STUDIES ON RIBONUCLEIC ACID POLYMERASE WITH SYNTHETIC POLYRIBONUCLEOTIDES AS TEMPLATES: EFFECT OF OLIGONUCLEOTIDES ON THE REACTIONS

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Synthetic polyribonucleotides serve efficiently as templates for the synthesis of complementary polyribonucleotides (Krakow and Ochoa, 1963; Fox et al. 1964; Stevens and Henry, 1964) with highly purified RNA polymerase. Using such well-defined systems, studies have been undertaken to investigate the mechanism of chain initiation by RNA polymerase. The stimulation of reactions involving poly U and poly A by complementary oligonucleotides is reported here. The studies suggest that the stimulation is due to the oligonucleotides acting as chain-initiators.

Materials and Methods

The purification of RNA polymerase from E. coli B has been described previously (Stevens and Henry, 1964). The studies reported here were carried out with the final density gradient fraction (Table I, Stevens and Henry, 1964).

Poly A and poly U (obtained from Miles Laboratories, Clifton, New Jersey) were observed to be homogeneous during ultracentrifugation in 0.15M NaCl-0.015M Na-citrate, pH 7.1, and the $S_{20,w}$ values were calculated to be about 10 and 5 respectively.

ATP-C^{14} and UTP-C^{14} were obtained from Schwarz Bioresearch, Inc., and were purified by paper chromatography before use.

Adenine oligonucleotides of type pApA were prepared from poly A by the action of a nuclease from A. agilis (Stevens and Hilmoe, 1960); those of type ApA were made by the action of E. coli alkaline phosphatase (Worthington Bio-

chemical Corp.) on type pApA; and type ApAp by controlled alkaline hydrolysis (Lane and Butler, 1959) of poly A. Uracil oligonucleotides of type UpUp were prepared by limited action of pancreatic ribonuclease on poly U (Heppel et al., 1957), and those of type UpU by the action of E. coli alkaline phosphatase on type UpUp. The oligonucleotides of different chain length were separated and purified by paper chromatography according to methods similar to Heppel et al. (1956, 1957).

TABLE I

Effect of Adenine Oligonucleotides on the RNA Polymerase Reaction with Different Templates

Oligonucleotide Added	C^{14}-ATP Incorporated		
	Poly A formation with Poly U template (Reacn. A) mμmoles	Homopolymer formation with heated T_2DNA (Reacn. B) mμmoles	Heteropolymer formation with T_2DNA (Reacn. C) mμmoles
None	0.27	6.3	9.3
pApA	0.41	5.6	-
pApApA	0.76	6.5	9.6
pApApApA	1.26	6.5	7.0
pApApApApA	4.86	6.8	-
pApApApApApA	6.57	6.5	7.1
ApApA	0.83	6.5	
ApApApA	3.03	6.2	
ApApApApA	4.61	6.4	
ApApApApApA	3.51	6.1	
ApAp	0.28		
ApApAp	0.23		

The complete reaction mixture (A) (0.2 ml) contained Tris buffer, pH 7.8, 4 μmoles; $MnCl_2$, 0.5 μmole; ATP-C^{14}, 50 mμmoles, (specific activity 7 x 10^5 cpm/μmole); β-mercaptoethanol, 4 μmoles; poly U, 10 μg; oligonucleotide (where added), 1 mμmole; and enzyme. The reaction mixtures (B) and (C) have been described previously (Stevens and Henry, 1964; Stevens, 1964). After a 10 minute incubation period at 37° C, the amount of isotope in acid-insoluble material was determined by the millipore filtration technique described previously (Stevens and Henry, 1964).

