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Chemically Synthesized Deoxypolynucleotides as Templates for Ribonucleic Acid Polymerase*

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The enzymatic synthesis of ribonucleic acid from the four ribonucleoside 5′-triphosphates in the presence of deoxyribonucleic acid has been documented in a number of laboratories (1–11). The reaction is catalyzed by an enzyme called RNA polymerase and the DNA determines the composition and the sequence of nucleotides in the synthesized RNA according to the base-pairing principle first recognized for DNA by Watson and Crick (7–11). The RNA synthesized in this manner stimulates the incorporation of amino acids into protein (12–15).

A deoxypolythymidylate fraction obtained by chemical synthesis (16) was tested previously by Hurwitz *et al.* (7, 11) and was found to bring about the synthesis of ribopolyadenylic acid. In the present work, purified synthetic deoxypolynucleotides of known size and sequence have been used to study further the mechanism of action of RNA polymerase. The results reported herein show that deoxyoligonucleotides as small as the pentanucleotide dT_5 can serve as templates for the synthesis of ribopolyadenylate.[1] The rate of the synthetic reaction increases with an increase in the size of the deoxypolythymidylate until a chain length of 14 is reached, and this polymer was actually more active than DNA. The product synthesized from deoxypolythymidylate of various sizes was invariably very much larger than the size of the template. Several lines of evidence showed that the enzyme initiated the synthesis of new chains, rather than causing esterification to the 3′-hydroxyl end of the deoxypolynucleotide chains. Finally, in these simpler systems, the incorporation of the ribonucleoside 5′-triphosphates again followed the Watson-Crick base-pairing principle, although some exceptions were noted. A brief report of these findings has already appeared (17).

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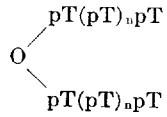
[1] All of the chemically synthesized polynucleotides used in the present work carry a 3′-hydroxyl group at one end and a 5′-phosphate group at the opposite end. For convenience, this class of homologous polynucleotides is simply designated in the text by the nucleoside initial with a subscript indicating the number of nucleosides in the chain. For example, deoxy-pTpTpTpTpT is dT_5 and deoxy-pCpCpCpCpCpC is dC_6, etc. A, G, C, U, and T stand for adenosine, guanosine, cytidine, uridine, and thymidine.

EXPERIMENTAL PROCEDURE

RNA polymerase was purified from *Escherichia coli* according to the procedure described by Chamberlin and Berg (10). *E. coli* phosphodiesterase (18) was a gift from Dr. I. R. Lehman. C^{14}-Ribonucleoside 5′-triphosphates were purchased from Schwarz BioResearch, Inc.

Deoxyribopolynucleotides—Deoxycytidine, deoxyadenosine, and deoxyguanosine oligonucleotides were prepared by published procedures (19–21). The homologous deoxythymidine polynucleotides dT_4 to dT_{11} were prepared as described previously (16, 22), and C^{14}-labeled thymidine polynucleotides were prepared by Mr. W. J. Connors by adaptation (23) of these published procedures (16, 22).

Pure thymidine polynucleotides dT_{12} to dT_{14} and a fraction containing members higher than dT_{14} were prepared from the 1 M triethylammonium bicarbonate fraction obtained in an experiment described previously (Khorana and Vizsolyi, Table I, and Fig. I (16)). The latter fraction, as mentioned earlier (16), was a complex mixture of polynucleotides containing apparently a high proportion of oligonucleotides linked together by pyrophosphate bridges between the terminal 5′-phosphomonoester groups, with general structure



The mixture (110 optical density units at 267 mμ) was treated in dry pyridine (2 ml) with 0.5 ml of acetic anhydride for 3 days in order to selectively cleave the pyrophosphate bonds, and the reaction mixture was worked up as described earlier (22). Chromatography on a DEAE-cellulose (carbonate form) column (50 × 1.2 cm) gave a series of peaks which were processed by the standard method developed earlier (16). As a result of cleavage of the pyrophosphate bonds, more than 50% of the ultraviolet-absorbing material was present as a mixture of oligonucleotides smaller than dT_{10}. The higher homologues were then applied to paper along with previously characterized dT_{11} as marker. The dT_{12}, dT_{13}, and dT_{14} traveled with progressively decreasing R_F values when chromatographed for 2 weeks in descending *n*-propyl alcohol-concentrated ammonia-water (55:10:35).

