The Polymerization of Guanosine Diphosphate by Polynucleotide Phosphorylase

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Polynucleotide phosphorylase catalyzes the reversible polymerization of nucleoside diphosphates (1). With enzyme preparations from *Escherichia coli* (2), *Azotobacter agile* (3), and *Micrococcus lysodeikticus* (4, 5) a reaction has been observed with single additions of adenosine, uridine, cytosine, or inosine diphosphate to form the corresponding homopolymer. However, the polymerization of guanosine diphosphate represents a special case. Littauer and Kornberg (2) observed no polymerization reaction upon incubating GDP with fractions from *E. coli*. In experiments with polynucleotide phosphorylase from *A. agile*, Grunberg-Manago et al. (3) noted a very slow release of P_i from GDP, but this reaction stopped far short of the equilibrium point found for ADP and UDP. In contrast, when mixed with other nucleoside diphosphates, GDP is well utilized and polymers such as poly AGUC[1] are formed (3).

The experiments described below show that GDP, when present alone, is not polymerized even after many hours by enzyme fractions from *A. agile* or *E. coli*. However, polymerization of GDP does take place in the presence of oligonucleotides such as ApApA and ApApU, and guanosine monophosphate units are added to the primer. No reaction occurs with GDP and oligonucleotides, such as ApApUp, that do not contain an unsubstituted hydroxyl group at carbon 3' of the terminal nucleoside residue. Highly purified fractions of *A. agile* polynucleotide phosphorylase, kindly supplied by Drs. Mii and Ochoa (6), catalyze the polymerization of ADP, UDP, CDP, and IDP only after a lag period. This lag can be overcome by ribonucleic acid and certain other polymers (6), as well as by oligoribonucleotides of various types, including pApApA, ApApU and ApApUp (see preceding paper (7)). Accordingly, GDP differs from other nucleoside diphosphates in that its polymerization requires the presence of oligonucleotides of the type that can be incorporated into the polymer.

MATERIALS AND METHODS

Most of the experimental procedures were the same as those the preceding paper (7) and only brief reference will be made them here.

[1] The abbreviations used are: EDTA, ethylenediaminetetraacetate. The biosynthetic polymers synthesized by the action polynucleotide phosphorylase are: poly A, polyadenylic acid; poly U, polyuridylic acid; poly C, polycytidylic acid; poly G, polyguanylic acid; poly AU, copolymer of adenylic and uridylic acids; poly AGUC, copolymer of adenylic, uridylic, guanylic, and cytidylic acids. R_{GDP} is the ratio of the R_F of a given compound that of GDP.

Materials—GDP was obtained from the Sigma Chemical Company and samples were usually stored at 3° for several months before use. The preparations were found to contain up to 15% of 5'-GMP and up to 4% of GTP by quantitative paper chromatography in Solvent 3 (see below) and the values stated for concentrations of GDP have been corrected. A sample of a chemically synthesized adenylic acid polymer (8) was kindly provided by Dr. A. M. Michelson, Arthur Guinness Son and Company, Ltd., Chemist's Laboratory, Dublin; it consists of short polynucleotide chains containing mixed 3'-5' and 2'-5' phosphodiester bridges and terminated by a cyclic 2',3'-phosphoryl group. A dinucleoside monophosphate with a 2'-5' phosphodiester bridge, namely adenylyl-(2'-5')-uridine (9) was also a gift from Dr. Michelson.

The preparation of polynucleotide phosphorylase from *E. coli* was a fraction carried through the first ethanol step in the procedure of Littauer and Kornberg (2); its specific activity in the "exchange" assay (3) was 15. Three highly purified preparations from *A. agile* (6) were given to us through the kindness of Drs. Mii and Ochoa; all of them were found to display a lag period in the polymerization of ADP and UDP which could be overcome by addition of a suitable primer. The fractions are designated by their specific activity in the exchange assay (3) as S.A. 70, S.A. 60, and S.A. 350. Two fractions (S.A. 40 and S.A. 160) that did not show well defined lag periods were also obtained from Drs. Mii and Ochoa. Snake venom phosphodiesterase was prepared by a modification of the procedure of Koerner and Sinsheimer (10). Phosphomonoesterase was fractionated from human seminal plasma (11).

Methods—The solvent systems used for descending chromatography were: Solvent 1, isopropanol-water (70:30, volume for volume) with NH_3 in the vapor phase (12); Solvent 2, saturated ammonium sulfate-isopropanol-1 M sodium acetate (80:2:18, volume for volume for volume) (13); Solvent 3, isobutyric acid-1 M NH_4OH-0.2 M EDTA[1] (100:60:0.8, volume for volume for volume) (14); Solvent 4, isopropanol, 170 ml, concentrated HCl, 44 ml, water up to 250 ml (15); Solvent 5, n-propanol-concentrated NH_4OH-water (60:30:10, volume for volume for volume) (16). Whatman No. 3MM paper was used with Solvents 1, 3, and 5, and Whatman No. 1 paper with Solvents 2 and 4.