Results and Discussion

As shown in Table I (Reacn. A) even at very low concentration (1 mμmole), adenine oligonucleotides (dinucleotide to the hexanucleotide) of types pApA and ApA (having a free 3'-hydroxyl end) greatly stimulate the formation of poly A with poly U as a template. The amount of stimulation increases with the chain length of the oligonucleotide. No stimulation is observed with oligonucleotides of type ApAp (having a 3'-phosphate end); instead, some inhibition is usually found at high concentrations of the oligonucleotide. Under the conditions employed, the oligonucleotides do not by themselves serve as templates for the formation of poly A, as tested by acid-insolubility and also by the paper chromatographic assay method of Falaschi, Adler, and Khorana (1963). The adenine oligonucleotides have no effect on reactions using poly A, poly C or poly AU as a template. Little or no effect is observed on the formation of either heteropolymer (Stevens and Henry, 1964) or homopolymer (poly A) (Stevens, 1964) with native T_2DNA and heated T_2DNA, respectively (Table I, Reacns. B and C).

Uracil oligonucleotides give results analogous to those above; UpUpU, for example, stimulates the formation of poly U with poly A as a template. Oligonucleotides of type UpUp do not stimulate. These results are shown in Table II.

TABLE II

Effect of Uracil Oligonucleotides on the Formation of Poly U with Poly A as Template

Oligonucleotide added	C^{14}-UTP Incorporated mμmoles
None	0.20
UpU	0.38
UpUpU	1.54
UpUpUpU	1.56
UpUp	0.21
UpU+UpUp	0.39
UpU (3 mμmoles) + UpUp	0.70

Reaction conditions same as described under Table I, except that 70 mμmoles of UTP-C^{14}, specific activity 5 x 10^5 cpm/μmole, and polyA, 10 μg, were used. Quantity of each oligonucleotide added was 1 mμmole except where shown.

It was of interest to determine whether the oligonucleotides were incorporated into the polymer formed. A small but significant amount of pApApApA, labeled with P^{32}, is incorporated into poly A in the poly U reaction (Reacn. A, Table I) with unlabeled ATP. The incorporation of the oligonucleotide is predominantly into chain ends as determined by radioactivity in adenosine-3',5'-diphosphate, isolated from alkaline hydrolysates of the product.

The results shown in Tables I and II indicate that a free 3'-hydroxyl end is essential for the stimulatory activity of the oligonucleotides; oligonucleotides with a 3'-phosphate end fail to stimulate. A free 5'-hydroxyl end is not required for stimulatory activity. The results along with the incorporation of the oligonucleotides into the chain ends of the product, suggest that the oligonucleotides stimulate the reaction by acting as primers.

The results shown in Tables I and II were obtained at suboptimal nucleoside triphosphate concentrations. The oligonucleotides have been found to have a striking effect on nucleoside triphosphate concentration curves. Substrate curves for poly U and poly A reactions are shown in Fig. 1, both in the absence and in the presence of complementary oligonucleotides. In the absence of oligonucleotide, the triphosphate concentration curves resemble those described for reactions in which the substrate also acts as an activator. Other interpretations, like a bimolecular reaction or allosteric effects, are possible. In the presence of stimulatory oligonucleotides a more usual type of substrate curve is obtained. The results are in line with the idea that the oligonucleotides act as chain-initiators. Further studies of this nature will be reported in detail elsewhere.

Fox et al. (1964) described the temperature dependency of the reactions with synthetic polyribonucleotides. Very similar temperature dependency has been found in our studies. The effect of oligonucleotides at different temperatures is very interesting. Formation of poly A with poly U as a template (minus oligonucleotide) proceeds rather slowly at 37° C and 45° C but quite well at lower temperatures. The oligonucleotide stimulation is maximal at 37° and 45° C (Fig. 2); at 25° C there is some stimulation, but at 15° C and lower, the oligo-