Acetylation of 3′-Hydroxyl End Groups in Penta- and Undecathymidylic Acids—An aqueous solution of ammonium salt of the polynucleotide (2 μmoles of thymidine) was passed through a column (1 × 2 cm) of Dowex 50 ion exchange resin (H^+) and

the total effluent and washings evaporated after addition of pyridine (1 ml). To the residue was added triethylamine (0.05 ml) and pyridine (2 ml) and the solution was re-evaporated with vacuum from an oil pump. The residue was rendered anhydrous by repetition of evaporation after addition of dry pyridine. Finally, dry pyridine (0.5 ml) and acetic anhydride (0.2 ml) were added and the sealed reaction mixture kept in the dark at room temperature for 4 hours. Water (2 ml) was then added and the total solution kept for 2 hours at room temperature. It was then evaporated under reduced pressure to an oil which was extracted with dry ethyl ether several times. The insoluble polynucleotide material was obtained as a fine solid deposit on the wall of the round bottom flask. It was dissolved in water and the aqueous solution was lyophilized. The solid residue was made up to 0.2 ml with water.

Resistance of Polynucleotides Bearing Terminal 3′-O-Acetyl Groups to E. coli Phosphodiesterase—An exonuclease purified from *E. coli* has been shown by Lehman (18) to degrade deoxyribopolynucleotides in a stepwise manner from the end bearing a 3′-hydroxyl group. The reaction produces deoxyribonucleoside 5′-phosphates until the chain length is reduced to the dinucleotide (18). In a control experiment, 0.17 μmole of the tetranucleotide d-pTpTpTpT was incubated at 37° in a 0.2-ml incubation mixture in the presence of 0.02 ml of 1 M Tris buffer (pH 7.5), 0.02 ml of 0.1 M magnesium chloride, and 100 units (18) of enzyme. Degradation to d-pT and d-pTpT was complete in under 2 hours as determined by paper chromatography of aliquots in descending ethyl alcohol-0.5 M ammonium acetate buffer, pH 3.8 (7:3, v/v). Incubation of the 3′-*O*-acetyl d-pTpTpTpTpT under identical conditions up to 4 hours showed complete resistance of the oligonucleotide to the enzyme. In a second experiment the use of a 3-fold higher concentration of the enzyme preparation under the above conditions showed likewise the absence of any degradation. On the other hand, the degradation of oligonucleotides bearing 3′-hydroxyl groups proceeded normally in the presence of the 3′-*O*-acetyl derivatives, showing that the latter were not inhibitory.

Assay of RNA Polymerase—The reaction mixture was exactly the same as that described by Chamberlin and Berg (10), except that synthetic deoxypolynucleotides usually replaced DNA. When DNA was used as primer, the C^{14}-RNA synthesized was measured after precipitation with perchloric acid, exactly as described by Chamberlin and Berg (10).

