POLYARABINOURIDYLIC ACID: PRIMORDIAL MESSENGER OR INHIBITOR?*

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Arabinonucleosides, and mono and diarabinonucleotides have demonstrated therapeutic potentialities (Cohen, 1966; Wechter, 1967; Smith, et al., 1967; Renis, et al., 1967). Polyarabinonucleotides, in addition to their potential therapeutic use, should contribute to our understanding of the role the carbohydrate moiety plays in polynucleotide structure and function.

No enzymatic synthesis of polyarabinonucleotides is currently available (Michelson et al., (1962). Schramm and Ulmer-Schürnbrand (1967) have reported that reaction of uridylic acid with polyphenylphosphate yields polyspongouridylate, their name for polyarabino U. A synthesis of polyarabino U by conversion of the ribo moiety of polyribo U to the arabino moiety in polyarabino U has also been announced (Nagyvary, 1967a, 1967b). This novel reaction is based on the ready formation of 2', 3', 5' cyclic triesters from ribopolymers of the pyrmidine bases, followed by the base catalyzed thermal rearrangement of this system to O^2, 2'-cyclouridine derivatives. Mild alkaline hydrolysis of these latter products yields oligoarabino-nucleotides of medium chainlength, with some ribo-uridine at the 3' end.

It is of interest to compare the biological properties of polyspongouridylate with those of polyarabino U synthesized by other syntheses. To date, polyspongouridylate has been analyzed (and evaluated as a pre-biological evolutionary agent) in terms of its ability to inhibit the action of pancreatic ribonuclease and its ability to function as a messenger-RNA (Schramm

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and Ulmer-Schürnbrand, 1967). This paper reports the biological properties of polyarabino U synthesized by conversion of polyribo U and by polymerization of O^2, 5'-cyclouridine-2',3'-cyclic phosphate (Nagyvary and Provenzale, 1968). Interestingly, these polyarabino U forms demonstrate biological properties in conflict with those reported for polyspongouridylate.

MATERIALS AND METHODS

The oligonucleotides $^{a}Up^{r}U$, $(^{a}Up)_2{}^{r}U$, $(^{a}Up)_3{}^{r}U$, $(^{a}Up)_4{}^{r}U$ and $(^{a}Up)_5{}^{r}U$ (the abbreviations ^{a}U and ^{r}U stand for 1-β-D-arabinofuranosyl uracil and uridine, respectively) were prepared from poly^{r}U (Nagyvary, 1967b). Degradation of these materials with snake venom phosphodiesterase and E. coli alkaline phosphatase gave ^{a}U and ^{r}U and indicated 10-20% of the products carried a 3' terminal ^{a}U instead of ^{r}U. The polyarabinouridylates $^{a}U_{10-20}$ and $^{a}U_{20-30}$ were obtained by a new solid state polymerization procedure from O^2, 5'-cyclouridine-2',3'-cyclic phosphate (Nagyvary and Provenzale, 1968). Polymer size (e.g., $^{a}U_{10}$ = decanucleotide of ^{a}Up) of these materials was determined by gel filtration on Sephadex G-75 (Hohn and Schaller, 1967), and confirmed in an analytical ultracentrifuge analysis (Yphantis, 1964). Degradation of these materials with snake venom phosphodiesterase and E. coli alkaline phosphatase yielded the expected amounts of ^{a}U and ^{r}U for structures terminating with a 3' terminal ^{r}U.

RNase inhibition. Cytidine-2', 3'-cyclic phosphate, Na^+ salt (Sigma) and pancreatic ribonuclease, 5 times recrystallized (Cal-Biochem), were used for studies of RNase action in the presence of the various arabinouridylate fractions. RNase was assayed on an Ionalyzer Model 801 pH-meter with a Jena glass combination electrode (Schott & Gen., Mainz) with a manually operated Manostat microbiuret.

