1, 351.
Marrack, J. R., and Richards, C. B. (1971), *Immunology 20*, 1019.
Metzger, H. (1970), *Annu. Rev. Biochem. 39*, 889.
Nezlin, R. S., Zagyansky, Y. A., and Tumerman, L. A. (1970), *J. Mol. Biol. 50*, 569.
Ohta, Y., Gill, T. J. III, and Leung, C. S. (1970), *Biochemistry 9*, 2708.
Pecht, I., Givol, D., and Sela, M. (1972), *J. Mol. Biol. 68*, 251.
Pilz, I. (1970), *Allg. Prakt. Chem. 21*, 21.
Pilz, I. (1973), *in* Physical Principles and Techniques of Protein Chemistry, Leach, S. J., Ed., New York, N. Y., Academic Press, in press.
Pilz, I., Kratky, O., and Karush, F. (1973), manuscript in preparation.
Pilz, I., Puchwein, G., Kratky, O., Herbst, M., Haager, O., Gall, W. E., and Edelman, G. M. (1970), *Biochemistry 9*, 211.
Porod, G. (1951), *Kolloid Z. 124*, 83.
Robbins, J. B., Haimovich, J., and Sela, M. (1967), *Immunochemistry 4*, 11.
Rotman, M. B., and Celada, F. (1968), *Proc. Nat. Acad. Sci. U. S. 60*, 660.
Sarma, R., Silverton, E. W., Davies, D. R., and Terry, W. D. (1971), *J. Biol. Chem. 246*, 3753.
Schechter, B., Conway-Jacobs, A., and Sela, M. (1971), *Eur. J. Biochem. 20*, 321.
Schechter, B., Schechter, I., and Sela, M. (1970), *J. Biol. Chem. 245*, 1438.
Schechter, J. (1971), *Ann. N. Y. Acad. Sci. 190*, 394.
Schechter, J., and Berger, A. (1966), *Biochemistry 5*, 3362.
Schechter, J., Schechter, B., and Sela, M. (1966), *Biochim. Biophys. Acta 127*, 438.
Steiner, L. A., and Lowey, S. (1966), *J. Biol. Chem. 241*, 231.
Tumerman, L. A., Nezlin, R. S., and Zagyansky, Y. A. (1972), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 19*, 290.
Warner, C., and Schumaker, V. (1970a), *Biochem. Biophys. Res. Commun. 38*, 125.
Warner, C., and Schumaker, V. (1970b), *Biochemistry 9*, 451.
Werner, T. C., Bunting, J. R., and Cathou, R. E. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 795.
Yguerabide, J., Epstein, H. F., and Stryer, L. (1970), *J. Mol. Biol. 51*, 573.
Zipper, P. (1969), *Acta Phys. Austr. 30*, 143.
Zipper, P. (1972), *Acta Phys. Austr. 36*, 27.

Preparation and Properties of the Repeating Sequence Polymer d(A-A-T)$_n$·d(A-T-T)$_n$†

Robert L. Ratliff,* D. Lloyd Williams, F. Newton Hayes, E. L. Martinez, Jr., and David A. Smith

ABSTRACT: The polymer d(A-A-T)$_n$·d(A-T-T)$_n$ has been prepared through a combination of organic chemical procedures and enzymatic synthesis with DNA polymerase from *Escherichia coli*. The physicochemical properties of the polymer have been compared with those of dA$_n$·dT$_n$ and d(A-T)$_n$·d(A-T)$_n$. Nearest neighbor analysis agrees with the assigned structure. Absorbance *vs.* temperature measurements as well as analytical buoyant density determinations in cesium chloride and cesium sulfate showed that d(A-A-T)$_n$·d(A-T-T)$_n$ has properties lying in between those of dA$_n$·dT$_n$ and d(A-T)$_n$·d(A-T)$_n$. Both strands of the d(A-A-T)$_n$·d(A-T-T)$_n$ polymer were found to be transcribed by the RNA polymerase from *E. coli* to give r(A-A-U)$_n$·r(A-U-U)$_n$.

The synthesis of polydeoxyribonucleotides has provided models for study of DNA and its biological functions. Single-stranded polymers with homo, multiblock, and random hetero sequences have been prepared by use of the terminal transferase from calf thymus (Bollum *et al.*, 1964; Ratliff *et al.*, 1967, 1968; Ratliff and Hayes, 1967; Kato *et al.*, 1967). Another procedure for obtaining single-stranded polydeoxyribonucleotides is separation of the complementary strands of double-stranded polymers (Wells and Blair, 1967). Double-stranded structures have been obtained by *de novo* synthesis, from primed reactions, and by the use of template. The *de novo* reactions using the DNA polymerase from *Escherichia coli* or *Micrococcus luteus* have yielded d(A-T)$_n$·d(A-T)$_n$ (Schachman *et al.*, 1960), dA$_n$·dT$_n$ (Burd and Wells, 1970), dC$_n$·dG$_n$ (Radding *et al.*, 1962), dC$_n$·dI$_n$ (Inman and Baldwin, 1964), and d(C-I)$_n$·d(C-I)$_n$ (Grant *et al.*, 1968). Primed amplification reactions have used complementary oligo- or polydeoxyribonucleotides that range from homo to repeating tetramer sequences (Wells *et al.*, 1965, 1967a,b, 1970; Morgan, 1970).

Seven of the possible repeating trinucleotide complementary double-stranded polydeoxyribonucleotides have been reported (Wells *et al.*, 1967a; Morgan, 1970). The five remaining are d(A-A-T)$_n$·d(A-T-T-)$_n$,[1] d(C-C-G)$_n$·d(C-G-G)$_n$, d(A-

† From the Cellular and Molecular Radiobiology Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87544. *Received August 22, 1973.* This work was performed under the auspices of the U. S. Atomic Energy Commission.

[1] The abbreviated notation for complementary double-stranded polydeoxyribotrinucleotides is in one of the two general forms allowed by the IUPAC–IUB Combined Commission on Biochemical Nomenclature (1970) and further incorporates an alphabetization rule that allows only one way to write the structure of each polymer. The left-hand member of the notation is stated as the alphabetically earliest three-letter set of the six possibilities in the polymer. The right-hand member is then fixed to be complementary in reverse order of letters.

TABLE I: R_F Values of Deoxyribonucleotides and Derivatives.