When synthetic deoxypolynucleotides were used, a new assay was devised in order to be able to detect any low molecular weight ribopolynucleotides that might be too small to precipitate in acid. The reaction was stopped with 0.02 ml of concentrated ammonium hydroxide and the entire mixture was then deposited in one pipetting onto the origin of a 2.5- × 57-cm strip of DEAE-cellulose[2] paper (Whatman DE-20). Descending chromatography was carried out in 0.3 M ammonium formate for $2\frac{1}{2}$ hours. Under these conditions polynucleotides as small as ribotetraadenylate remain at the origin, whereas unused nucleoside triphosphates move away. The strip was then left to dry in air. The area containing the C^{14}-ribopolynucleotides (from 2.5 cm below to 2.5 cm above the origin) was cut out, folded in half at the origin, placed folded up in a scintillation vial containing solvent (3 g of 2,5-diphenyloxazole and 100 mg of 1,4-bis-2-(5-phenyloxazolyl)benzene per liter of reagent grade toluene), and counted at 2-80 (875 volts) and window settings of 10, 50, and 100 in a Packard Tri-Carb liquid scintillation spectrometer.

Determination of Size of Ribopolynucleotides—After the area containing the C^{14}-ribopolynucleotide had been counted as described above, it was cut into small pieces and incubated in 2 ml of 0.3 N NaOH for 20 hours at 37° to hydrolyze the ribopolynucleotide. The liquid was then filtered and chilled in ice. It was neutralized by adding slowly small amounts of dry Dowex 50 (H^+) resin; after each addition, about 5 minutes with occasional stirring were allowed before the pH was measured. When the pH reached 7, 5 μl of 1 N NaOH were added in order to avoid any dephosphorylation which could occur if the pH became less than 7 during the subsequent manipulations. The supernatant liquid, combined with 0.1 N ammonium hydroxide washings of the resin, was concentrated by lyophilization and spotted on a strip, 2.5 cm × 57 cm, of DEAE-cellulose paper. Descending chromatography in 0.2 M ammonium formate was carried out for 6 hours until the front reached the end of the paper. This served to separate added (as markers) adenosine, adenosine 3′-phosphate, and adenosine 2′(3′),5′-diphosphate. The dried strip was cut at 1-cm intervals and these pieces were put into scintillation vials and counted as described above. The ratio of radioactivity in adenylic acid to adenosine or in adenylic acid to the adenosine diphosphate was considered the average size of the ribopolynucleotide.

RESULTS

Deoxypolythymidylate as Template for Synthesis of Ribopolyadenylate

Effect of Chain Length on Rate of Synthesis—The initial rate of incorporation of adenylate varied with the size of the deoxypolythymidylate at saturating concentrations of each polymer, as shown in Fig. 1.

With dT_3 no activity was observed under the conditions used, and with dT_4 there was occasional activity. But dT_5 always brought about significant, although small, incorporation of adenylate. With further increase in size the effectiveness of the polymers then rose, at first slowly up to dT_7, then with a big leap upward between lengths of 8 and 11. The activity reached

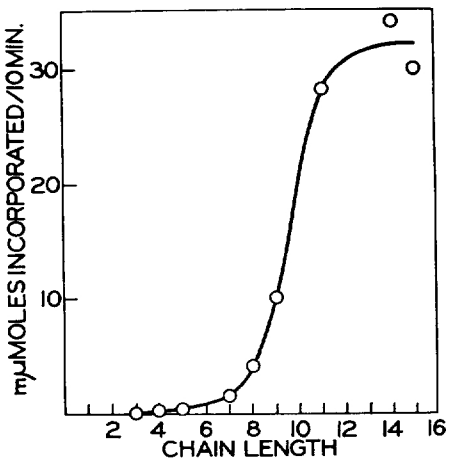


FIG. 1. Effect of chain length on the activity of deoxypolythymidylate. The experimental procedure for measuring the incorporation of adenylate from C^{14}-ATP is described under "Assay of RNA Polymerase."

[2] The form DEAE- refers to diethylaminoethyl-.

TABLE I

Efficiency of DNA and deoxypolythymidylate in stimulating RNA polymerase

The experimental procedure is described under "Assay of RNA Polymerase."

