THE EFFECT OF POLYNUCLEOTIDES ON AMINOACYL-RNA SYNTHETASES

I. INHIBITION BY SYNTHETIC POLYNUCLEOTIDES

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The initial steps of protein biosynthesis are known to involve the activation of an amino acid to form an aminoacyl adenylate and the transfer of this activated amino acid to a specific s-RNA (Hoagland et al., 1958). However, despite the immense amount of work that has been devoted to the study of these reactions, very little is actually known about the structural features of the s-RNA molecule which determine the specificity of its interaction with aminoacyl-RNA synthetases.

Several approaches have been used in order to learn more about the mechanism of this interaction. These include: 1) complete nucleotide sequence analyses of specific s-RNA molecules (McCully and Cantoni, 1962), and 2) selective modification of the s-RNA structure by chemical (Yu and Zamecnik, 1963) or enzymatic (Nishimura and Novelli, 1964) means. A third approach, which has received relatively little attention, (Hayashi and Miura, 1964) is the use of natural or synthetic oligo- or polynucleotides as inhibitors of the synthetase reaction. The observation of specific competition between inhibitor and s-RNA molecules may reveal the nature of the site on the s-RNA which is interacting with the enzyme.

The specific inhibitory properties of various synthetic polyribonucleotides on the attachment of glutamic acid to s-RNA by a partially purified glutamyl-RNA synthetase are reported in this paper. Preliminary results with other aminoacyl-RNA synthetases are also presented.

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Materials and Methods: The synthetic polynucleotides were products of the Miles Chemical Co., Elkhart, Ind. They were dissolved in H_2O and used either directly or after extensive dialysis against LiCl, EDTA, and H_2O. The Poly G, prepared by Dr. M. Grunberg-Manago, was a gift from Dr. J. Marmur and was dissolved in .15M NaCl-.015M Na Citrate.

S-RNA was prepared from rabbit liver by direct phenol extraction according to the method of Rosenbaum and Brown (1961). It was further purified on DEAE-cellulose. Glutamyl-RNA synthetase was partially purified from rat liver by precipitation at pH 5.5, alumina Cγ treatment and ammonium sulfate fractionation. The final specific activity of the preparation was 4-5 mμmules glutamate transferred to s-RNA/min/mg protein. Details of the purification procedure will be published elsewhere. The crude seryl- and leucyl-RNA synthetases were pH 5.5 fractions from rat liver.

Results: The data in Table 1 show that several of the polynucleotides tested were able to inhibit the attachment of glutamic acid to s-RNA. Among the homopolymers, Poly G and Poly I were found to be the most effective inhibitors even at very low concentrations. Poly U inhibited to an intermediate degree. However, neither Poly A nor Poly C had any inhibitory properties. Extensive dialysis of Poly U and Poly I did not alter their inhibitory effects. In the copolymer series it is seen that only Poly UG 3:1 and Poly UI were effective inhibitors. Poly CA 5:1 did not inhibit, in agreement with the results obtained with the homopolymers. It is also interesting to note that in the series of copolymers containing all 4 bases, the only polynucleotide which inhibited was the one in which U predominated.

These results indicate that the inhibition of the reaction by synthetic polynucleotides is specific for certain bases and is not merely due to the presence of excess polynucleotide. In addition, uracil, uridine, 5'-UMP, 2',3'-UMP and UDP, at levels of .25 μmoles, each inhibited less than 5% (which is the limit of error of the assay).

Table I

Relative Percent Inhibition of Glutamyl-RNA Synthetase by Polynucleotides*

Polynucleotide**	µmoles Nucleotide Added***	% Inhibition
Poly A	.15	<5
Poly U	.25	35
Poly C	.25	<5
Poly G	.008	93
Poly I	.011	75
Poly UG 3:1	.175	67
Poly UG 1:1	.300	17
Poly UA 2:1	.265	22
Poly UC 2:1	.256	12
Poly UI (1:1)	.095	48
Poly CA 5:1	.187	<5
Poly AGUC 1:1:1:7	.110	<5
Poly AGUC 7:1:1:1	.187	<5
Poly AGUC 1:1:7:1	.204	47

* Since these results were obtained at single concentrations of s-RNA and polynucleotide, only relative percent inhibitions are given.

