dApdApdApdA | 3.0 | 3.1 |
| Penta-dA | dApdApdApdApdA | 4.0 | 4.6 |

[a] Venom phosphodiesterase digests of all oligo(Am) samples contained some residual AmpAm (see also Gray and Lane, 1967), and this material was included in the calculation of the product ratio.

*Oligoadenylates with 5′-Terminal Phosphate.* The preparation and characterization of pApApA (tri-pA) have been described (Rottman and Nirenberg, 1966). The muskmelon endonuclease of Muschek and Fairley (1970) was employed to digest poly(Am) to oligonucleotides which included pAmpAmpAm, AmpAmpAm, and AmpAmpAmpAm (tri-pAm, tri-Am, and tetra-Am, respectively). They were separated on a DEAE-cellulose column (Whatman DE23) with an ammonium formate gradient, followed by paper electrophoresis in 0.1 M ammonium bicarbonate (pH 7.8). Their purity was confirmed by electrophoresis on Whatman DEAE paper with 0.1 M Tris-phosphate (pH 6.6) (Yaron and Sober, 1965). The tri-pAm was characterized by digestion with snake venom phosphodiesterase, producing a single product which migrated as pAm in the ethanol–ammonium acetate system described above. Incubation of tri-pAm with alkaline phosphatase yielded a product with the electrophoretic mobility of tri-Am. The latter trinucleoside diphosphate was freed of alkaline phosphatase by paper chromatography in the ethanol–ammonium acetate system. It was then characterized by digestion with snake venom phosphodiesterase as described above, giving a ratio of pAm to Am of 1.9:1.

*Binding Assay. E. coli* B ribosomes and unfractionated L-[$^{14}C$]lysyl-tRNA were prepared as has been described

[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: Am, 2′-O-methyladenosine; di-Am, AmpAm; tri-Am, AmpAmpAm; tri-pAm, pAmpAmpAm.

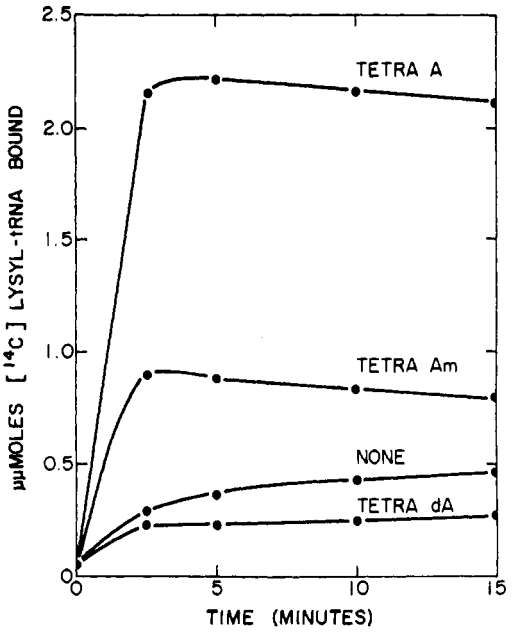


FIGURE 1: Time dependence of the binding of lysyl-tRNA to ribosomes in the presence of tetra-A (4.1 mμmoles), tetra-Am (2.3 mμmoles), or tetra-dA (3.9 mμmoles). Assay mixtures (50 μl) contained 23 mM magnesium acetate and were incubated at 23°.

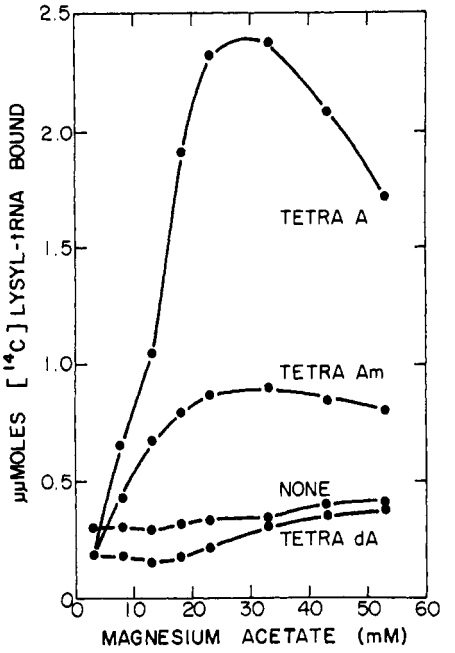


FIGURE 2: Dependence on magnesium ion concentration of the binding of lysyl-tRNA to ribosomes in the presence of tetra-A (3.3 mμmoles), tetra-Am (3.5 mμmoles), and tetra-dA (3.9 mμmoles). Incubations were for 15 min at 23°.