Inorganic phosphate was determined by the method of Fiske and SubbaRow (17), with the use of the Klett colorimeter with a No. 66 filter. The higher concentrations of oligonucleotide used in this study caused an interfering turbidity upon addition of ammonium molybdate. This difficulty was overcome and satisfactory assay of P_i was obtained by the following procedure. An aliquot of the incubation mixture containing from 0.015 to 0.060
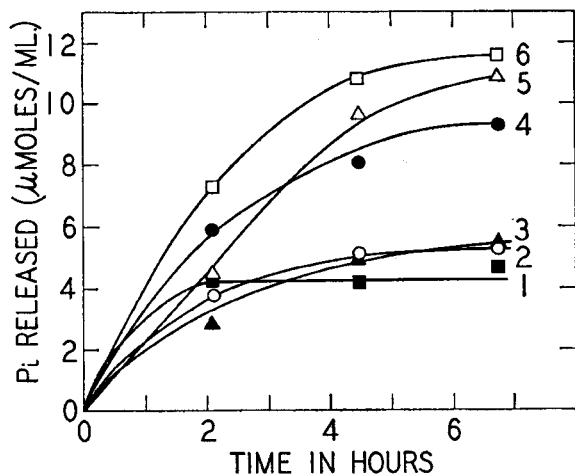
FIG. 1. Polymerization of GDP as measured by formation of P$_i$; effect of primers. The incubation mixture (0.05 ml) contained 328 µg per ml of enzyme for *Curve 3* and 164 µg per ml for all of the other curves. The concentration of GDP was 6.8 mM for *Curves 1, 2, 3,* and 13.7 mM for *Curves 4, 5,* and *6*. Primer additions: *Curve 1*, 4.6 mM pApApA; *Curve 2*, 3.8 mM pApA; *Curve 3*, 1.3 mM pApA; *Curve 4*, 1.8 mM pApApA; *Curve 5*, 3.8 mM pApA; *Curve 6*, 7.1 mM pApA. Aliquots of 0.01 ml were removed for P$_i$ analysis. In control experiments, without primer, the concentration of P$_i$ was <0.1 mM after as long as 20 hours.
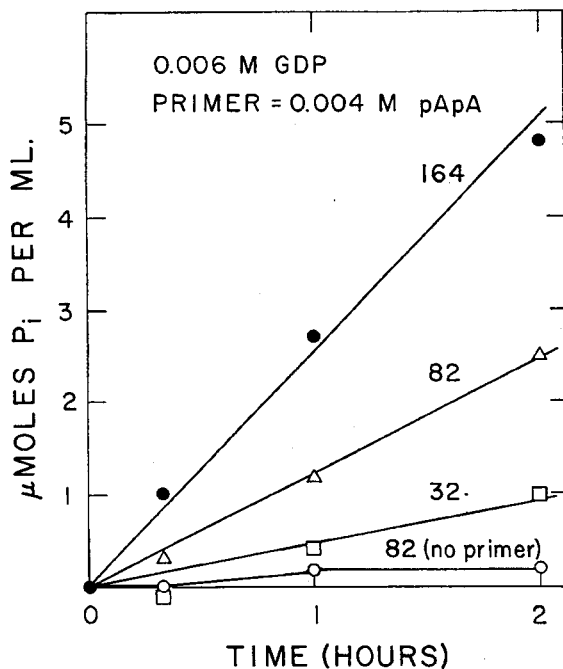

FIG. 2. Polymerization of GDP with different amounts of enzyme. The incubation mixtures contained the amounts of enzyme shown in the figure (µg per ml), and the following in mM: GDP, 6; pApA, 4.0.

µmole of P$_i$ is made up to 0.5 ml with cold perchloric acid whose strength is such that the final concentration is 2.5%. After 5 minutes at 0° the mixture is centrifuged and 0.3 ml of the supernatant fluid is mixed with 0.8 ml of 1 N H$_2$SO$_4$, 0.26 ml of water, 0.16 ml of 2.5% ammonium molybdate, and 0.08 ml of reducing reagent. The mixture is centrifuged for 5 minutes at 1,500 × *g* in the International No. 1 centrifuge. The clear, blue, super-

natant fluid is decanted carefully into a Klett tube and read in the instrument, together with appropriate phosphate standards and blanks, 10 minutes after addition of the reducing agent.