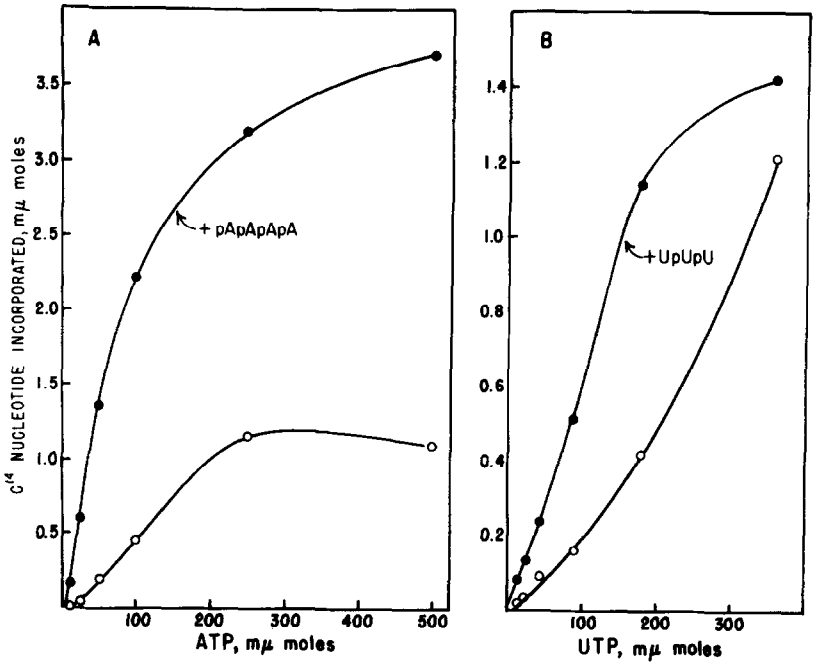

Fig. 1. *Effect of oligonucleotides on substrate (nucleoside triphosphate) concentration curves.*

A. Formation of poly A with poly U template (in the absence and presence of pApApApA). Reaction conditions similar to those described under Table I (Reaction A), using different concentrations of C^{14}-ATP. Amount of pApApApA where added was 1 mμmole.

B. Formation of poly U with poly A template in the absence and presence of UpUpU. Reaction conditions similar to those described under Table II, using different concentrations of C^{14}-UTP. Amount of UpUpU added was 1.6 mμmoles.

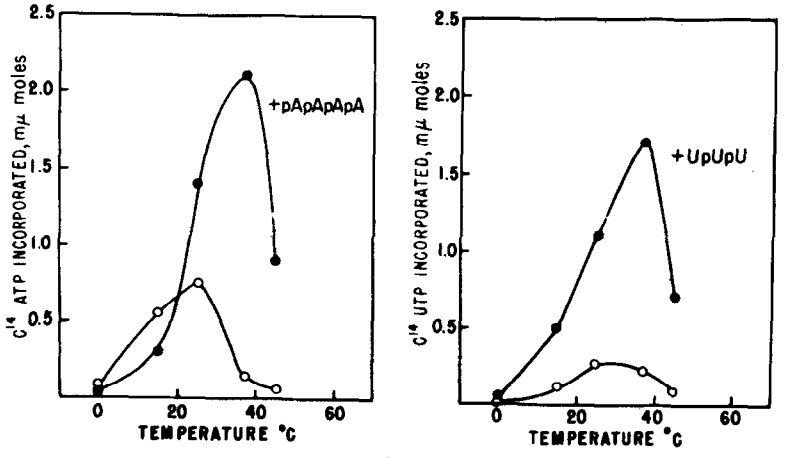

Fig. 2. Effect of oligonucleotides on temperature dependency curves.

A. Formation of poly A with poly U template. Reaction mixture similar to those described under Table I (Reacn. A). Amount of pApApApA where added was 1 mμmole.

B. Formation of poly U with poly A template. Reaction mixture similar to those described under Table II. Amount of UpUpU where added was 1.6 mμmoles.

nucleotides have some inhibitory effect. Poly U formation with poly A as a template (minus oligonucleotide) proceeds maximally at 25° C though the rate is not much higher than that at 37° C. Maximal stimulation with uracil oligonucleotides occurs at 37° C and at 45° C, although appreciable stimulation occurs also at lower temperatures (Fig. 2). The studies suggest a temperature dependency of chain initiation.

The studies reported in this paper open up possibilities of investigating various aspects of the mechanism of the RNA polymerase reaction with well-defined templates and primers. Such studies are in progress and will be reported in detail elsewhere.

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