Reagents used for biological assays. E. coli ribosomes were isolated from early log phase cells by the method of Nirenberg et al. (1962) and subsequently washed 3 times by suspension in (Potter Elvehjem homogenizer) and sedimentation from (120,000 x g) 10 ml of cold 0.01 M Tris pH 7.8,

0.01 M magnesium acetate, 0.06 M KCl, 0.006 M mercaptoethanol (Buffer A). Final 3X washed ribosomes were resuspended in Buffer A (48 mg ribosome/ml) and stored at 0.2° C until use.

[^{14}C]phenylalanyl-t-RNA of E. coli (82,500 cpm/mg) was prepared from [^{14}C]L-phenylalanine (360 mc/m mole, New England Nuclear Corp., Boston, Mass.) and E. coli t-RNA (Schwartz BioResearch Inc., Orangeburg, N. Y.) by the method of von Ehrenstein and Lipmann (1961). Polyribo U was obtained from Miles Lab, Elkhart, Ind. Messenger-RNA free, 30,000 x g supernatant (S-30) for use in protein synthesis studies was prepared from early log phase E. coli cells by the method of Clark et al. (1965).

Assay of aminoacyl-t-RNA, m-RNA, ribosome complex formation. The binding of aminoacyl-t-RNA to m-RNA containing ribosomes was measured by a modification of the method of Nirenberg and Leder (1964). In this assay, 0.25 ml reaction mixtures containing 40 μ moles of NH_4Cl, 12.5 μ moles Tris-Cl, pH 7.4, 3 μ moles 2-mercaptoethanol, 2.5 μ moles of $MgCl_2$, 0.2 mg of [^{14}C]phenylalanyl-t-RNA, 1.44 mg of 3X washed ribosomes and quantities of polyribo U or polyarabino U as indicated were incubated at 30° for 20 minutes (i.e., until equilibrium was achieved). Reaction mixtures were then diluted with 5 ml of cold (0-2°) 0.16 M NH_4Cl, 0.05 M Tris-Cl, pH 7.4, 0.01 M $MgCl_2$, 0.012 M 2-mercaptoethanol (binding buffer). The diluted solutions were vacuum filtered through 7/8 inch diameter type HAWP Millipore filters. These filters, containing adsorbed aminoacyl-t-RNA, m-RNA, ribosome complexes, were washed with 4 consecutive 5 ml washes of cold binding buffer before being dried (90°, 10 min) and subsequently counted by liquid scintillation counting in 0.4% PPO, 0.01% POPOP in toluene.

Assay of coding properties of m-RNAs during protein biosynthesis. The ability of polyribo U and polyarabino U to support the incorporation of [^{14}C]phenylalanine into polypeptide material was assayed according to the method of Clark et al. (1965) except that natural m-RNA was replaced with varying amounts of polyribo U or polyarabino U and [^{14}C]L-phenylalanine

(0.5 μc of 360 mc/m mole material, New England Nuclear Corp., Boston, Mass.) was the only amino acid added to the reaction.

RESULTS AND DISCUSSION

Oligoarabino-(3' ribo)-uridylates demonstrate a chainlength dependent inhibition of pancreatic RNase (Fig. 1) as previously suggested by Schramm