EXPERIMENTAL AND RESULTS

Effect of pApA and its Homologues on Polymerization of GDP—
There is no polymerization reaction when GDP alone is incubated with polynucleotide phosphorylase from *A. agile*. Thus, no release of P$_i$ (<0.02 µmole) from GDP occurs in 4 hours, with amounts of enzyme which would form 10 to 20 µmoles of P$_i$ from ADP per hour.[2] Over 30 experiments were carried out with 6 different lots of enzyme, with 0.005 to 0.05 M GDP and incubation times that varied from 4 to 24 hours. In addition, study of the reaction mixture by paper chromatography in Solvents 1, 2, and 3 affords no evidence for the formation of polynucleotide material. However, in the presence of pApA, pApApA, or pApApApA one observes formation of P$_i$ at a rate that is nearly linear with time until equilibrium is approached. The equilibrium point is not well defined by the data but it appears to correspond to the conversion of from 70 to 80% of GDP to P$_i$ and polynucleotide (Fig. 1).

It can be seen from Fig. 2 that the initial rate of formation of P$_i$ is proportional to the concentration of enzyme, in the presence of 6 mM GDP and 4 mM pApA. A similar rate was obtained at 13.7 and 30 mM GDP, suggesting that the enzyme is saturated with GDP at a concentration of less than 6 mM. Because of insufficient material it was not possible to determine the concentrations of the various primers required to saturate the enzyme. Thus the rate of P$_i$ formation is 0.9 and 2.0 µmoles per hour per ml of reaction mixture in the presence of 4.3 and 7.0 mM pApA, respectively (GDP, 13.7 mM; enzyme, S.A. 70, 82 µg per ml). The trinucleotide is effective at lower concentrations; under the same conditions mentioned above 2.8 mM pApApA gives a rate of 3 µmoles of P$_i$ per hour per ml. It should be noted that concentrations of pApA and pApApA that do not saturate the GDP system are more than enough to give maximal stimulation of the rate of polymerization of ADP and UDP (7). The tetranucleotide, pApApApA, was tested at concentrations of 2.1 mM, 0.74 mM, and 0.44 mM; these levels were found to be approximately equivalent to 2.9 mM, 0.97 mM, and 0.58 mM pApApA, respectively. Thus, the tetranucleotide is effective at somewhat lower concentrations than required for the trinucleotide.

There is no detectable formation of P$_i$ when any of the enzyme fractions are incubated with pApA, pApApA, or pApApApA in the absence of GDP. Also, there is no reaction in the absence of enzyme.

Similar results were obtained with the *E. coli* polynucleotide phosphorylase. Again, in the absence of pApA or pApApA, no material with an R_F of zero was detected on paper chromatograms nor were any oligonucleotides containing guanosine formed. The curve of P$_i$ formation plotted against time was also quite flat except for a small initial burst. A minor contaminant of GDP preparations appears to be rapidly dephosphorylated by the *E. coli* enzyme but not by *A. agile* fractions. It might also be mentioned that a primer requirement for GDP polymerization could be demonstrated with cruder enzyme prep-

[2] In the case of enzyme fractions that showed a lag phase in the polymerization of ADP this comparison is based on the rate achieved after the lag phase, or on the initial rate in the presence of saturating amounts of poly A.

arations from *A. agile*, preparations that showed no primer requirement for the polymerization of ADP and UDP.

Effect of Other Materials That Were Tested as Primers—Table I gives a list of nucleotides that did not induce polymerization of GDP. In addition, no effect was noted with an unknown material that occurs in filter paper and can be eluted with water, although in experiments with ADP or UDP a small but significant stimulation was obtained in this way (7).

Three oligonucleotides containing no phosphomonoester end group were tested with 13.7 mM GDP and 82 μg of S.A. 70 enzyme per ml. The results were as follows: (a) ApA, at 3.6 mM, resulted in a stimulation of P_i release equivalent to that obtained with 4.2 mM pApA, (b) ApApU (2 mM) had exactly the same effect as pApApA (2 mM). ApApA was less effective, but the significance of this is doubtful, since only a single preparation was tested. Several lots of the other oligonucleotides were used.

Two thymidine oligonucleotides, namely, pTpT and pTpTpT, were inactive as primers.

Products Formed from Polymerization of GDP in Presence of pApApA—When GDP is incubated with polynucleotide phosphorylase and pApApA for a brief interval the major reaction product is pApApApG. Oligonucleotides corresponding to the addition of 2 and 3 guanosine monophosphate residues also appear, but in smaller amounts. More highly polymerized material, with an R_F of zero in various solvent systems, cannot be detected until later in the course of incubation. These observations are illustrated in the ultraviolet photograph of a chromatogram showing components of a typical reaction mixture at different time intervals (Fig. 3).

Evidence for the structure of pApApApG includes the following: The material moves as a single band in Solvents 1 and 3, and its R_F is consistent with the structure assigned. Hydrolysis

TABLE I

Compounds that failed to stimulate polymerization of guanosine diphosphate

The incubation mixtures contained the following, in μmoles per ml: Tris buffer, pH 8.2, 150; $MgCl_2$, 10; EDTA, 0.4; GDP 10 or 14; and other additions as indicated below. Incubation temperature, 37°. Formation of P_i was determined at three time intervals over a period of 4 hours, and the values were corrected for P_i present at zero time. Purified *A. agile* polynucleotide phosphorylase (S.A. 70) was used.