** The numbers after the polynucleotide refer to the input ratios in the polynucleotide phosphorylase reaction.

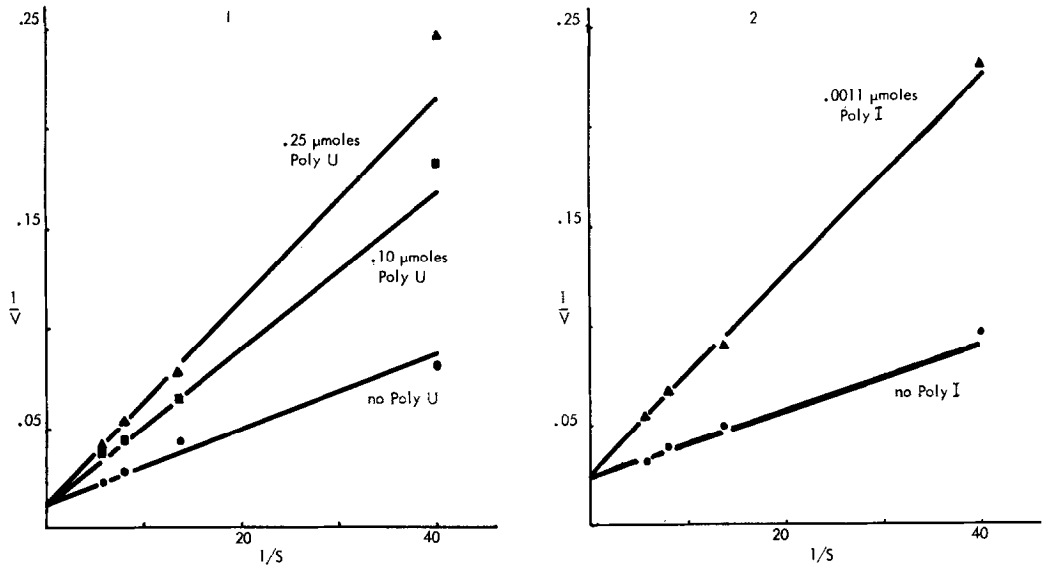
*** The amount of polynucleotide added is expressed in µmoles P for the copolymers and in µmoles nucleotide for the homopolymers. The latter values were calculated from extinction coefficients for the homopolymers given in the literature (Sigler et al, 1962; Fresco, 1963).

The reaction mixtures contained in 200 µl: 40 µmoles TRIS, 4 µmoles Na ATP, 5 µmoles $MgCl_2$, 125 µg s-RNA, 10 mµmoles C^{14}-glutamate = 220,000 dpm, approximately 5 µg enzyme and the indicated amounts of polynucleotide; all adjusted to pH 7.0. The mixture was incubated at 37° for 3 min. The reaction was stopped by addition of 2 ml of cold 10% TCA containing 1 µmole glutamate/ml. The precipitate was collected on a Millipore filter and washed with about 20 ml of cold 5% TCA containing .5 µmoles glutamate/ml. The filter was air dried and radioactivity was measured in a Tri-Carb scintillation counter. Efficiency of counting was 70%.

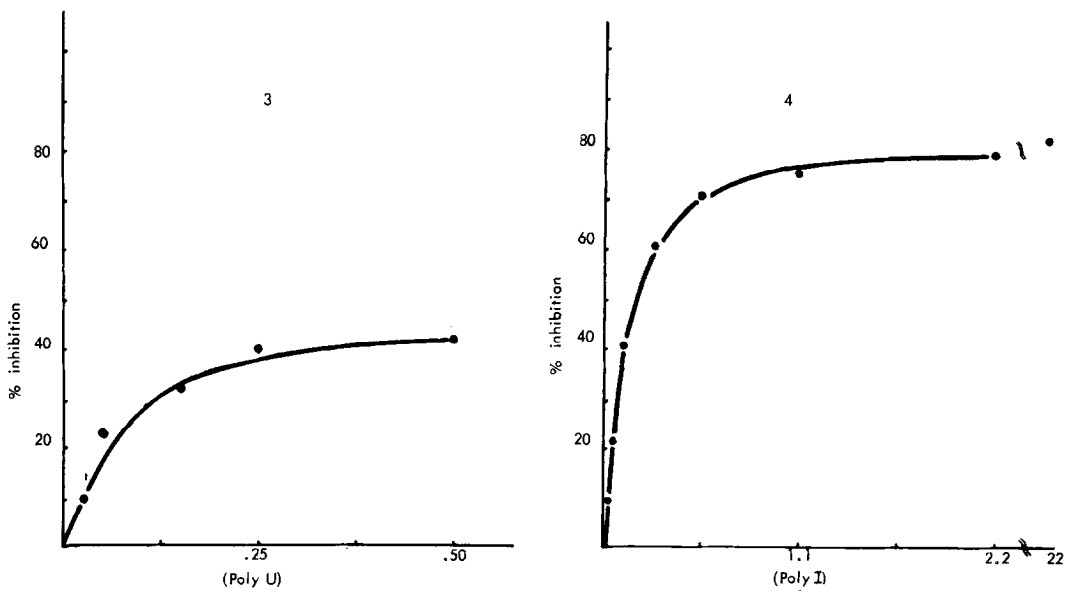
Although similar results were obtained with inosine and 5'-IMP, the same concentration of IDP did inhibit 10%. It may be concluded that a sequence of bases, and not just a single base, is involved in the inhibition.