(Nirenberg, 1963; Rottman and Nirenberg, 1966), except that the ribosomes were washed in 2 M KCl as described by Nishimura *et al.* (1965). The filter assay of Nirenberg and Leder (1964) for ribosomal-bound aminoacyl-tRNA was employed. It involved incubation of ribosomes and oligonucleotides for 5 min at 0°, followed by the addition of aminoacyl-tRNA and incubation for 15 min at 23°. The 50-μl assay mixtures contained 1.16 $A_{260}$ units of ribosomes, 0.1 M Tris-acetate (pH 7.2), 0.05 M potassium acetate, and 0.023 M magnesium acetate. Each reaction mixture contained 5.2 μμmoles of [$^{14}C$]lysyl-tRNA (0.17 $A_{260}$ unit, 231 μCi/μmole). Dried filters were counted in scintillation fluid containing 15.1 g of 2,5-bis-2-(5-*tert*-butylbenzoxazolyl)thiophene/gal. of toluene. Background radioactivity obtained in the absence of ribosomes (about 50 cpm) has been subtracted from the data presented. Oligonucleotide concentrations were calculated by use of the extinction coefficients of Singer *et al.* (1962).

*Other Materials.* Neomycin B was a gift from Dr. W. Sokolski of the Upjohn Co. Streptomycin sulfate was purchased from Sigma Chemical Co. Sheep kidney endonuclease was prepared by the method of Kasai and Grunberg-Manago (1967). The *Azotobacter agilis* endonuclease, which was a gift from Dr. A. Stevens, was a Cγ gel fraction from Stevens and Hilmoe (1960).

## Results

*Time Course.* Figure 1 shows the amount of lysyl-tRNA bound to ribosomes as a function of incubation time. As found previously (Nirenberg and Leder, 1964; Rottman and Nirenberg, 1966; Moon *et al.*, 1966), the binding stimulated by adenosine-containing oligonucleotides is very rapid and reaches a stable maximum value. Furthermore, the presence of an oligonucleotide which fails to function as a template (tetra-dA in this case) results in a reduction of the background level of binding of aminoacyl-tRNA observed in the absence of added oligonucleotide. Both tetra-Am and tetra-A stimulated maximal lysyl-tRNA binding by 2.5 min but the level of binding obtained with a similar concentration of tetra-Am was lower.

*Magnesium Dependence.* As observed previously (Nirenberg and Leder, 1964; Moon *et al.*, 1966), the binding of aminoacyl-tRNA to ribosomes is dependent on the $Mg^{2+}$ concentration, with an optimum in the 20–30 mM range (see Figure 2). Tetra-dA did not act as a template, even at high $Mg^{2+}$ concentrations. Tetra-Am stimulated lysyl-tRNA binding with a $Mg^{2+}$ dependence similar to that found with tetra-A, but again tetra-Am gave a lower maximum level of binding. In similar studies (data not shown), a high level of $Mg^{2+}$ (43 mM) did not influence the binding observed in the presence of tri-Am or penta-Am.

*Oligonucleotide Chain Length and Concentration.* Figure 3 shows the binding of lysyl-tRNA to ribosomes as a function of the concentration of oligonucleotide template. Trimers, tetramers, and pentamers containing adenosine, 2′-*O*-methyladenosine, and 2′-deoxyadenosine were employed. They all possessed free 3′- and 5′-hydroxyl groups.

As indicated in Figure 3, tri-A stimulated lysyl-tRNA binding approximately sixfold. However, tri-Am and tri-dA were inactive. Tetra-A gave results similar to tri-A, and tetra-dA was inactive. In contrast to the trimer, tetra-Am was active, stimulating binding at approximately one-third the level observed with tetra-A. Penta-A stimulated binding similar to that observed with tri-A and tetra-A. Penta-dA was inactive. However, penta-Am gave binding at about one-fifth the level observed with penta-A.