Except where specifically indicated, the same incubation conditions and the same enzyme preparation were used in the other experiments reported.

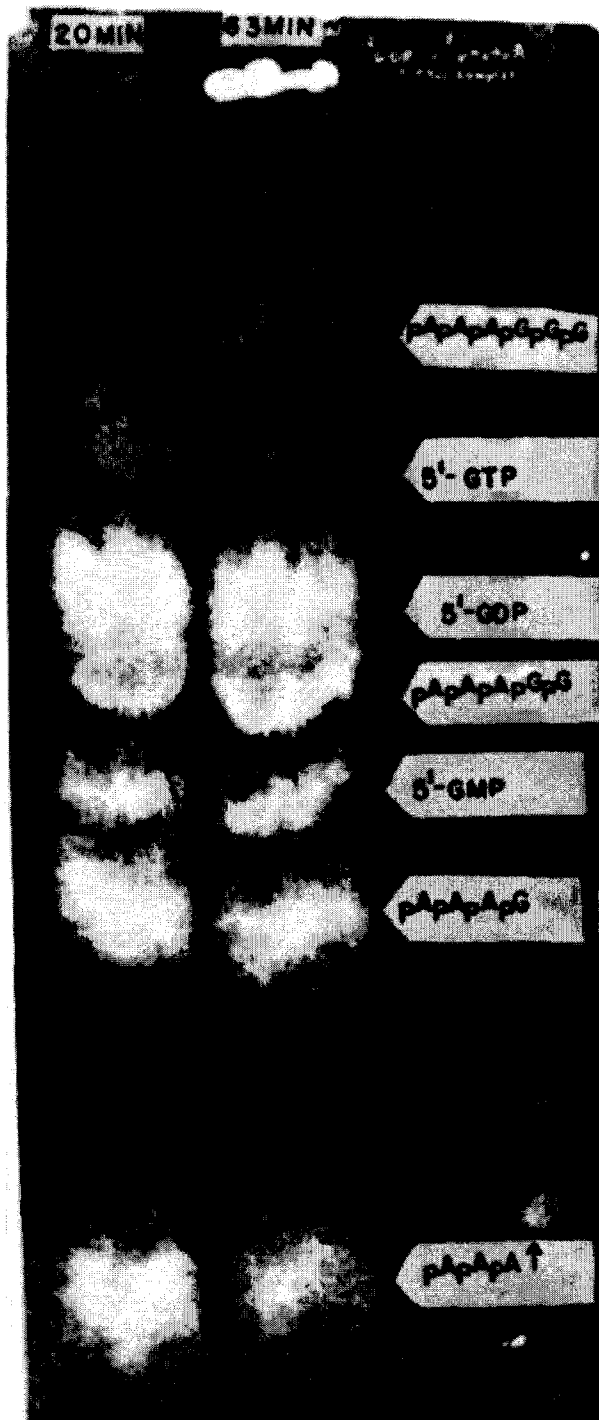| Compound	Concentration
	mM
ApUp	5.8
ApApUp	0.8
Adenosine	5.0
AMP	6.6
Adenosine 5'-benzyl phosphate	5.0
Adenylyl-(2'-5')-uridine	6.3
	mg/ml
Poly A	1.2
Poly AGUC	1.0
Poly C	3.0
Poly U	3.0
Polyadenylic acid, chemically prepared (8)	1.2

FIG. 3. Ultraviolet photograph of a chromatogram run in Solvent 3. This is from an experiment similar to that in Fig. 4. At zero time (not shown) the only visible densities corresponded to pApApA, GMP, GDP and GTP. This photograph shows partial disappearance of pApApA as the incubation proceeds, as well as the formation of pApApApG, pApApApGpG, pApApApGpGpG, and polymer (visible at 63 minutes, near top of photograph).