Polymer	Incorporation of nucleotide	
	CTP, GTP, UTP, and C^{14}-ATP present	Only C^{14}-ATP present
	mμmoles/10 min	
Calf thymus DNA	14.1	0.82
Heated calf thymus DNA*	4.8	3.05
Deoxypolythymidylate, 1 M fraction		20.0
dT_{14}		34.0

* Thymus DNA was heated by immersion in a boiling water bath for 10 minutes, then chilled in ice.

a maximum at dT_{14} and dT_{15}. Table I shows that dT_{14} is actually more active for ribopolyadenylate synthesis than DNA is for the synthesis of RNA in the presence of all four nucleoside triphosphates.

Hurwitz *et al.* (7, 11) had already shown that the 1 M triethylammonium bicarbonate fraction referred to above is active for the synthesis of ribopolyadenylate. We have now confirmed the activity of the same 1 M fraction and have found it to be about 60% as active as dT_{14} (Table I). This lesser activity is probably due to the presence of less active polymers or inhibitors in the mixture. As mentioned above, the 1 M fraction contains polymers of various sizes larger than dT_{11} and also oligonucleotides linked together by pyrophosphate bridges. It seems very likely that such pyrophosphate compounds are inhibitors for the priming action of polynucleotides.

RNA polymerase will catalyze the synthesis of ribopolyadenylate when DNA is present together with ATP as the only nucleoside triphosphate (10). Denatured DNA is a preferred primer for this activity (24).[3] Table I shows a confirmation of these results and a comparison of DNA with the activity of the 1 M fraction and dT_{14}. For the synthesis of ribopolyadenylate dT_{14} is the most active polymer.

In order to be sure that the comparison of the results with polynucleotides of different sizes was meaningful, it was necessary to check that the primer did not undergo degradation during the incubation with RNA polymerase (see also below). C^{14}-Labeled dT_7 was used in the usual reaction mixture except that ATP was omitted. The mixture was incubated as usual and then put on a DEAE-cellulose carbonate column (0.6 × 27 cm) and eluted with a linear gradient of triethylammonium bicarbonate from 0 to 0.48 M (total volume, 500 ml). More than 99.7% of the radioactivity appeared as a single peak in the position corresponding to dT_7. This ruled out the possibility of detectable breakdown of the polymer by the enzyme preparation.

Effect of Chain Length on Saturating Concentration of Template—A saturating concentration was determined for each polymer that stimulated the synthesis of ribopolyadenylate in the experiment of Fig. 1. For dT_7, dT_{11}, and dT_{14} the results are plotted in Fig. 2 according to the method that Lineweaver and Burk have used for substrates (25). The polymer concentrations which gave half-maximal rates with dT_7, dT_{11}, and dT_{14} were found to be 50×10^{-6}, 20×10^{-6}, and 2.0×10^{-6} M, respectively. Increasing the size of the polymer not only increases the maximal velocity of the reaction (as shown also by Fig. 1) but decreases very strikingly the concentration of polymer required for half-saturation. Apparently the affinity of the deoxypolythymidylate for the enzyme increases markedly with size between dT_7 and dT_{14}.

Size of Ribopolyadenylate Formed—The C^{14}-ribopolyadenylate formed in the presence of deoxypolythymidylate of various sizes was hydrolyzed with sodium hydroxide to C^{14}-adenosine, C^{14}-adenosine 2′(3′)-phosphate, and a C^{14}-material that resembled adenosine 3′,5′-diphosphate but was not further characterized. The details are described under "Experimental Procedure." The ratio of radioactivity in adenylic acid to adenosine and in adenylic acid to the adenosine diphosphate was taken to be the average size of the ribopolyadenylate. Table II lists the results for the size of the products formed from dT_7, dT_8, dT_9, dT_{11}, and dT_{14}; for smaller thymidine oligonucleotides too little product was available to provide significant results. The estimate of the chain length of the products is only approximate, owing to the inaccuracy of counting on DEAE paper the small amounts of radioactivity in the adenosine and in the adenosine diphosphate region. The most striking feature of the results is that the product is of a much larger size than the deoxypolythymidylate. No marked differences are apparent between the sizes of the products obtained with different sized deoxypolythymidylates.