The Lineweaver-Burk plots shown in Figures 1 and 2 reveal that the inhibition by both Poly U and Poly I is competitive with respect to s-RNA. However, as can

be seen from Figures 3 and 4, the inhibition is not complete, i.e., does not reach 100%.



Figs. 1 and 2. Competitive nature of Poly U and Poly I inhibition. Conventional double reciprocal plots of glutamate incorporation (expressed in μμmoles/3 min^{-1}) at different s-RNA concentrations (expressed in mg^{-1}). Incubations were carried out as described in Table 1.



Figs. 3 and 4. Effect of Poly U and Poly I concentration on the percent inhibition. The inhibitor concentration is expressed in μmoles for Poly U and μmoles x 10^{-2} for Poly I. Incubations were carried out as described in Table 1.

Poly U inhibition levels off at about 50% and Poly I inhibition levels off at about 85%. The Poly I inhibition, however, requires much lower concentrations of polynucleotide to achieve the same % inhibition as Poly U. These results, and those obtained from I/V vs. I and I/i vs. I/I plots (i is the fractional inhibition and I the inhibitor concentration), reveal that the inhibition is a partially competitive type.

The specificity of the inhibition was studied further in several experiments in which two polynucleotides were added to the incubation mixture.

Table II

Effect of Two Polynucleotides on the Inhibition of Glutamyl-RNA Synthetase

Polynucleotide	% Inhibition
.0011 μmoles Poly I	45
.0011 μmoles Poly I + .15 μmoles Poly A	<5
.0011 μmoles Poly I + .25 μmoles Poly C	<5
.25 μmoles Poly U	45
.25 μmoles Poly U + .15 μmoles Poly A	<5
.25 μmoles Poly U + .25 μmoles Poly C	43

The incubations were carried out as described in Table 1.

Table II shows that when the two synthetic polynucleotides present were able to form a complex, such as Poly I + Poly A, Poly I + Poly C, or Poly A + Poly U, the inhibition was completely abolished. However, a mixture of Poly U and Poly C, which are not known to form a stable complex, did not lead to a diminution of inhibition. These results suggest that the inhibitory polynucleotides are unable to inhibit glutamyl-RNA synthetase when they are bound in a stable complex with a non-inhibitory polynucleotide.

Preliminary results obtained with other aminoacyl-RNA synthetases demonstrate that the pattern of inhibition is dependent upon the enzyme used. For example, neither Poly A, Poly U, nor Poly C inhibited a crude serine enzyme from liver or a purified serine enzyme from yeast (kindly furnished by Dr. M. Makman and Dr. G. L. Cantoni). In contrast to these results, Poly A and Poly C inhibited a crude leucine

enzyme from liver to a small extent, but Poly U had no effect.

The data presented in this paper demonstrate that synthetic polyribonucleotides can specifically inhibit the glutamyl-RNA synthetase reaction and that the inhibition is competitive with respect to s-RNA. However, since Poly U and Poly I seem to be partial competitive inhibitors, it is possible that they are binding to a different site on the enzyme than the s-RNA with a concomitant lowering of the affinity of the enzyme for this substrate. These results may be related to the 'allosteric' effects on aminoacyl-RNA synthetases recently reported by Hele (1964). The data may also have a bearing on the inhibition of cell-free protein synthesis by homopolynucleotides reported by Moller and von Ehrenstein (1963).

The results presented here suggest that the interaction of synthetic oligo- or polynucleotides with aminoacyl-RNA synthetases may serve as a model which may lead to an increased understanding of the mechanism of interaction between these enzymes and s-RNA.

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