Compound	Solvent I	II	III	IV	V
d-pA	0.128		0.195		
d-p-BzA	2.6[a]				
d-p-BzA(Ac)	3.8[a]				
d-CNEt-p-BzA	5.0[a]				
d(CNEt-p-BzAp-BzA)	4.0[a]		2.8[a]		
d(p-BzAp-BzA)			2.0[a]		
d(pApA)			0.3[a]	0.88[a]	
d(p-BzAp-BzApT)			1.2[a]		
d(pApApT)			0.24[a]	0.57[b]	
d(ApApT)				1.8[b]	1.5[b]
d-pT	0.099	0.49	0.41		0.15
d-pT(Ac)	1.95[b]				
d-CNEt-pT	2.8[b]				3.0[b]
d(CNEt-pTpT)	1.5[b]		1.4[b]		
d(pTpT)	0.31[b]	0.70[b]	0.57[b]		
d(pTpTpA)			0.28[b]		0.27[b]
d(TpTpA)					1.3[b]
d-Tp					0.19
d-Ap					0.11
d-Thd					0.59
d-Ado					0.56

[a] Relative to d-pA. [b] Relative to d-pT.

C-C)$_n$·d(G-G-T)$_n$, d(A-C-G)$_n$·d(C-G-T)$_n$, and d(A-G-C)$_n$·d(G-C-T)$_n$. We present here the synthesis and characterization of d(A-A-T)$_n$·d(A-T-T)$_n$ and a comparison of its physicochemical properties with the previously reported dA$_n$·dT$_n$ and d(A-T)$_n$·d(A-T)$_n$.

Experimental Procedures

Chemicals and Reagents. There were obtained from Schwarz/Mann deoxythymidine 5′-phosphate, deoxyadenosine 5′-phosphate, deoxyadenosine 5′-triphosphate, [^{14}C]-deoxythymidine 5′-triphosphate, [^{14}C]adenosine 5′-triphosphate, and [^{14}C]uridine 5′-triphosphate; from P-L Biochemicals, Inc., adenosine 5′-triphosphate and uridine 5′-triphosphate; from International Chemical and Nuclear Corp. [α-^{32}P]deoxyadenosine 5′-triphosphate and [α-^{32}P]deoxythymidine 5′-triphosphate; from New England Nuclear Corp. [α-^{32}P]adenonsine 5′-triphosphate and [α-^{32}P]uridine 5′-triphosphate; and from Aldrich Chemical Co., Inc., 2-mesitylenesulfonyl chloride, 2,4,6-triisopropylbenzenesulfonyl chloride, and dicyclohexylcarbodiimide. Each of the two sulfonyl chloride reagents was recrystallized from petroleum ether before every use.

Hydracrylonitrile was obtained from Eastman Kodak Co. and was fractionally distilled under vacuum. Pyridine used as solvent in the chemical syntheses was distilled successively from chlorosulfonic acid and solid KOH and was then dried over a molecular sieve (Linde, type 3A). Cesium chloride and cesium sulfate were obtained from the Harshaw Chemical Co. Agarose for column chromatography (Bio-Rad Laboratories) was obtained from Calbiochem.

Analytical Procedures. Paper chromatography was done on Whatman No. 40 paper by the descending technique using the following solvent systems: (I) 1 M NH_4OAc (pH 6.0)–2-propanol (2:5); (II) 0.5 M NH_4OAc (pH 3.9)–ethanol (2:5); (III) 1 M NH_4OAc (pH 7.5)–ethanol (2:5); (IV) 1-propanol–concentrated NH_4OH–water (55:10:35); (V) 2-propanol–concentrated NH_4OH–water (7:1:2); and (VI) isobutyric acid–concentrated NH_4OH–H_2O (66:1:33). Chromatographic mobilities of various nucleotides and protected derivatives are given in Table I.

Paper electrophoretic separation of nucleotides was performed on strips of Whatman No. 3MM paper using a Savant high-voltage, tank-type apparatus with isoparaffinic coolant and formate buffer (pH 3.8).

Size distribution of synthetic polynucleotides was obtained by chromatography through Bio-Gel A-50m (8 cm^2 × 82.4 cm) in an LKB Model 4901A column, equilibrated with 50 mM triethylammonium bicarbonate (pH 8.5).

Analytical buoyant density centrifugation was carried out in a Spinco Model E ultracentrifuge equipped with ultraviolet optics and a photoelectric scanner with a multiplexer attachment. Double-sector, 12-mm Kel-F centerpieces and the four-hole An-F Ti rotor were used. Ultracentrifuge runs were carried out at 44,000 rpm and 25°. All samples run in cesium chloride included a marker of *E. coli* DNA with a density of 1.703 g/ml (Vinograd *et al.*, 1963; Wells and Blair, 1967). All runs in cesium sulfate included a marker of T-4 DNA with density of 1.443 g/ml (Erikson and Szybalski, 1964). Density values were calculated by the equation

$$\rho_{sample} = \rho_{marker} + (\omega^2/2\beta_0)(r_s^2 - r_m^2)$$

where r_m and r_s are the distances in centimeters from the axis of rotation to the center of the marker and sample bands, respectively. For cesium chloride runs a value of 1.19×10^9 was used for β (Ifft *et al.*, 1961); for cesium sulfate runs β was 5.934×10^8 (Chamberlin and Berg, 1964). Density values were calculated as the average of several scans made in each run.

Enzymes. Micrococcal DNase was purchased from Mann Research Laboratories. Alkaline phosphatase was obtained from Worthington Biochemical Corp., and its solution in 0.05 M glycylglycine buffer (pH 8.0) was heated at boiling water bath temperature for 10 min to destroy endonuclease activity. Spleen phosphodiesterase was prepared by the methods of Hilmoe (1960) and Richardson and Kornberg (1964). *Escherichia coli* DNA polymerase was prepared according to the procedure of Richardson *et al.* (1964), and both the DEAE-cellulose fraction and the phosphocellulose fraction were used The preparations had specific activities of 300–1300 units/mg of protein when assayed using d(A-T)$_n$·d(A-T)$_n$ as template. RNA polymerase from *E. coli* was purified by a modification of the procedure of Chamberlin and Berg (1962), as described previously (Ratliff *et al.*, 1964), and had a specific activity of 3000 units/mg of protein.