in 1 N HCl (18) followed by quantitative chromatography in Solvent 4 yielded adenine and guanine in a ratio of 3.2:1.0 (theory, 3.0:1.0). Hydrolysis in 0.3 N KOH (19), followed by chroma-
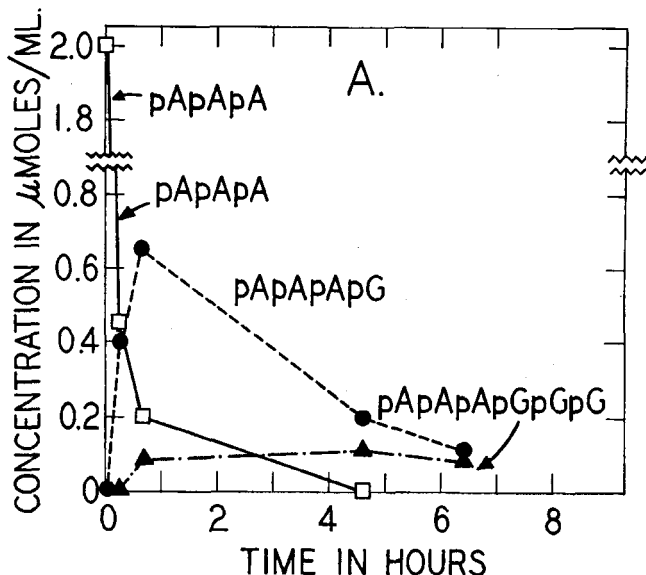
FIG. 4. Polymerization of GDP in the presence of pApApA. The incubation mixture (0.2 ml) contained 82 μg per ml of enzyme, and the following, in μmoles per ml: GDP, 13.7 and pApApA, 2. Aliquots of 0.03 ml were chromatographed in Solvent 3 and the various bands were quantitatively transferred and run in Solvent 1. Quantitative elution was again carried out and the concentration of the various nucleotides was determined.[3] No polymer could be detected after 15 minutes but it was found in samples removed after 40 minutes of incubation. Thereafter the concentration of polymer increased progressively; it reached a value equivalent to 8 μmoles of base per ml in 6.5 hours, when it accounted for almost all of the GDP which disappeared.

tography in Solvent 1 gave adenosine 3′,5′-diphosphate (mixed with 2′,5′ isomer), 3′-AMP (mixed with 2′-AMP), and guanosine, the expected products. No guanylic acid was formed. The results of partial hydrolysis by purified snake venom phosphodiesterase were also informative. This fraction has been shown to cleave both oligodeoxyribonucleotides (20) and polyribonucleotides (21) in stepwise fashion, beginning at that end of the molecule bearing an unsubstituted hydroxyl group at carbon 3′. From pApApApG one would expect 5′-GMP and pApApA to be the major early products, followed later by pApA and 5′-AMP. When enzymic hydrolysis of pApApApG was carried to the extent of 40% the following were found by quantitative elution from paper chromatograms: pApApA, 0.023 μmole; 5′-GMP, 0.020 μmole; 5′-AMP, 0.010 μmole; pApA, 0.002 μmole.

Evidence for the structure of pApApApGpG is the following: It moves as a single band in Solvents 1 and 3, with a lower R_F than the tetranucleotide just discussed. Acid hydrolysis yielded adenine and guanine in a ratio of 1.7:1.0 (theory, 1.5:1.0). Alkaline hydrolysis gave adenosine 3′,5′-diphosphate (and the 2′,5′ isomer), 3′-AMP (and 2′-AMP), 3′-GMP (and 2′-GMP), and guanosine.

The hexanucleotide, pApApApGpGpG, was obtained in small amounts. It was found to contain adenylic and guanylic acid residues but there was insufficient material for accurate quanti-

[3] As in the preceding paper (7), the assumption is made that the molar extinction coefficient of an oligonucleotide is approximated by the sum of the extinction coefficients of its constituent nucleotides. Values used for the molar extinction coefficients, at 257 mμ and pH 2, for AMP and GMP are 15,100 and 12,200, respectively. The approximation does not account for any hypochromic effect.

tative analysis. The structure is therefore only tentatively assigned.

The following values for R_{GDP} were obtained for pApApApG, pApApApGpG and pApApApGpGpG: in Solvent 1, 0.52, 0.26, and 0.10, respectively; in Solvent 3, 1.60, 1.17, and 0.49, respectively. The ratio of absorbancy (pH 2) at 280 mμ over that at 260 mμ was found to be 0.33 for pApApApG, 0.42 for pApApApGpG, and 0.58 for pApApApGpGpG.

Structure of "poly G"—This term is restricted to material that is precipitated by 2 volumes of ethanol, that is insoluble in cold 2.5% HClO₄, and that remains at the origin upon chromatography in Solvents 1, 2, or 3. Alkaline hydrolysis of such material yields mostly 3′-GMP and 2′-GMP, identified by their R_F values in Solvents 1 and 2, and by the fact that they were readily hydrolyzed by phosphomonoesterase purified from human seminal plasma but not by 5′-nucleotidase. Smaller amounts of adenine-containing nucleotides derived from the incorporated primer are also obtained.