TABLE II: Binding of Lysyl-tRNA to Ribosomes in the Presence of Neomycin B or Streptomycin Sulfate.[a]

| Template | Antibiotic (10 μg/ml) | Oligonucleotide | | | | |
|---|---|---|---|---|---|---|
| | | None | Dimer | Trimer | Tetramer | Pentamer |
| Oligo(A) | None | 0.37 | 0.54 | 2.26 | 1.97 | 1.85 |
| | Neomycin B | 0.43 | 0.53 | 1.55 | 1.34 | 1.39 |
| | Streptomycin | 0.36 | 0.50 | 1.73 | 1.35 | 1.21 |
| Oligo(Am) | None | 0.37 | 0.43 | 0.43 | 0.69 | 0.56 |
| | Neomycin B | 0.43 | 0.47 | 0.44 | 1.05 | 0.79 |
| | Streptomycin | 0.36 | 0.44 | 0.43 | 0.96 | 0.65 |
| Oligo(dA) | None | 0.37 | 0.40 | 0.38 | 0.29 | 0.29 |
| | Neomycin B | 0.43 | 0.47 | 0.43 | 0.27 | 0.36 |
| | Streptomycin | 0.36 | 0.40 | 0.32 | 0.30 | 0.30 |

[a] Mixtures were incubated for 15 min at 23° with 23 mM $Mg^{2+}$ and the following amounts (millimicromoles) of dimer, trimer, tetramer, or pentamer: oligo(A) (10.4, 4.7, 3.3, and 2.1), oligo(Am) (5.8, 5.5, 2.3, and 3.1), or oligo(dA) (8.4, 5.9, 3.9, and 2.8). Results are expressed as micromicromoles of lysyl-tRNA bound.

Figure 4 shows the results of additional experiments which summarize these comparisons of chain length for oligomers of A, Am, and dA. The dimer, di-A, slightly stimulated binding, whereas di-Am and di-dA did not. It is again apparent that, while tri-Am was inactive, tetra-Am was somewhat active and penta-Am was less active than tetra-Am.

*Effect of Neomycin B and Streptomycin.* Table II summarizes experiments on the effect of neomycin B and streptomycin sulfate on the ability of these adenine-containing oligonucleotides to serve as templates. Both neomycin B and streptomycin sulfate inhibited the activities of tri-A, tetra-A, and penta-A. These antibiotics did not induce any activity with the oligodeoxyriboadenylates nor with tri-Am. However, both antibiotics stimulated the binding observed in the presence of tetra-Am and penta-Am.

*Competition of Trinucleotides.* Since tri-Am and tri-dA did not stimulate lysyl-tRNA binding alone, they were checked as possible inhibitors of the template activity of a nonsaturating level of tri-A. The results presented in Table III show no significant inhibition in the binding of lysyl-tRNA by tri-A in the presence of 2.5 to 3 times as much tri-Am or tri-dA.

*Oligoadenylates with a Terminal 5′-Phosphate.* Since AmpAmpAm was inactive as a template while AmpAmpAmpAm stimulated lysyl-tRNA binding to ribosomes, it was possible that the activity of the latter was due to the presence of an additional nucleotide residue or perhaps the phosphate moiety.

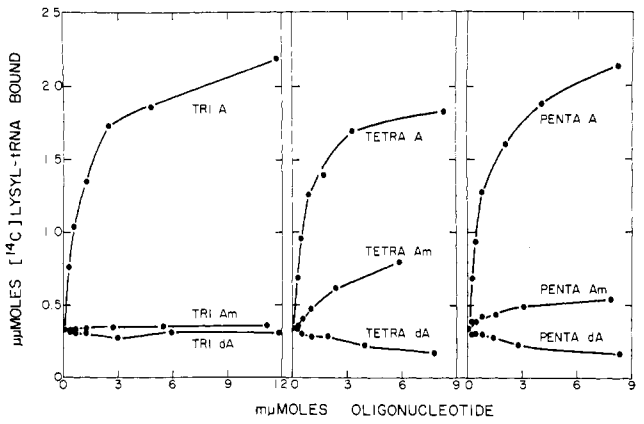


FIGURE 3: Dependence of lysyl-tRNA binding to ribosomes on the concentration of trinucleotide, tetranucleotide, and pentanucleotide template. Incubations were performed in 23 mM $Mg^{2+}$ at 23° for 15 min.