Preparation of Intermediates. 2-Cyanoethyl 5′-thymidylate was prepared by the method of Schaller and Khorana (1963) and isolated in quantitative yield as the calcium salt. $O^{3'}$-Acetylthymidine 5′-phosphate was prepared according to Khorana and Vizsolyi (1961). *N*-Benzoyldeoxyadenosine 5′-phosphate was prepared by the procedure of Ralph and Khorana (1961). $O^{3'}$-Acetyl-*N*-benzoyldeoxyadenosine 5′-phosphate was prepared by the procedure of Weimann *et al.* (1963). 2-Cyanoethyl *N*-benzoyl-5′-deoxyadenylate was prepared by a procedure similar to that of Ohtsuka *et al.* (1965); *N*-benzoyldeoxyadenosine 5′-phosphate (2.3 mmol) was allowed to react with hydracrylonitrile (230 mmol) and di-

cyclohexylcarbodiimide (23 mmol) in 15 ml of anhydrous pyridine for 48 hr. After the addition of 25 ml of water, the reaction mixture was kept at 2° for 2 days. Dicyclohexylurea was removed by filtration, and the filtrate was concentrated under vacuum with additions of pyridine. Chromatography in solvent I showed a major spot at R_F 0.51 (5.1 relative to d-pA at R_F 0.10) and a trace of material at R_F 0.42 (d-p-BzA reference, R_F 0.25). To the residual solution of d-CNEt-p-BzA in hydracrylonitrile were added 0.5 M $CaBr_2$ in 95% ethanol (2.15 mmol) and 200 ml of acetone which resulted in partial precipitation of the calcium salt. With stirring, 400 ml of ether was added, and the mixture was cooled at 2° for 16 hr. The solid calcium salt was collected in a fritted funnel and washed with dry acetone several times (total volume 125 ml). A solution of the solid in dry methanol (100 ml) was added with agitation to 1500 ml of ether. After 12 hr at 2°, the precipitated calcium salt was collected in a medium fritted funnel and dried under vacuum; weight 1.2682 g. Ultraviolet analysis (ϵ_{280} 18.3 × 10^3) indicated 2.295 mmol of Ca d-CNEt-p-BzA; the acetone wash liquor (125 ml) contained 0.06 mmol.

Chemical Synthesis and Polymerization of Deoxytrinucleotides. d(A-A-T) SERIES. Dinucleotide d(CNEt-p-BzAp-BzA). An anhydrous pyridine (8 ml) solution of pyridinium d-CNEt-p-BzA (2.0 mmol), pyridinium d-p-BzA (2.64 mmol), and 2.73 g (13.2 mmol) of dicyclohexylcarbodiimide was shaken with 1 g of pyridinium Dowex 50W-X4 resin for 4.5 days at room temperature. With cooling an equal volume of water was added, and the solution was refrigerated for 16 hr. Dicyclohexylurea was removed by filtration and washed repeatedly with 30% pyridine. The combined filtrate, after extraction of dicyclohexylcarbodiimide with petroleum ether, was concentrated to 75 ml, cooled in ice, and treated with cold 2 N NaOH (75 ml) for 20 min. The cold solution was poured onto excess pyridinium Dowex 50W-X4 resin (240 mequiv), with stirring. The resin was removed by filtration and thoroughly washed with 5% pyridine (200 ml). The total filtrate was reduced under vacuum to 200 ml, and the pH was adjusted to 7.0 with 0.1 N triethylamine. The desired dinucleotide was isolated in two parts by anion exchange chromatography on a DEAE-cellulose column (6.5 × 50 cm, bicarbonate form) with a linear gradient of triethylammonium bicarbonate (ph 7.5), 0.005–0.3 M in 12 l. The yield of pure d(p-BzAp-BzA) was 19,300 A_{280} units (ϵ_{280} 18 × 10^3 based on phosphorus analysis). Paper chromatography in solvent II showed a major species with R_F 0.65 and a trace of material at R_F 0.5. After hydrolysis with concentrated NH_4OH, the resulting d(pApA) was homogeneous in two solvents (R_F 0.47 in solvent IV and R_F 0.52 in solvent VI).

An anhydrous pyridine (15 ml) solution of d(p-BzAp-BzA) (0.882 mmol), hydracrylonitrile (88.2 mmol), and dicyclohexylcarbodiimide (8.82 mmol) was shaken at room temperature for 54 hr. With cooling, water (35 ml) was added; after 16 hr at 2°, dicyclohexylurea was removed by filtration and dicyclohexylcarbodiimide extracted with ether. The aqueous pyridine filtrate was evaporated with additions of pyridine, and the calcium salt of d(CNEt-p-BzAp-BzA) was prepared as described above for d-CNEt-p-BzA. The isolated product (0.86 mmol of dimer based on phosphorus analysis) was homogeneous in solvent II, R_F 0.69.

Trinucleotide d(p-BzAp-BzApT). An anhydrous pyridine (20 ml) solution of trihexylammonium d(CNEt-p-BzAp-BzA) (0.63 mmol), pyridinium d-pT(Ac) (3.78 mmol), and triisopropylbenzenesulfonyl chloride (9.45 mmol) was shaken for 6 hr at room temperature. The reaction mixture was cooled in ice, water was added (20 ml), the pH was adjusted to 7.2 with 1 N NH_4OH, and the solution was stored at 2° for 15 hr. To effect removal of the cyanoethyl and $O^{3'}$-acetyl groups, the aqueous pyridine solution (75 ml) was cooled in ice, and cold 2 N NaOH (75 ml) was added. After 20 min at 0°, the solution was poured with stirring onto an excess of pyridinium Dowex 50W-4X resin. The resin was collected and washed thoroughly with 25% pyridine. The total filtrate was reduced in volume under vacuum, the pH was adjusted to 7.0, and the mixture was chromatographed on a DEAE-cellulose column (5.2 × 60 cm, bicarbonate) at 4°. Elution was with a linear gradient of triethylammonium bicarbonate (pH 7.5), 0.005–0.4 M in 16 l. The yield of the desired trinucleotide was 3800 A_{279} units (0.09 mmol). Paper chromatography in solvent III gave a major species at R_F 0.199 with a trace of material at R_F 0.092. After removal of the *N*-benzoyl groups with 9 N NH_4OH at 60° during 2 hr, only one spot was apparent at R_F 0.030 (solvent III). Following removal of the 5′-phosphate group with alkaline phosphatase, the d(ApApT) (R_F 0.51, solvent IV) was degraded with spleen phosphodiesterase. The ratio of d-Ap to d-Thd was 2.06:1.00.