Evidence for Noninvolvement of Terminal 3′-Hydroxyl Groups of Polythymidylate in Ribopolyadenylate Formation—During chromatography of the total alkaline hydrolysate of the ribopolyadenylate (see above), no significant amount of radioactivity

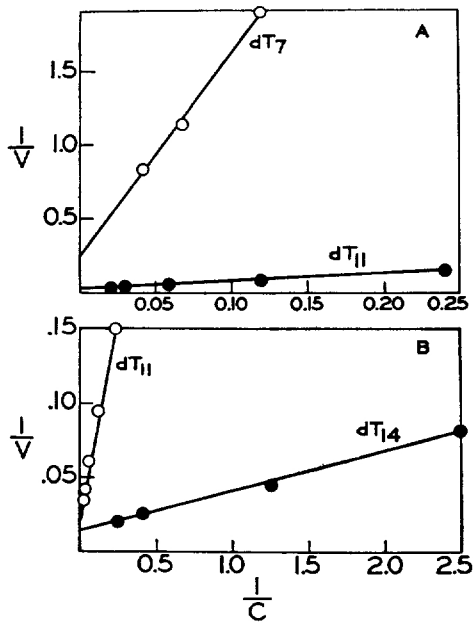


FIG. 2. Reciprocal plot of the effect of concentration of deoxypolythymidylate on the incorporation of adenylate. *A* compares dT_7 and dT_{11}; *B* compares dT_{11} and dT_{14}. The concentration of polymer is expressed as millimicromoles of polymer (not nucleotide equivalents) per ml; *v*, millimicromoles of adenylate incorporated from ATP per 10 minutes, as measured by the DEAE-cellulose paper assay described under "Assay of RNA Polymerase."

[3] M. Chamberlin and P. Berg, unpublished observations.

remained at the origin of the chromatogram where the deoxyribopolythymidylate remains adsorbed. This result indicated that ribopolyadenylate synthesis was not initiated by esterification of the terminal 3′-hydroxyl group in deoxypolythymidylate to form a phosphodiester linkage. Such a linkage would have been resistant to alkaline hydrolysis and a product of the general structure (Diagram I) would have resulted.

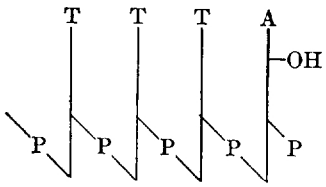


DIAGRAM I

To test further whether the 3′-hydroxyl group is essential in the synthesis of ribopolynucleotides, 3′-*O*-acetyl-dT_{11} was prepared for testing as a primer. Table III (Lines 1 and 3) shows that 3′-*O*-acetyl-dT_{11} is about as active as dT_{11}. The concentration of 3′-*O*-acetyl-dT_{11} required to half-saturate the enzyme was very similar to the concentration already found for dT_{11}. This diminished the possibility that a small amount of unacetylated dT_{11} was the active component in the 3′-*O*-acetyl-dT_{11} preparation. Furthermore, 3′-*O*-acetylthymidylate was found to be stable when incubated with the RNA polymerase preparation; this served to exclude any acylase activity.

In order to ensure that none of the unacetylated dT_{11} was present, the preparation of the acetylated polynucleotide was preincubated with the *E. coli* phosphodiesterase. Acetylation of the 3′-hydroxyl group in thymidine oligonucleotides confers resistance toward this enzyme (see above). Table III (Lines 2 and 4) compares dT_{11} and 3′-*O*-acetyl-dT_{11} after treatment with phosphodiesterase. This enzyme abolishes nearly all of the activity of dT_{11} whereas most of the activity of 3′-*O*-acetyl-dT_{11} remains resistant to phosphodiesterase.