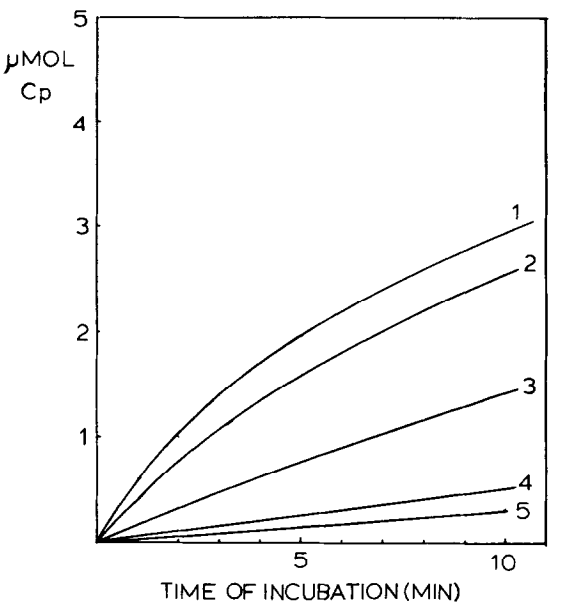

Fig. 1. Chainlength dependence of inhibition. Incubation mixtures at 20° contained 10 μmoles of cytidine-2',3'-cyclic phosphate, 5 μmoles of inhibitor, 50 μg RNase and 0.2 mmole of NaCl in 1 ml. The pH was kept constant by (7.00±0.05) the continuous addition of 0.1 N KOH. Lines 1. no inhibitor, 2. $^{a}Up^{r}U$, 3. $(^{a}Up)_2{}^{r}U$, 4. $(^{a}Up)_3{}^{r}U$, 5. $(^{a}Up)_5{}^{r}U$

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and Ulmer-Schürnbrand. The inhibition by $(^{a}Up)_3{}^{r}U$ is competitive on a Lineweaver-Burk diagram (Fig. 2). But in contrast to polyspongouridylate, our $^{a}U_{10-20}$ does not bind [^{14}C]phenylalanyl-t-RNA onto washed E. coli ribosomes under the conditions where polyribo U actively partakes in this reaction. Further, polyarabinouridylic acid does not support the incorporation of [^{14}C]L-phenylalanine into polypeptide material under conditions where polyriboudirylic acid codes for [^{14}C]polyphenylalanine synthesis

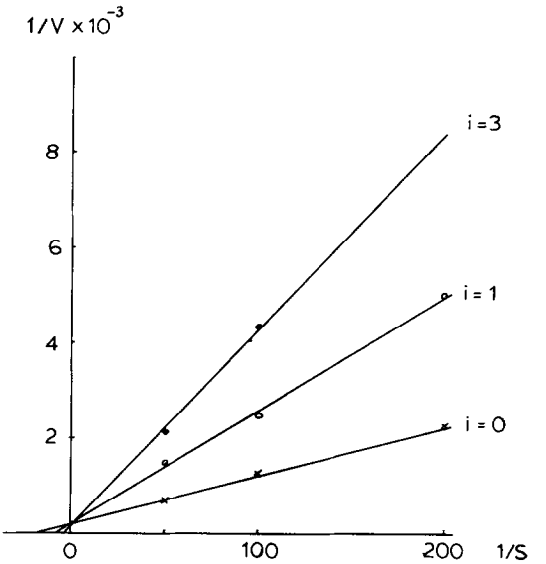

Fig. 2. Lineweaver-Burk diagram of RNase inhibition by the tetranucleotide $(^{a}Up)_3{}^{r}U$. The concentration of cytidine-2',3'-cyclic phosphate is expressed in moles/liter, the initial velocities in moles/liter/min. The incubation mixtures at 37° contained 100 μg RNase and no inhibitor, 1 μmole and 3 μmoles of inhibitor, respectively, in 1 ml.

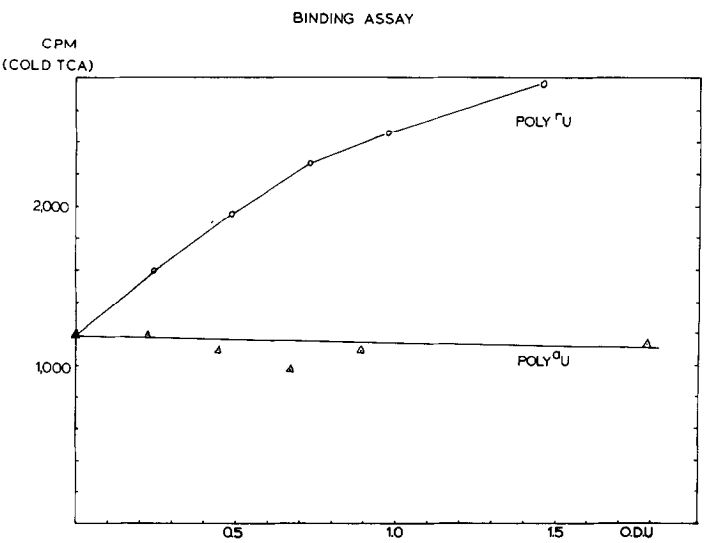

Fig. 3. Binding studies with polyribo U and polyarabino U.