Polytrimer d(A-A-T)$_{\geq 5}$. Polymerization of the protected trinucleotide was done according to the procedure of Narang *et al.* (1967) except that the 25% of $O^{3'}$-protected trinucleotide was not included. The solution of trihexylammonium d(p-BzAp-BzApT) (3180 A_{279} units, 0.076 mmol) in dry pyridine was made anhydrous by repeated evaporations of dry pyridine (5 × 1 ml). The residue was dissolved in dry pyridine (1 ml), mesitylenesulfonyl chloride (0.684 mmol) was added, and the volume of solution was reduced by half. After 5 hr at room temperature, 25% pyridine (1 ml) and triethylamine (1 ml) were added. After 24 hr at 2°, the benzoyl groups were removed with concentrated NH_4OH (39 hr), and the solution was evaporated under vacuum to a small volume (5 ml, pH 8.4). The mixture was then chromatographed on a DEAE-cellulose column (3.8 × 77 cm, bicarbonate) with a linear gradient of triethylammonium bicarbonate (pH 7.5) from 0.005 to 0.4 M in 12 l. After emergence of the probable dodecamer peak, the column was washed with 1 M triethylammonium bicarbonate to remove together all the longer oligomers.

d(A-T-T) SERIES. The following synthesis of the trinucleotide d(pTpTp-BzA) was prepared essentially according to the procedure of Ohtsuka and Khorana (1967).

Dinucleotide d(CNEt-pTpT). To an anhydrous pyridine (6 ml) solution of the pyridinium salts of d(CNEt-pT) (1.5 mmol) and d-pT(Ac) (1.5 mmol) was added 7.5 mmol of dicyclohexylcarbodiimide. After 4 days at room temperature water (10 ml) was added, and the solution was left for 16 hr. The aqueous pyridine solution was diluted to 25 ml with water, filtered to remove dicyclohexylurea, and extracted with petroleum ether to remove dicyclohexylcarbodiimide. The aqueous phase was made 9 N in NH_4OH and heated at 60° for 3 hr. It was then cooled, concentrated to 20 ml under vacuum, and filtered to remove additional dicyclohexylurea. The filtrate was applied to a DEAE-cellulose column (6.5 × 46 cm, bicarbonate); elution was with a linear gradient of triethylammonium bicarbonate (pH 7.5), 0.005–0.6 M in 16 l. The yield of d(pTpT) was 13,200 A_{267} units (0.713·mmol). Paper chromatography in solvent III indicated a small contaminant at R_F 0.11 in the product, R_F 0.21. To eliminate the contaminant, the product was rechromatographed on the same DEAE-cellulose column. The purified product amounted to 12,200 A_{267} units and was homogeneous in solvent I.

An anhydrous pyridine (7 ml) solution of pyridinium d(pT-pT) (0.66 mmol), hydracrylonitrile (66 mmol), and dicyclo-

hexylcarbodiimide (7.26 mmo) containing dry pyridinium Dowex 50W-X4 resin (0.5 g) was kept at room temperature for 3 days. The reaction mixture was diluted to 40 ml with water and kept at room temperature for 24 hr; it was then filtered to remove dicyclohexylurea and resin and extracted with petroleum ether to remove dicyclohexylcarbodiimide. Paper chromatography at this stage, solvent I, indicated a major product at R_F 1.2 relative to d(pTpT) and a minor product at R_F 3.6 relative to d(pTpT). The high R_F material was proven be an ester of the type bis(CNEt)-d(pTpT) (Narang *et al.*, 1967) by complete deblocking with 9 N NH_4OH. This resulted in a single product at R_F 0.22 in solvent III, corresponding to d(pTpT). Triethylamine equivalent to the phosphate anion was added to the solution which was then concentrated to a syrup under vacuum. The residue was dried by evaporation of pyridine (2 × 10 ml), dissolved in dry pyridine (10 ml), and added with stirring to 400 ml of ether and left at 2° for 15 hr. After centrifugation, the mother liquor was decanted from the precipitate which was resuspended in 400 ml of ether and collected and freed of ether under vacuum. The dry ester was dissolved in 50% ethanol (30 ml) and assayed, amounting to 11,700 A_{267} units (0.632 mmol).

Trinucleotide d(pTpTp-BzA). An anhydrous pyridine (6 ml) solution of trihexylammonium d(CNEt-pTpT) (0.318 mmol), pyridinium d-p-BzA(Ac) (1.5 mmol), and triisopropylbenzenesulfonyl chloride (3.75 mmol) was shaken for 8 hr. With cooling water was added, the pH was adjusted to 7.3 with 1 N NH_4OH, and the solution was filtered. To the filtrate (52 ml) cooled in ice was added cold 2 N NaOH (52 ml). After 20 min at 0°, the solution was poured onto excess pyridinium Dowex 50W-X4 resin (130 ml) with stirring. The resin was collected and washed with 5% pyridine. The total filtrate was applied to a DEAE-cellulose column (4.4 × 75 cm, bicarbonate) and chromatographed at 4° with a linear gradient of triethylammonium bicarbonate (pH 7.5), 0.005–0.4 M in 16 l. The yield of the desired trinucleotide was 2590 A_{274} units (0.075 mmol), with the following spectral properties at pH 8.4: λ_{max} 274 nm, λ_{min} 240 nm. After removal of the benzoyl group with 9 N NH_4OH, d(pTpTpA), R_F 0.061 in solvent III, was dephosphorylated with alkaline phosphatase. The resulting d(TpTpA), R_F 0.212 in solvent V, was degraded with spleen phosphodiesterase. The observed ratio of d-Tp to d-Ado was 1.93:1.00.

Polytrimer d(A-T-T)$_{\geq 5}$. Polymerization of the protected trinucleotide was according to Narang *et al.* (1967), with the exception noted above. To an anhydrous pyridine (1 ml) solution of trihexylammonium d(pTpTp-bzA) (0.075 mmol) was added mesitylenesulfonyl chloride (0.8 mmol), and the volume was immediately reduced by half. After 5 hr at room temperature 25% pyridine (1 ml) and 9 N NH_4OH (5 ml) were added, and the mixture was left for 7 days. After evaporation of NH_4OH under vacuum, the reaction mixture was chromatographed on a DEAE-cellulose column (3.8 × 77 cm, bicarbonate) with a linear gradient of triethylammonium bicarbonate (pH 7.5), 0.005–0.4 M in 12 l. After emergence of the probable dodecamer peak, the column was washed with 1 M triethylammonium bicarbonate to remove together all longer oligomers.