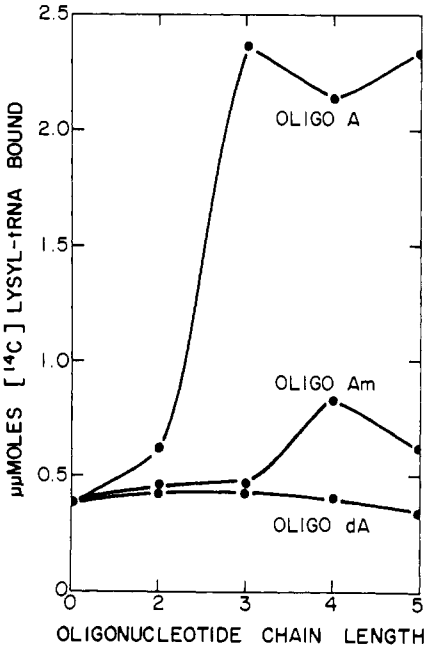


FIGURE 4: Effect of oligonucleotide chain length on the binding of lysyl-tRNA to ribosomes. Incubation mixtures contained the following amounts (millimicromoles) of dimer, trimer, tetramer, or pentamer as template: oligo(A) (10.4, 4.6, 3.3, and 4.2); oligo(Am) (7.3, 5.4, 4.5, and 4.7); or oligo(dA) (11.1, 5.9, 3.9, and 4.2). Incubations were performed in 23 mM $Mg^{2+}$ for 15 min at 23°.

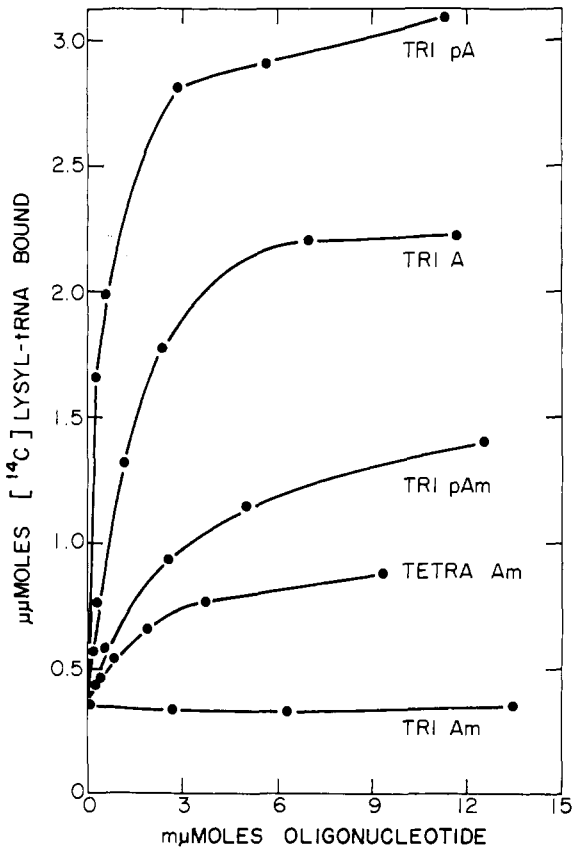


FIGURE 5: Dependence of binding of lysyl-tRNA to ribosomes on the concentration of oligonucleotide templates with or without 5′-phosphates. Incubations were performed in 23 mM $Mg^{2+}$ at 23° for 15 min.

To discriminate between these two alternatives it became necessary to synthesize pAmpAmpAm. Several nucleases which digested poly(A) to oligoadenylates bearing 5′-phosphates were tested and found to be unable to degrade poly(Am); these endonucleases were isolated from sheep kidney (Kasai and Grunberg-Manago, 1967), *Azotobacter agilis* (Stevens and Hilmoe, 1960), and rat liver nuclei (Lazarus and Sporn, 1967). However, an endonuclease preparation from muskmelon seeds (Muschek and Fairley, 1970) was found to hydrolyze poly(Am) slowly to oligonucleotides bearing 5′-phosphate residues and some without terminal phosphates.

The results of binding assays which compare the template activity of these oligonucleotides are presented in Figure 5. As found previously (Nirenberg and Leder, 1964; Rottman and Nirenberg, 1966), tri-pA was more active than tri-A. Tri-pAm was active and stimulated binding about 40% as well as tri-pA. Tri-pAm was more active than tetra-Am.