The following experiment illustrates the preparation of "poly G," of short average chain length, formed by addition of guanosine monophosphate units to pApApA. The incubation mixture contained 50 μg of *E. coli* polynucleotide phosphorylase, 125 μmoles of Tris buffer, pH 8.2, 10 μmoles of MgCl₂, 0.4 μmole of EDTA, 1.31 μmoles of pApApA, and 27.4 μmoles of GDP, in a total volume of 1.0 ml. The mixture was incubated at 37° for 25.5 hours, with toluene added after 6 hours. The formation of P_i amounted to 8 μmoles. A chromatogram run in Solvent 3 showed complete incorporation of pApApA and all of the reaction products had an R_F of zero. Two volumes of cold ethanol were added and after 3 hours at 2° the precipitate was collected by centrifugation and dissolved in distilled water. The solution was dialyzed against 1 liter of cold 0.001 M EDTA for 24 hours and then against cold, running distilled water for 48 hours. The yield of polymer, based on measurement of optical density, was 56% of what could be expected from the amount of P_i that had been formed. Paper chromatographic examination of the product after dialysis showed complete removal of GDP. Hydrolysis of a sample of the dialysis residue in 1 N HCl (18) followed by chromatography in Solvent 4 gave a guanine-adenine ratio of 2.8:1, corresponding to an average chain length of 11.4. This indicates the addition of 8.4 guanylic acid residues, on the average, to every molecule of pApApA. The material was acid insoluble, and was nondialyzable.

In later experiments, with the use of much larger amounts of enzyme, poly G that had an average chain length of 30 was synthesized. Further, with the use of pUpUpU as a primer and treating the resultant polymer with pancreatic ribonuclease, it was possible to remove uridylic acid from the preparation and so obtain poly G free of bases other than guanine. In a typical experiment the incubation mixture (0.5 ml) contained 27.4 μmoles of GDP, 75 μmoles of Tris buffer, pH 8.2, 5 μmoles of MgCl₂, 0.2 μmole of EDTA, 0.9 μmole of pUpUpU, and 320 μg of a gel eluate fraction from *A. agile* (S.A. 160). The mixture was kept at 37° for 6.5 hours. The polymer was precipitated by the addition of 1.0 ml of cold 5% HClO₄ and collected by centrifugation. It was washed with 2 ml of 3% HClO₄ and then with two portions (2 ml each) of 0.01 N HCl. The precipitate was suspended in 1 ml of water and dissolved by addition of sufficient 1 N NH₄OH to bring the pH to 7.0. An opalescent, distinctly viscous solution was obtained. An aliquot was hydrolyzed with 0.3 N KOH (19) and the products were separated in Solvent 5 and
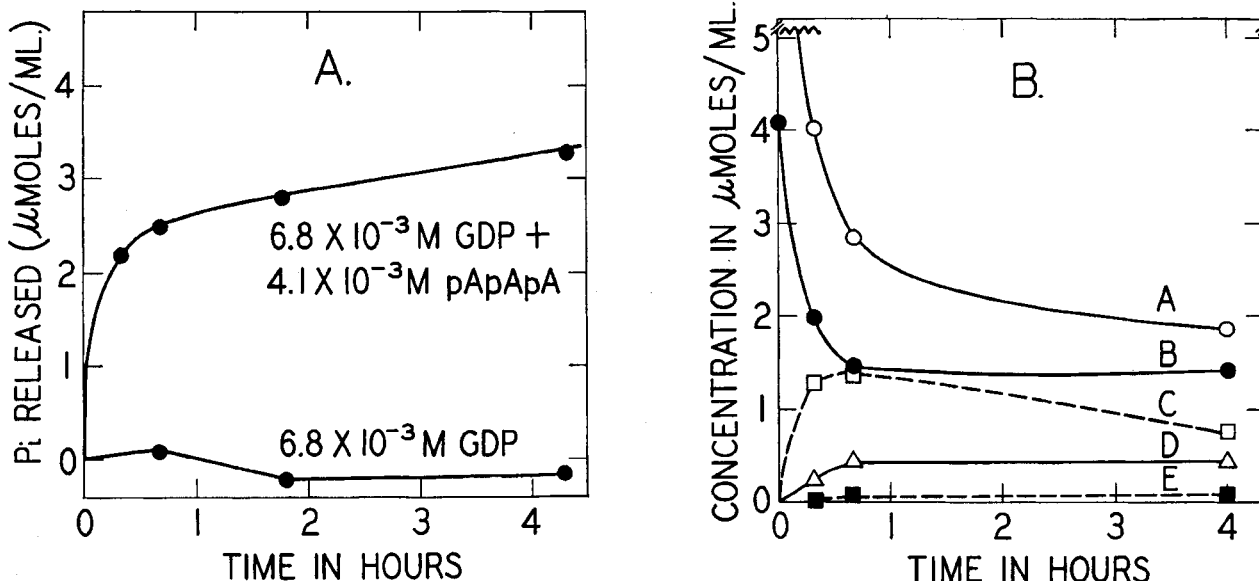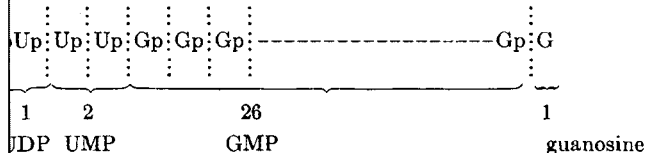
FIG. 5. Polymerization of GDP in the presence of pApApA. The incubation mixture (0.2 ml) contained 82 μg per ml of enzyme and the following, in μmoles per ml: GDP, 6.8; pApApA, 4.1. Samples of 0.03 ml were removed at intervals for chromatography on Solvent 3. In A, formation of P_i plotted against time. In B, changes in the concentration of GDP and various oligonucleotides