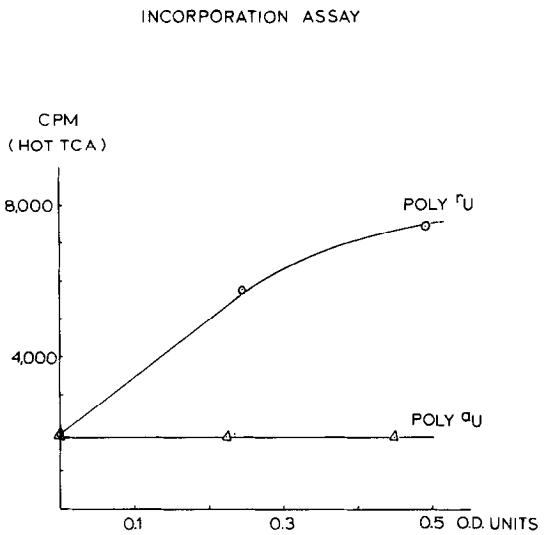

Fig. 4. Incorporation of [^{14}C]phenylalanine in presence of polyribo U and polyarabino U.

(Fig. 4). Further, mixing of $^{a}U_{20-30}$ with poly A does not cause any hypochromicity, therefore the formation of a double stranded structure seems to be excluded. This finding is substantiated by studies with molecular models which clearly show that the parallel stacking of bases within polyarabinonucleotides is sterically impossible.

We share the view of Schramm and Ulmer-Schürnbrand of the prebiological occurrence of these polymers, and we believe that such hypothesis is further accentuated by the convertibility of a polyribonucleotide into a polyarabinonucleotide. On the basis of our present findings, and the generally inhibitory activities of arabinonucleosides, we envision the prebiotic evolutionary role of polyarabinonucleotides as that of inhibitors instead of as primeval templates. This latter conclusion disagrees with that of Schramm and Ulmer-Schürnbrand (1967).

REFERENCES

Clark, J. M., Jr., Chang, A. Y., Spiegelman, S., and Reichmann, M. E., Proc. Nat. Acad. Sci. 54, 1193 (1965).

Cohen, S. S., Progress in Nucleic Acid Research and Molecular Biology, ed. by Davidson and Cohn, Academic Press, 5, 2 (1966).

von Ehrenstein, G., and Lipmann, F., Proc. Nat. Acad. Sci. 47, 941 (1961).

Hohn, T., and Schaller, H., Biochim. Biophys. Acta 138, 466 (1967).

Michelson, A. M., Dondon, J., and Grunberg-Manago, M., Biochim. Biophys. Acta 55, 529 (1962).

Nagyvary, J., Fed. Proc. 26, No. 2, 292 (1967a).

Nagyvary, J., XXI. Congress of IUPAC (1967b), Prague, Abstract N-48.

Nagyvary, J. and Provenzale, R., Science (In press) (1968).

Nirenberg, M. W., Matthaei, J. H., and Jones, O. W., Proc. Nat. Acad. Sci. 48, 104 (1962).

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

Renis, H. E., Hollowell, C. A., and Underwood, G. E., J. Med. Chem. 10, 777 (1967).

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

Smith, C. G., Buskirk, H. H., and Lummis, W. L., J. Med. Chem. 10, 774 (1967).

Wechter, W. J., J. Med. Chem. 10, 762 (1967).

Yphantis, D., Biochemistry 3, 297 (1964).