## Discussion

On the basis of earlier experiments (Nirenberg and Leder, 1964; Rottman and Nirenberg, 1966; Moon *et al.*, 1966), it was suggested that free 2′-hydroxyl groups in the template oligomer may be essential for the binding of aminoacyl-tRNA to ribosomes. Morgan *et al.* (1967) apparently confirmed these results with poly(dA), which was shown to be inactive and unable to inhibit the activity of poly(A) in the binding of lysyl-tRNA to ribosomes and in the incorporation of lysine into polypeptides. However, our experiments indicate that 2′-hydroxyl groups are not required for oligoadenylates to stimulate lysyl-tRNA binding to ribosomes. Tri-pAm, tetra-Am, and penta-Am, which have all of their 2′-hydroxyl groups blocked as methyl ethers, are active as templates, although they function less efficiently than their unsubstituted analogs. The time course, magnesium dependence, and oligonucleotide concentration dependence (see Figures 1, 2, and 3) of the stimulation of binding of lysyl-tRNA appear to be similar for tetra-A and tetra-Am, although tetra-Am gives only about one-third the maximum level of binding observed with tetra-A.

TABLE III: Binding of Lysyl-tRNA to Ribosomes with Tri-A in the Presence of Tri-Am or Tri-dA.[a]

| Trinucleotide (mμmoles) | | | Lysyl-tRNA bound (μμmoles) |
|---|---|---|---|
| Tri-A | Tri-Am | Tri-dA | |
| 0 | 0 | 0 | 0.33 |
| 1.1 | 0 | 0 | 1.52 |
| 0 | 2.3 | 0 | 0.36 |
| 0 | 0 | 3.0 | 0.35 |
| 1.1 | 1.1 | 0 | 1.45 |
| 1.1 | 2.7 | 0 | 1.52 |
| 1.1 | 0 | 1.6 | 1.52 |
| 1.1 | 0 | 3.0 | 1.47 |

[a] Incubations were performed with 23 mM $Mg^{2+}$ for 15 min at 23°.

It is unclear why tri-Am fails to function as a template, while tetra-Am and penta-Am do stimulate binding (see Figures 3, 4, and 5). We are not aware of a similar instance in which the trinucleoside diphosphate in a homologous series of oligomers is inactive while the tetranucleoside triphosphate is active. We suggest that this trinucleoside diphosphate may be inherently weak in its binding to ribosomes. This suggestion is supported by the observations (Table III and Figure 3) that tri-dA and tri-Am failed to inhibit the template activity of tri-A and failed to reduce the background level of lysyl-tRNA binding, whereas tetra-dA and penta-dA did reduce it. These data may indicate that three phosphate residues may be required in certain oligonucleotides for stable interaction with the ribosome. Thus the presence of a 5′-terminal phosphate on AmpAmpAm, either in mono- or diester linkage, converts a template-inactive oligonucleotide into one which is active.

Our earlier results on the physical structure of poly(Am) (Bobst *et al.*, 1969a–c) demonstrated that this polynucleotide is similar to poly(A) in its conformation and its ability to form single-stranded helices with partially stacked bases at neutral pH. These conclusions were based on circular dichroism data and ultraviolet absorption spectra under conditions of different pH and temperature. However, the double-helical form of poly(Am) is stable to higher pH and tem-

perature conditions than that of poly(A). This increased interaction of 2′-O-methyladenosine residues may explain the decrease in lysyl-tRNA binding observed with penta-Am *vs.* tetra-Am (see Figure 4) and the absence of lysine incorporation into polypeptides with poly(Am) *vs.* poly(A) as a template (B. E. Dunlap and F. Rottman, manuscript in preparation).

Oligo(dA) and poly(dA) have structures quite different from the corresponding riboadenylate compounds. The former may have an altered plane of the bases allowing interaction only in base pairs rather than in the cooperative stacks found in poly(A) (Vournakis *et al.*, 1967; Ts'o *et al.*, 1966; Bush and Scheraga, 1969; Adler *et al.*, 1969). In agreement with previous results (Nirenberg and Leder, 1964), we have found oligomers of 2′-deoxyadenylate to be inactive as templates for the binding of lysyl-tRNA to ribosomes. Even the high $Mg^{2+}$ concentrations which were found to induce activity with the mixed oligonucleotide, dApdApA (Moon *et al.*, 1966), were not effective with tetra-dA (see Figure 2).