quantitatively eluted. The products were: uridine 3′,5′- (and 3′,5′)-diphosphate, 0.05 μmole; 3′ (and 2′)-GMP, 1.28 μmoles; 3′ (and 2′)-UMP, 0.105 μmole; guanosine, 0.045 μmole. From the total amount of compounds containing guanosine isolated in this experiment the yield of poly G was calculated to be 50% of that expected from P_i release. The results are close to what one would expect from a polymer with an average chain length of 30. This can be represented diagrammatically, with vertical dotted lines showing the points of alkaline cleavage:

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Up:Up:Up:Gp:Gp:Gp: ------------------Gp:G
                                         
 1    2          26                     1
UDP  UMP         GMP                  guanosine
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Since no guanosine 3′,5′-diphosphate was found, and guanosine was equal to uridine diphosphate, it is evident that all of the polymer chains were built onto pUpUpU as a primer.

The remainder of the preparation of poly G was shaken with volume of CHCl₃ and $\frac{1}{10}$ volume of isoamyl alcohol (22), and centrifuged. The aqueous layer was removed and re-extracted in order to complete the removal of enzyme protein. The organic solvents were removed by extraction with diethyl ether, after which the aqueous solution was aerated at 40°. The polymer solution was then treated with 500 μg of pancreatic ribonuclease for 4 hours, at 37°, in order to cleave off uridylic acid units. The treatments with organic solvents and precipitation with perchloric acid were repeated. Alkaline hydrolysis of the resultant polymer gave, as expected, only 3′ (and 2′)-GMP and guanosine. From the ratio of GMP to guanosine the average chain length was found to be 24. The removal of 3 uridylic acid residues from the original polymer, of chain length 30, would be expected to result in an average chain length of 27. The discrepancy is probably due to experimental error.

plotted against time (Curve A, GDP; Curve B, pApApA; Curve C, pApApApG; Curve D, pApApApGpG; Curve E, pApApApGpGpG). Two other products were found, but only in the sample removed at 240 minutes, namely pApA (0.35 mM) and polymer (equivalent to 2.0 mM mononucleotide).

Time Course of Polymerization Reactions with Different Concentrations of GDP and Primer—Fig. 4 shows the results of an experiment in which GDP and pApApA were incubated with the A. agile enzyme. At various time intervals, aliquots of the incubation mixture were removed and its components were determined by quantitative chromatography. It is evident from Fig. 4 that most of the primer is utilized within 15 minutes and all of it disappears within several hours. The concentration of pApApApG rises to nearly 0.7 mM in 40 minutes and after 6 hours falls to 0.1 mM. The pentanucleotide, pApApApGpG, accumulates only in trace amounts (not shown), whereas the hexanucleotide reaches a level of 0.11 mM in 4.5 hours. No polymer is noted after 15 minutes but it can be found in an aliquot removed after 40 minutes and thereafter its concentration rises (see legend to Fig. 4). An experiment with 13.7 mM GDP and 1 mM pApApA gave similar results.

An experiment was carried out under identical conditions except that the concentration of pApApA was increased to 4 mM. This led to a more rapid accumulation of pApApApG, so that its concentration was 1.6 mM after 20 minutes. Also there was a less rapid decline with time, compared with the experiment mentioned above. A substantial amount of pApApApGpG was formed, as well as smaller amounts of pApApApGpGpG. No polymer was evident in a sample removed after 20 minutes but it was found after 1 hour, and thereafter increased with time of incubation. In this experiment pApApA was not completely utilized; in fact, its concentration showed a small rise between 1 and 6 hours. Possible explanations are considered below.

The results of an experiment in which the concentration of GDP (6.8 mM) was brought close to that of pApApA (4.1 mM) are presented in Figs. 5A and B. The changes to be observed between 40 and 240 minutes illustrate several points. First, there is a substantial decrease in the concentration of pApApApG. Also, there is evidence of a continued forward reaction,

namely: (*a*) decrease in GDP (−0.9 mM), (*b*) increase in P_i (+0.6 mM), and (*c*) appearance of polymer (equivalent to 2.0 mM GMP). There is also evidence for a net reaction in the reverse direction, occurring simultaneously. Thus, no pApA is present after 20 and 40 minutes, but 0.35 mM[4] is noted after 240 minutes. This simultaneous phosphorolysis of pApApA and continued polymerization may explain why the decrease in GDP is greater than the net increase in P_i.