Enzymatic Synthesis of d(A-A-T)$_n$·d(A-T-T)$_n$. The synthesis of d(A-A-T)$_n$·d(A-T-T)$_n$ using the oligonucleotide fractions d(A-A-T)$_{\geq 5}$ and d(A-T-T)$_{\geq 5}$ as template was initially accomplished with the DEAE-cellulose fraction of the DNA polymerase from *E. coli*. The reaction mixture, in 2 ml of total volume, contained 50 μmol of potassium phosphate buffer (pH 7.5), 10 μmol of $MgCl_2$, 2 μmol of 2-mercaptoethanol, 5 μmol each of dATP and dTTP, 50 mono-nmol[2] of each of the oligodeoxyribonucleotides as templates, 80 units of *E. coli* DNA polymerase, and [^{14}C]dTTP as the labeled substrate. The reaction temperature was 15°, and the course of polymer synthesis was examined by radioisotope incorporation into acid-insoluble material (Hayes *et al.*, 1966). The utilization of triphosphates amounted to 0.7% in 24 hr, 4% in 48 hr, and 34% in 72 hr, at which time reaction was stopped by addition of 0.1 vol of 1% sodium dodecyl sulfate, and protein was extracted with phenol as described previously (Smith *et al.*, 1970). After chromatography through a Bio-Gel A-50m column, the isolated d(A-A-T)$_n$·d(A-T-T)$_n$ (2.32 mono-μmol, 23-fold net synthesis) was used as template in a large-scale reaction. The composition of the reaction mixture (total volume 40 ml) was the same as above except that 150 units of the phosphocellulose fraction of the DNA polymerase from *E. coli* and 50 mono-nmol of the d(A-A-T)$_n$·d(A-T-T)$_n$ were used per milliliter of the reaction mixture. The isolated yield of polymer was 45.8 mono-μmol.

The synthesis kinetics of each of the three polymers containing deoxyadenosine and deoxythymidine were measured by incorporation of [^{32}P]deoxythymidylic acid from α-labeled triphosphate into acid-insoluble material. To follow the synthesis of d(A-A-T)$_n$·d(A-T-T)$_n$, a 1-ml aliquot was removed from the large-scale reaction mixture described above, and [α-^{32}P]dTTP was added. The rates of formation of dA$_n$·dT$_n$ and d(A-T)$_n$·d(A-T)$_n$ were also determined in 1-ml reaction mixtures.

Nearest neighbor analysis was done for each of the polymers according to the procedures of Josse *et al.* (1961) and Ratliff *et al.* (1968). Size distributions were determined as described by Hayes and Mitchell (1969) and by Jang and Bartl (1971). The absorbance–temperature transitions were determined using the procedure and apparatus previously reported by Hayes *et al.* (1970). The method for following the kinetics of transcription of each of the deoxyribopolymers by RNA polymerase from *E. coli* was essentially as described by Morgan (1970).

Results

The initial synthesis of d(A-A-T)$_n$·d(A-T-T)$_n$ using the complementary chemically synthesized oligodeoxyribonucleotides as template and the DEAE-cellulose fraction of DNA polymerase from *E. coli* was done at 15°. All attempts to obtain synthesis at 37° resulted either in no reaction or in formation of the alternating polymer d(A-T)$_n$·d(A-T)$_n$. The long incubation period is attributed to both the low enzyme concentration (1.2 mono-nmol of template to 1 unit of DNA polymerase) and the temperature (15°). The highest yields of primed synthesis using d(A-A-T)$_n$·d(A-T-T)$_n$ were obtained with the phosphocellulose fraction of the DNA polymerase from *E. coli*, and this enzyme fraction was used for large-scale preparation of d(A-A-T)$_n$·d(A-T-T)$_n$ as well as for kinetic studies and preparation of material for nearest neighbor analysis of all three polymers.

The synthesis of d(A-T)$_n$·d(A-T)$_n$ reached a maximum of 36% utilization of deoxyribonucleoside triphosphates present in 48 hr, as measured by incorporation of [α-^{32}P]dTTP into acid-insoluble material (Figure 1). The extent of synthesis of d(A-A-T)$_n$·d(A-T-T)$_n$ was 43% in 48 hr, while that of dA$_n$·

[2] The quantities of oligomers or polymers are referred to in terms of moles of mononucleotide residues as determined by phosphorus analyses.

TABLE II: Nearest Neighbor Analysis of Each of the Three Isomeric Polymers.

Template	α-^{32}P-Labeled Radioact. in Deoxyribonucleoside 3′-Phosphate[a]					α-^{32}P-Labeled Radioact. in Ribonucleoside 2′(3′)-Phosphate[b]				
	Labeled Triphosphate	3′-Deoxyadenylate cpm	%	3′-Deoxythymidylate cpm	%	Labeled Triphosphate	2′(3′)-Adenylate cpm	%	2′(3′)-Uridylate cpm	%
dA$_n$·dT$_n$	dATP	42,500	99.5	200	0.5					
	dTTP	500	0.3	143,800	99.7					
d(A-T)$_n$·d(A-T)$_n$	dATP	100	0.2	45,800	99.8					
	dTTP	147,200	99.7	500	0.3					
d(A-A-T)$_n$·d(A-T-T)$_n$	dATP	12,800	32.6	26,500	67.4	rATP	15,600	33.8	30,500	66.2
	dTTP	85,300	65.5	44,900	34.5	rUTP	43,100	66.4	21,800	33.6

[a] For analysis of d(A-A-T)$_n$·d(A-T-T)$_n$, two 1-ml aliquots were removed from the large reaction mixture to one of which was added [α-^{32}P]dATP and [α-^{32}P]dTTP to the other. For the other two polymers, the reaction mixtures and temperatures were the same as described for kinetic reactions (Figure 1) except that two different preparations were performed: one with [α-^{32}P]-dATP and the second with [α-^{32}P]dTTP. The ^{32}P-labeled dA$_n$·dT$_n$ reactions were stopped after 24 hr, while those of d(A-T)$_n$·d(A-T)$_n$ and d(A-A-T)$_n$·d(A-T-T)$_n$ were stopped after 72 hr. The reactions were terminated by placing them in a boiling water bath for 10 min. The samples were cooled and centrifuged, and the supernatants were dialyzed against 0.01 M sodium pyrophosphate and then distilled water to remove unreacted deoxyribonucleoside triphosphates. The polymers were degraded to their 3′-mononucleotides using micrococcal DNase and spleen phosphodiesterase and were separated by electrophoresis on paper (Josse *et al.*, 1961; Ratliff *et al.*, 1968). The radioactive spots were located with a Packard radiochromatogram scanner, cut out, and counted in a Packard Tri-Carb liquid scintillation counter. All counts per minute (cpm) have been rounded off to the nearest 100. [b] Two 0.2-ml aliquots were removed from a 5-ml transcription reaction containing 5 mono-μmol of d(A-T-T)$_n$·d(A-T-T)$_n$, 5 μmol each of rATP and rUTP, 200 μmol of Tris-HCl buffer (pH 8.0), 20 μmol of magnesium chloride, 5 μmol of manganese chloride, 60 μmol of 2-mercaptoethanol, and 2000 units of RNA polymerase. To one aliquot was added [α-^{32}P]rATP and to the other [α-^{32}P]rUTP. Incubation was at 37° for 4 hr. The ^{32}P-labeled reactions were terminated by placing them in a boiling water bath for 10 min. The samples were cooled and centrifuged, and the supernatants were dialyzed against 0.01 M sodium pyrophosphate and then distilled water. The retentates were made 0.3 N in KOH by adding 0.06 ml of 5 N KOH and adjusting the total volume to 1.0 ml with water. Incubation at 37° for 18 hr was followed by neutralization through addition of 0.06 ml of 5 N HCl to each solution. The 2′(3′)-ribonucleotides were separated by paper electrophoresis according to the procedure of Markham and Smith (1952). The total results are averages of results from four separate runs.