In contrast, Morgan *et al.* (1967) found that poly(d-CA), poly(d-TG), and poly dT efficiently directed the binding of the same aminoacyl-tRNA species as the corresponding polyribonucleotides. However, neomycin B was required to permit the subsequent translation of these polydeoxyribonucleotides into polypeptides. This was in agreement with the earlier results of McCarthy *et al.* (1966) using various aminoglycoside antibiotics for translation of single-stranded natural DNA. Pestka *et al.* (1965) found that 11 μg/ml of streptomycin gave a 30–40% stimulation of binding of lysyl-tRNA to ribosomes in the presence of tri-A and tri-pA. In our experiments (see Table II), neomycin B and streptomycin sulfate had no effect on lysyl-tRNA binding to ribosomes in the presence of oligomers of 2′-deoxyadenosine, and they inhibited the binding observed with adenosine oligonucleotides. However, these antibiotics stimulated the binding observed with tetra-Am and penta-Am. They did not induce any template activity with tri-Am. It remains to be demonstrated whether this stimulation of binding with tetra-Am is relevant to the induction by these antibiotics of template activity in tRNA and rRNA (McCarthy *et al.*, 1966) which have very low levels of 2′-O-methylnucleotides, few of which are adjacent (Starr and Sells, 1969).

We conclude that free 2′-hydroxyl groups are not essential for this early step in translation, *i.e.*, codon recognition at the ribosomal level. This raises the interesting possibility that 2′-*O*-methyl groups in RNA may not block translation and may be present in RNA molecules which are translated *in vivo*. Recent studies on the template properties of synthetic heteropolymers containing 2′-*O*-methylnucleotides in cell-free protein-synthesizing systems are consistent with this possibility (B. E. Dunlap and F. Rottman, manuscript in preparation).

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## References

Adler, A., Grossman, L., and Fasman, G. D. (1969), *Biochemistry 8*, 3846.

Bobst, A. M., Cerutti, P. A., and Rottman, F. (1969a), *J. Amer. Chem. Soc. 91*, 1246.

Bobst, A. M., Rottman, F., and Cerutti, P. A. (1969b), *J. Amer. Chem. Soc. 91*, 4603.

Bobst, A. M., Rottman, F., and Cerutti, P. A. (1969c), *J. Mol. Biol. 46*, 221.

Bush, C. A., and Scheraga, H. A. (1969), *Biopolymers 7*, 395.

Gray, M. W., and Lane, B. G. (1967), *Biochim. Biophys. Acta 134*, 243.

Kasai, K., and Grunberg-Manago, M. (1967), *Eur. J. Biochem. 1*, 152.

Lazarus, H. M., and Sporn, M. B. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 1386.

McCarthy, B. J., Holland, J. J., and Buck, C. A. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31*, 683.

Moon, M. W., Nishimura, S., and Khorana, H. G. (1966), *Biochemistry 5*, 937.

Morgan, A. R., Wells, R. D., and Khorana, H. G. (1967), *J. Mol. Biol. 26*, 477.

Muschek, L. D., and Fairley, J. L. (1970), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 29*, 535.

Nagyvary, J., Provenzale, R., and Clark, J. M., Jr. (1968), *Biochem. Biophys. Res. Commun. 31*, 508.

Nirenberg, M. W. (1963), *Methods Enzymol. 6*, 17.

Nirenberg, M., and Leder, P. (1964), *Science 145*, 1399.

Nishimura, S., Jones, D. S., Ohtsuka, E., Hayatsu, H., Jacob, T. M., and Khorana, H. G. (1965), *J. Mol. Biol. 13*, 283.

Pestka, S., Marshall, R., and Nirenberg, M. (1965), *Proc. Nat. Acad. Sci. U. S. 53*, 639.

Ralph, R. K., and Khorana, H. G. (1961), *J. Amer. Chem. Soc. 83*, 2926.

Rottman, F., and Heinlein, K. (1968), *Biochemistry 7*, 2634.

Rottman, F., and Johnson, K. L. (1969), *Biochemistry 8*, 4354.

Rottman, F., and Nirenberg, M. (1966), *J. Mol. Biol. 21*, 555.

Schramm, G., and Ulmer-Schürnbrand, I. (1967), *Biochim. Biophys. Acta 145*, 7.

Singer, M. F., Heppel, L. A., Rushizky, G. W., and Sober, H. A. (1962), *Biochim. Biophys. Acta 61*, 474.

Starr, J. L., and Sells, B. H. (1969), *Physiol. Rev. 49*, 623.

Stevens, A., and Hilmoe, R. J. (1960), *J. Biol. Chem. 235*, 3016.

Ts'o, P. O. P., Rapaport, S. A., and Bollum, F. J. (1966), *Biochemistry 5*, 4153.

Vournakis, J. N., Poland, D., and Scheraga, H. A. (1967), *Biopolymers 5*, 403.

Yaron, A., and Sober, H. A. (1965), *Anal. Biochem. 12*, 173.