It may be concluded that 3′-*O*-acetyl-dT_{11} is active for the synthesis of ribopolyadenylate, and that a free 3′-hydroxyl group of deoxypolythymidylate is not essential for ribopolyadenylate synthesis to occur. It follows that addition of adenylate to the 3′-hydroxyl end of deoxypolythymidylate is not a necessary part of ribopolyadenylate synthesis.

TABLE II

Size of ribopolyadenylate formed from deoxypolythymidylate templates

The experimental procedure is described under "Size of Ribopolyadenylate Formed."

Polymer	Radioactivity			Chain length		
	Adenosine diphosphate (A)	Adenylic acid (B)	Adenosine (C)	B/A	B/C	Average
	c.p.m.					
dT_7	29	2,550	96	73	27	50
dT_8	80	10,100	135	125	74	100
dT_9	70	4,680	32	67	146	107
dT_{11}	34	3,590	86	101	46	74
dT_{14}	114	9,080	63	79	144	111

TABLE III

Acetylated deoxypolythymidylate as template for RNA polymerase

Treatment with *E. coli* phosphodiesterase was carried out at 37° in a 0.15-ml reaction mixture containing 100 units (18) of enzyme, 0.02 ml of 1 M Tris buffer (pH 7.5), 0.01 ml of 0.1 M magnesium chloride, and 0.4 μmole of nucleotide equivalent of polynucleotide. Then 0.02-ml aliquots of this were added to an RNA polymerase reaction mixture without prior inactivation of the diesterase, since it could be shown that the presence of diesterase does not interfere with the synthesis of ribopolyadenylate.

Polymer	Treatment with phosphodiesterase	Incorporation of adenylate from ATP
		mμmoles/30 min
dT_{11}	−	2.30
dT_{11}	+	0.04
3′-*O*-acetyl-dT_{11}	−	2.37
3′-*O*-acetyl-dT_{11}	+	1.32

TABLE IV

Deoxypolycytidylate as template for synthesis of ribopolyguanylate

The experimental procedure is described under "Assay of RNA Polymerase."

Polymer	Incorporation of guanylate from GTP	Incorporation of adenylate from ATP
	mμmole/10 min	*mμmoles/10 min*
dC_5	<0.05	
dC_6	<0.05	
dC_8	<0.05	
dC_9	0.15	
dC_{10}	0.17	
dT_9		3.2

Deoxypolycytidylate as Template for Synthesis of Ribopolyguanylate

Incubation of deoxypolycytidylate with RNA polymerase and GTP led to the formation of ribopolyguanylate, and the rate of the reaction depended on the size of the deoxypolycytidylate (Table IV). Whereas a chain length of 5, 6, or 8 proved too small to be effective, dC_9 and dC_{10} showed significant activity. The dependence of the reaction on the concentration of dC_{10} is shown in Fig. 3; half-saturation occurred at 5.4×10^{-6} M. A comparison of the deoxypolycytidylate series with the deoxypolythymidylate series shows that lower homologues are more effective in the latter series (Table IV) but a lower concentration of the deoxypolycytidylate is required for saturating the enzyme. Deoxypolycytidylate of much higher molecular weight than used here, prepared from the deoxypolycytidylate-deoxypolyguanylate polymer (26), has been shown by Chamberlin and Berg to be active for the synthesis of ribopolyguanylate (27).

Experiments with Deoxypolyguanylate and Deoxypolyadenylate

In the deoxypolyguanylate series, only dG_6 was tested. It proved to be inactive for the incorporation of nucleotide from CTP. This may be explained by the finding that even small homologues of deoxypolyguanylate have a high tendency to form aggregates of very large molecular weight (28). Chamberlin and Berg (27) have reported that deoxypolyguanylate prepared from the deoxypolycytidylate-deoxypolyguanylate polymer is also inactive for the incorporation of cytidylate.