In similar experiments, except that more enzyme was present, it was found that the composition of the reaction mixture changed to a considerable extent *after* the formation of P_i and the utilization of GDP came to a halt. Disappearance of pApApApG and pApApApGpG was noted, together with an increase in the concentration of polymer and of smaller oligonucleotides. These observations led us to incubate pApApA and pApApApA with polynucleotide phosphorylase in the absence of added nucleoside diphosphate or P_i. A transnucleotidation reaction was found to occur, with the simultaneous formation of smaller and larger polynucleotides. This reaction does not appear to involve the participation of ADP or P_i, at least not in the free state. A detailed study will be reported in a future publication.

The incorporation of the dinucleotide, pApA, into poly G was less extensively investigated, but here too it was found that oligonucleotides containing guanine accumulated early in the course of the reaction. At later time intervals these were observed to decrease in concentration and material with an R_F of zero was the major product.

DISCUSSION

The present results help to explain why no polymerization reaction has been observed with GDP and polynucleotide phosphorylase in the past, even though guanosine monophosphate units have been incorporated into polynucleotide chains when a mixture of nucleoside diphosphates was used. The data indicate that a polynucleotide cannot be synthesized *de novo* from GDP; it is only possible for the enzyme to catalyze the addition of guanosine monophosphate units to a preformed oligonucleotide primer. These results were obtained with every sample of polynucleotide phosphorylase that was tested. Some of these enzyme fractions showed no primer requirement at all with the other nucleoside diphosphates. Thus, it should be emphasized that these results differ from those reported in the preceding paper (7), where a lag period in the polymerization of ADP or IDP is overcome by compounds such as pApApA or ApApUp. With GDP the requirement for an oligonucleotide of suitable structure appears to be absolute; without it no reaction can be detected even after many hours.

The primer must have an unsubstituted hydroxyl group at carbon 3′ of the terminal nucleoside residue in order for a 3′-5′ phosphodiester bridge to be established. Thus, ApUp and ApApUp are inactive. Compounds that contained adenosine or uridine as the terminal nucleoside residue were used in the present study.

A suitable primer for the polymerization of GDP must have at least one 3′,5′ phosphodiester bond. It is of considerable interest that ApA is an effective primer, whereas adenylyl-(2′-5′)-uridine (which contains a 2′-5′ phosphodiester bond) is not.

[4] It was not possible to obtain an accurate measure of the ADP formed by phosphorolysis along with pApA because it appeared as a very diffuse density on the chromatogram.

No reaction could be demonstrated with poly A, poly U, poly C, or poly AGUC. Here there may have been inhibitory interactions, similar to the suppression of poly A synthesis by poly U (6). It is also possible that the additions of polymer that were employed provided too low a concentration of terminal nucleoside residues with free hydroxyl groups at C-3′. An effort was made to detect some reaction in an incubation mixture containing 10 mg per ml of poly A. Relatively large aliquots were removed for estimation of P_i, but the results were inconclusive. It is probable that a primer must be a ribose derivative since pTpT and pTpTpT were inactive.

In the presence of 4 mM pApApA the rate of P_i formation from GDP with purified *A. agile* fractions was one-fourth of that obtained with ADP. It is likely that even faster rates could have been observed with higher concentrations of primer. In other studies[5] it was shown that under suitable conditions the rate of the GDP-P_i exchange reaction is nearly equivalent to that observed with other nucleoside diphosphates. It therefore appears that GDP is an effective substrate for the enzyme. These observations are consistent with the findings of Mii and Ochoa (6), with highly purified fractions from *A. agile*, that indicate that a single enzyme is active with all of the ribonucleoside diphosphates.

At present one may only speculate as to why there is an absolute requirement for a primer in the polymerization of GDP by polynucleotide phosphorylase whereas the same preparations of enzyme, when tested with other nucleoside diphosphates, may show only a lag period, after which a rapid reaction occurs in the absence of added primer.

SUMMARY

No polymerization of guanosine diphosphate occurs when polynucleotide phosphorylase is incubated with this compound alone. However, a polymerization reaction does take place in the presence of an oligoribonucleotide containing an unsubstituted hydroxyl group at carbon 3′ of the terminal nucleoside residue. Such oligoribonucleotides include ApA, ApU, ApApU, pApA, and pApApA.

The oligonucleotide serves as a primer and successive guanosine monophosphate units are added to it, beginning with esterification of the hydroxyl group at carbon 3′. Thus, with a trinucleotide containing adenosine serving as primer, polynucleotides were recovered whose structures corresponded to the addition of 1, 2, and 3 guanosine monophosphate residues. A polymer that is precipitated by acid and by 2 volumes of ethanol is also formed. It is nondialyzable and its composition indicates that, on the average, up to 27 guanosine monophosphate residues have been added to each molecule of primer.

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