dT$_n$ reached a maximum of 34% in 24 hr followed by a rapid drop to 12% in 36 hr.

The nearest neighbor analyses for each of the three isomeric polymers containing exclusively deoxyadenosine and deoxythymidine are given in Table II. The results show that, in polymers prepared using [α-^{32}P]dATP, all radioactivity was found in d-Ap from dA$_n$·dT$_n$, in d-Tp from d(A-T)$_n$·d(A-T)$_n$, and two-thirds in d-Tp and one-third in d-Ap from d(A-A-T)$_n$·d(A-T-T)$_n$. When [α-^{32}P]dTTP was the labeled deoxyribonucleoside triphosphate, 100% of the label was found in d-Tp from dA$_n$·dT$_n$, 100% in d-Ap from d(A-T)$_n$·d(A-T)$_n$, and two-thirds in d-Ap and one-third in d-Tp from d(A-A-T)$_n$·d(A-T-T)$_n$. The nearest neighbor results show that the dinucleotide sequences in d(A-A-T)$_n$·d(A-T-T)$_n$ were one-third each d(ApT) and d(TpA) and one-sixth each d(ApA) and d(TpT), as expected. When ribonucleoside [α-^{32}P]triphosphates were used with d(A-A-T)$_n$·d(A-T-T)$_n$ and RNA polymerase, the nearest neighbor results (Table II) in the transcribed ribopolymer demonstrated dinucleotide sequences that were one-third each r(ApU) and r(UpA) and one-sixth each r(ApA) and r(UpU).

When the highest molecular weight fraction (see Figure 2) of d(A-A-T)$_n$·d(A-T-T)$_n$ isolated from the large-scale reaction was used in a subsequent reaction for synthesis of more of the same polymer, the nearest neighbor results were incorrect such that, when [α-^{32}P]dATP was used as one of the substrates, 22% of the label was recovered in d-Ap and 78% in d-Tp. When [α-^{32}P]dTTP was used as one of the substrates, 56% of the label was found in d-Ap and 44% in d-Tp. However, when d(A-A-T)$_n$·d(A-T-T)$_n$ with an average size of 129 base pairs was used as primer, product was obtained corresponding in quantity to 44-fold synthesis and with nearest neighbor analyses correct for d(A-A-T)$_n$·d(A-T-T)$_n$.

Figure 2 gives the results of fractionation according to mo-

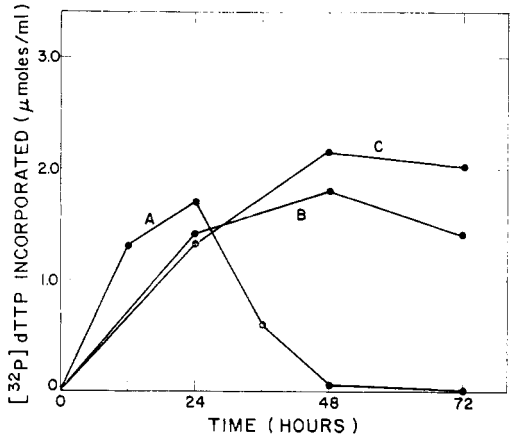

FIGURE 1: Kinetics of polymer-directed synthesis of dA$_n$·dT$_n$ (A), d(A-T)$_n$·d(A-T)$_n$ (B), and d(A-A-T)$_n$·d(A-T-T)$_n$ (C). Each reaction mixture contained the following components in a total volume of 1 ml: 25 μmol of potassium phosphate buffer (pH 7.5), 5 μmol of $MgCl_2$, 1 μmol of 2-mercaptoethanol, 2.5 μmol each of dATP and dTTP, [α-^{32}P]dTTP as labeled substrate, 50 mono-nmol of the polydeoxyribonucleotide template, and 150 units of *E. coli* DNA polymerase (phosphocellulose fraction). The incubation temperature was 15°, and aliquots were analyzed as described under Experimental Procedure.

TABLE III: Physical Properties of the Three Isomeric Polymers.

Polymer	T_m (°C) (0.05 M Na^+)	$\Delta\lambda$ (nm) (Helix → Coil)	λ (nm) (A_{max})	Slope, m ($T_m = m \ln M + k$)	Buoyant Density In Cesium Chloride (g/ml)	Buoyant Density In Cesium Sulfate (g/ml)
$dA_n \cdot dT_n$	61.8	+2.0	258	8.25	1.638	1.419
$d(A\text{-}T)_n \cdot d(A\text{-}T)_n$	54.5	−2.0	262	8.97	1.672	1.427
$d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$	56.7	−0.5	260.5	7.99	1.666	1.420
DNA (100% A·T)	58.6			7.95	1.653[a]	

[a] See Wells and Blair (1967).

lecular size by chromatography through a Bio-Gel A-50m column using 0.05 M triethylammonium bicarbonate as eluent. The homopolymer $dA_n \cdot dT_n$ gave the largest molecular weight and sharpest size distribution followed, in turn, by $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ and $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ which came from the large reaction.

Figure 3 gives representative melting curves for each of the polymers in 0.05 M Na^+. The molarity dependence of the T_m of $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ was determined with high molecular weight polymer across a 100-fold range of Na^+ concentrations at neutral pH. The linear least-squares fit to the data, expressed in degrees Celsius and moles per liter of Na^+, is $T_m = 7.99 \cdot (\ln M) + 79.4$. The slope ($m = 7.99$) in this relationship is entered in Table III along with corresponding m values for $dA_n \cdot dT_n$ and $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ derived from the data of Wells *et al.* (1970) and for DNA with 100% A·T pairs calculated from the equation of Frank-Kamenetskii (1971). The T_m values in 0.05 M Na^+ for the three isomeric polymers as determined in Figure 3 are also entered in Table III along with the calculated T_m in 0.05 M Na^+ (Frank-Kamenetskii, 1971) for DNA with 100% A·T pairs. Also entered in Table III are spectrophotometric data for the three isomeric polymers indicating λ at A_{max} in the helix form and the change in λ at A_{max} as a result of melting the helix into the separated coils.