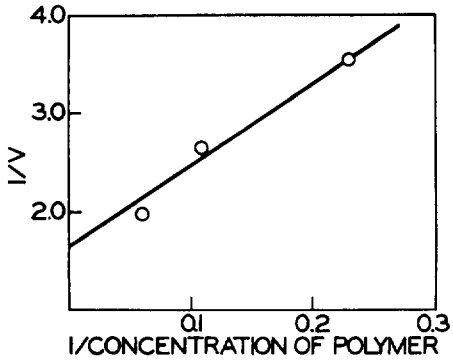


FIG. 3. Reciprocal plot of the effect of concentration of dC_{10} on the incorporation of guanylate. The experimental procedure is described under "Assay of RNA Polymerase." The concentration of polymer is expressed as millimicromoles of polymer (not nucleotide equivalents) per ml; v, millimicromoles of guanylate incorporated from GTP in 10 minutes, as measured by the DEAE-cellulose paper assay described under "Assay of RNA Polymerase."

TABLE V

Specificity of incorporation of nucleotide

The experimental procedure is described under "Assay of RNA Polymerase."

Polymer	Nucleotide incorporated			
	ATP only	GTP only	CTP only	UTP only
	mμmoles/10 min			
Poly dT, 1 M fraction	8.7	<0.05-0.65	<0.05	<0.05
dC_{10}	<0.05	0.69	<0.05	<0.05
Poly dA, 1 M fraction	1.4	<0.05	<0.05	<0.05
dG_6			<0.05	

Deoxypolyadenylate homologues of sizes up to dA_8 and a 1 M fraction that contained a mixture of homologues larger than dA_8 failed to show significant incorporation of nucleotides from UTP. Possibly, conditions other than those used so far might be effective for the synthesis of ribopolyuridylate.

Specificity of Incorporation of Nucleotides

In the presence of the 1 M fraction of deoxypolythymidylate, no significant incorporation of CTP or UTP was observed in 10 minutes, under conditions in which 8.7 mμmoles of adenylate from ATP were incorporated (Table V, Line 1). Surprisingly, 0.65 mμmole of guanylate from GTP was incorporated under the same conditions. As the enzyme preparation became older, the incorporation of guanylate disappeared at a time when the incorporation of adenylate had decreased by only 60%. Furth, Hurwitz, and Anders in one experiment (7) also noted a small incorporation of guanylate.

Deoxypolycytidylate brought about specific incorporation of guanylate (Table V, Line 2). With dC_{10}, 0.69 mμmole of nucleotide was incorporated from GTP but there was no significant incorporation of nucleotide from ATP, CTP, or UTP.

With the 1 M fraction of deoxypolyadenylate, the incorporation of 1.4 mμmoles of nucleotide from ATP was unexpectedly observed (Table V, Line 3); in this experiment there was no significant incorporation of nucleotide from UTP, CTP, or GTP.

DISCUSSION

The present work has demonstrated that short chain deoxypolynucleotides serve as templates for the synthesis of ribopolynucleotides in the presence of RNA polymerase. The effectiveness of the homologous members in the reaction increases with an increase in chain length, the maximal rate in the case of the polythymidylate series being reached with dT_{14}. It is noteworthy that the maximal rate here obtained was higher than the rate normally obtained when all the four ribonucleoside triphosphates and DNA are used. The saturating concentrations of the thymidine polynucleotides decreased with an increase in chain length and the enzyme showed high affinity even for the short chain length dT_7.

A major point of interest established by the present work has been that the ribopolynucleotide synthesis does not begin by adding nucleotides to the terminal 3′-hydroxyl group of the deoxyribonucleotide. This conclusion is based primarily on two findings: alkaline hydrolysis of the ribopolyadenylate product leaves no detectable adenylate in the deoxypolythymidylate, and dT_{11} carrying a 3′-*O*-acetyl group is still effective in the synthesis of ribopolyadenylate. Throughout this paper we have referred to the deoxypolynucleotides as "templates" for RNA polymerase rather than "primers" because the synthesis of the product involves the formation of new complementary chains instead of elongation of chains. Since addition to the end of the deoxypolynucleotide is not necessary, it may be that replication can begin anywhere along the template; this has significance at the biological level, for it allows the synthesis of messenger RNA from any one of the genes in a DNA molecule without requiring synthesis from all.

Although in the present work the Watson-Crick type of base-pairing was ordinarily observed, the slight incorporation of deoxyguanylate in the presence of thymidine polynucleotides was noted, an observation which has also been made previously (7). A further noteworthy exception, which merits further study, was the formation of ribopolyadenylate when deoxyribopolyadenylates were used as templates. The reaction resembles the previously documented ribopolyadenylate-primed synthesis of ribopolyadenylate (29), and its biological significance remains unknown. Under the conditions tested no polyuridylate synthesis occurred when deoxypolyadenylate was used as template. The failure is perhaps due to the lack of appropriate conditions, since the polyriboadenylate-dependent polyuridylate synthesis has already been found to be very sensitive to temperature and to the presence of a critical concentration of manganous ions (30).

RNA polymerase brings about the synthesis of ribopolyadenylate when DNA is present and ATP is the only substrate (10, 24). The reaction proceeds best when denatured DNA is used (24).[3] The size of the ribopolyadenylate formed has been estimated to be in the range of 60 to 70 (10) and 400 (24). To explain that the expected short runs of polythymidylates in DNA could bring about the synthesis of much larger sized ribopolyadenylate, Chamberlin and Berg (10) postulated a "slippage" mechanism whereby a run of AMP residues would slip along the sequence of thymidylate residues, leading to an elongation of the polyadenylate chain. The present work indicates that it would take a run of only five to seven thymidylates in DNA in order to synthesize ribopolyadenylate of a much larger size.

All evidence points to the involvement of a single enzyme for the DNA-dependent synthesis of RNA and of ribopolyadenylate

(24).[3] Since the synthesis of ribopolynucleotides observed here is so similar to the DNA-dependent polyriboadenylate synthesis, and since the same purified enzyme (10) was used here, it seems most probable that the syntheses studied in this work are due actually to RNA polymerase rather than some contaminating enzyme.

The assay used in the present work involved separation of the product from the unreacted labeled nucleoside triphosphate by anion exchange chromatography on a DEAE-cellulose paper. Oligonucleotides as short as the tetranucleotide could be separated from the nucleoside triphosphates under the conditions used. This assay was developed to detect low molecular weight polynucleotides, which could be missed by the usual assay (10) that depends on acid insolubility of polynucleotides. The assay may be generally applicable in studies on polynucleotides, for example in determining the initial events in DNA and RNA synthesis. A related method based on the use of DEAE-cellulose paper has been previously reported by Bollum (31).

The ribopolynucleotides obtained by the use of chemically synthesized deoxypolynucleotides and RNA polymerase may be expected to bring about the specific incorporation of amino acid into protein when added to an amino acid-incorporating system (32, 33). For example, the ribopolyadenylate synthesized here would be expected to lead to incorporation of lysine (33).

SUMMARY

Short chain thymidine polynucleotides serve as templates for the synthesis of ribopolyadenylate in the presence of RNA polymerase. The effectiveness as template increases markedly with size. Thus, thymidine pentanucleotide shows detectable activity whereas the maximal activity is reached with tetradecanucleotide, the latter being more active for ribopolyadenylate synthesis than thymus DNA is for ribopolyadenylate or RNA synthesis. The product formed from the different sized templates has in each case an average chain length of 50 to 100. The synthesis of ribopolyadenylate has been shown not to involve addition to the 3′-hydroxyl ends of polythymidylate.

Deoxypolycytidylate larger than the octanucleotide brings about the synthesis of ribopolyguanylate. Deoxypolyadenylate has failed so far to give synthesis of polyuridylate. With deoxypolyadenylate the synthesis of ribopolyadenylate is noted.

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