The length dependence for the T_m of $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ was determined in 0.05 M Na^+ across values of B ranging from 24 to 1000 base pairs. The linear least-squares fit to the data, expressed in degrees Kelvin and base pairs, is $10^3/T_m = 3.021 + 2.076/B$.

A scan of a buoyant density experiment in which *E. coli* DNA and $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ were centrifuged to equilibrium in neutral cesium chloride is shown in Figure 4. Using a value of 1.703 g/ml for the buoyant density of *E. coli* DNA (Vinograd *et al.*, 1963; Wells and Blair, 1967), the buoyant density of $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ was calculated to be 1.666 g/ml. Figure 4 also shows the buoyant densities of $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ and $dA_n \cdot dT_n$. These polymers and marker *E. coli* DNA were run in separate cells in the four-hole An-F Ti rotor simultaneously with the cell which contained $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ and *E. coli* DNA. The values of 1.672 g/ml for $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ and 1.638 g/ml for $dA_n \cdot dT_n$ agree with the values determined by Wells and Blair (1967). The buoyant densities of three different preparations of $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ were determined (average of several scans in each run) to be 1.6660, 1.6662, and 1.6652 g/ml in neutral cesium chloride. The buoyant density of $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ in cesium sulfate was found to be 1.420 g/ml compared to 1.427 g/ml for $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ and 1.419 g/ml for $dA_n \cdot dT_n$. These values also agree with previous

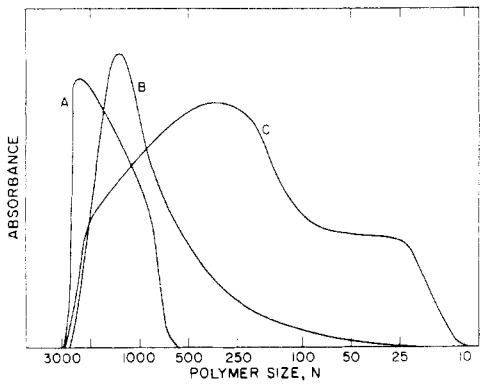

FIGURE 2: Size distributions. The newly synthesized polydeoxyribonucleotides were chromatographed using Bio-Gel A-50m (8 $cm^2 \times 82.4$ cm) in an LKB Model 4901A separation column equilibrated with 50 mM triethylammonium bicarbonate (pH 8.5). This same buffer served as eluent at a flow rate of 25 ml/hr. The eluate was monitored with an ISCO Model 222 ultraviolet analyzer and collected in tubes, each of which was filled to 20.2 ml. The cumulative elution volume, V, at the one-half filled point for any given tube was converted to an average number of base pairs per molecule (N) for these double-stranded molecules by use of the equation: $\log N = 4.49 - 0.00565V$, which was obtained by calibration of the column with polydeoxyribonucleotides of known length, as in Hayes and Mitchell (1969). The absorbance of each tube was read on a Unicam Model SP-800 spectrophotometer. The absorbance scale is linear, but the relationships between separation runs are normalized arbitrarily. The amounts applied to the column were 25 A_{260} units of $dA_n \cdot dT_n$ (A) and 100 A_{260} units each of $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ (B) and $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ (C).

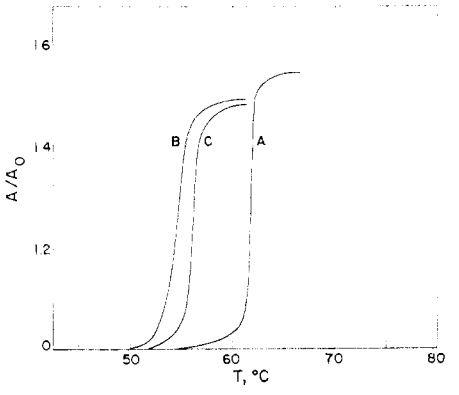

FIGURE 3: Thermal hyperchromicity profiles. Solutions of $dA_n \cdot dT_n$ (A), $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ (B), and $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ (C) were prepared in 0.04 M NaCl and 0.005 M Na_2HPO_4 (pH 7.0), adjusted with H_3PO_4, by extensive dialysis. For all three polymers the fraction used had an average size of $N = 1000$. Approximately 1.2 ml with A_{260} units of 0.7–0.9 were heated in the apparatus described by Hayes *et al.* (1970). A Unicam Model SP-1800 spectrophotometer was used, and five points were recorded per degree of temperature rise at 260 nm. The T_m values are 61.8 (A), 54.5 (B), and 56.7° (C). The standard deviation of these T_m values is ±0.3°.

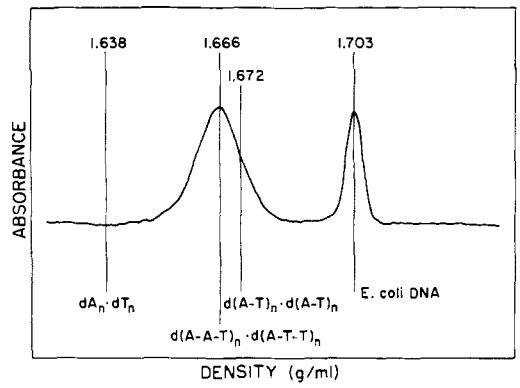

FIGURE 4: Cesium chloride buoyant density centrifugation of d(A-A-T)$_n$·d(A-T-T)$_n$. The solvent for cesium chloride was 0.01 M Tris (pH 7.0)–0.001 M EDTA. Centrifugation was at 25° for 40 hr at 44,000 rpm. A density marker of *E. coli* DNA was added. Also shown are the locations and buoyant densities (in g/ml) of d(A-T)$_n$·d(A-T)$_n$ and dA$_n$·dT$_n$ which were run in different cells in the same An-F Ti rotor simultaneously with d(A-A-T)$_n$·d(A-T-T)$_n$.

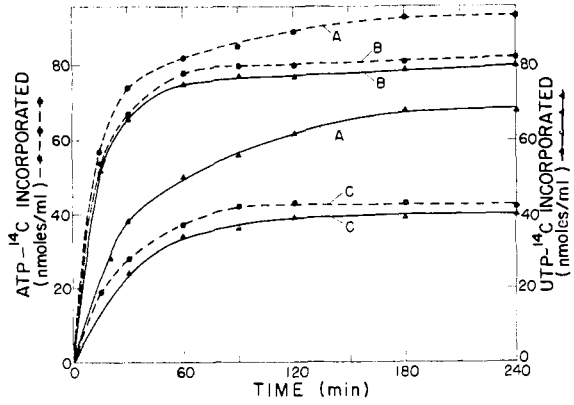

FIGURE 5: Kinetics of transcription of each of the deoxyribonucleotide polymers by the RNA polymerase from *E. coli*. Each reaction mixture contained 40 μmol of Tris-HCl buffer (pH 8.0), 4 μmol of magnesium chloride, 1 μmol of manganese chloride, 12 μmol of 2-mercaptoethanol, 100 nmol of each ribonucleoside triphosphate, 100 mono-nmol of a polydeoxyribonucleotide template, and 300 units of RNA polymerase in a final volume of 1 ml. The reactions were incubated at 37°, and measurement of incorporation of labeled ribonucleoside triphosphate into acid-insoluble material was carried out by the glass filter disc assay procedure. Transcription of dA$_n$·dT$_n$ gave rA$_n$·rU$_n$ (A), transcription of d(A-T)$_n$·d(A-T)$_n$ gave r(A-U)$_n$·r(A-U)$_n$ (B), and transcription of d(A-A-T)$_n$·d(A-T-T)$_n$ gave r(A-A-U)$_n$·r(A-U-U)$_n$ (C).

determinations (Riley *et al.*, 1966; Morgan and Wells, 1968). It is interesting that in cesium chloride d(A-A-T)$_n$·d(A-T-T)$_n$ has a buoyant density more like d(A-T)$_n$·d(A-T)$_n$ than dA$_n$·dT$_n$, while in cesium sulfate the situation is reversed. The buoyant densities are entered in Table III along with an extrapolated value for DNA with 100% A·T pairs (Wells and Blair, 1967).

Several attempts were made to separate the strands of d(A-A-T)$_n$·d(A-T-T)$_n$ in alkaline cesium chloride and alkaline cesium sulfate. However, strand separation was not achieved. Practical strand separations of other repeating trinucleotides (Wells *et al.*, 1967b) also are difficult to obtain (R. Wells, personal communication). Although it is possible that the d(A-A-T)$_n$ sequences and d(A-T-T)$_n$ sequences are covalently linked to each other, available evidence does not support this notion. The DNA polymerase preparation used to synthesize d(A-A-T)$_n$·d(A-T-T)$_n$ contains the strand separation factor described by Paetkau (1969) and by Morgan and Paetkau (1972), since d(A-C)$_n$·d(G-T)$_n$ made with this enzyme preparation contained completely separable stands. Also, brief digestion of d(A-A-T)$_n$·d(A-T-T)$_n$ with DNase I did not produce separable strands.

The kinetics of transcription of each of the deoxyribonucleotide polymers by the RNA polymerase from *E. coli* is given in Figure 5. Transcription of d(A-T)$_n$·d(A-T)$_n$ to give r(A-U)$_n$·r(A-U)$_n$ reached a maximum of 80% polymerization of ribonucleoside triphosphate present in 90 min with equal amounts of rATP and rUTP being utilized and a twofold synthesis over the amount of DNA polymer present. Under the same conditions, transcription of d(A-A-T)$_n$·d(A-T-T)$_n$ to give r(A-U-U)$_n$·r(A-A-U)$_n$ gave a onefold synthesis of the ribopolymer with equal amounts of rATP and rUTP being utilized. Transcription of dA$_n$·dT$_n$ gave a rapid synthesis of rA$_n$, 90% polymerization in 90 min, with a much slower transcription of the dA$_n$ strand to give rU$_n$, 55% utilization of rUTP in 90 min.

Discussion

Wells *et al.* (1967b) have shown previously that complementary short-chain deoxyribopolynucleotides containing repeating trinucleotide sequences can be successfully amplified to large molecular weight, double-stranded polymers by the *E. coli* DNA polymerase-catalyzed polymerization of appropriate deoxyribonucleoside triphosphates. Absorbance–temperature transition, analytical buoyant density, and circular dichroism studies also have been reported by Wells *et al.* (1970) on 4 of the possible 12 repeating trinucleotide polymers.

Preparation of the trinucleotide polymer d(A-A-T)$_n$·d(A-T-T)$_n$ has allowed us to compare its physicochemical properties with the homopolymer dA$_n$·dT$_n$ and the dinucleotide polymer d(A-T)$_n$·d(A-T)$_n$. The nearest neighbor analysis of d(A-A-T)$_n$·d(A-T-T)$_n$ showed that the dinucleotide sequences d(ApT) and d(TpA) made up equally two-thirds of the sequences in the polymer, while the dinucleotide sequences d(ApA) and d(TpT) made up equally the other one-third of the sequences. That the structure of the polymer is clearly distinct from both dA$_n$·dT$_n$ and d(A-T)$_n$·d(A-T)$_n$ was further verified by the absorbance–temperature transition and buoyant density studies (data shown in Table III). All three comparisons with DNA in Table III show that d(A-A-T)$_n$·d(A-T-T)$_n$ is more like DNA than are its isomeric synthetic polymers. Attempts to calculate T_m and buoyant density values were made by summing one-third of the dA$_n$·dT$_n$ value plus two-thirds of the corresponding d(A-T)$_n$·d(A-T)$_n$ value. This treatment is one case of the first-neighbor approximation originally described by Cantor and Tinoco (1965) and later used by Wells *et al.* (1970) and Gray and Tinoco (1970) for computation of circular dichroism spectra.

The calculated d(A-A-T)$_n$·d(A-T-T)$_n$ values are 0.2° high for T_m in 0.05 M Na^+, 0.004 g/ml high for buoyant density in cesium sulfate, and 0.005 g/ml low for buoyant density in cesium chloride. The agreements between calculated and measured values are poor in both buoyant density systems where measurements carry a precision of ±0.001 g/ml. The agreement in T_m is within the precision of measurement. Circular dichroism studies of d(A-A-T)$_n$·d(A-T-T)$_n$ and r(A-A-U)$_n$·r(A-U-U)$_n$ by Gray *et al.* (1973) showed poor agreement between observed and calculated spectra. However, agreement became excellent after melting the polymers. Perhaps taking into consideration interstrand nucleotide inter-

action due to base pairing, which would be related to T_m of the polymer, in determining buoyant density and circular dichroism properties would give better agreement between observed and calculated values. When the T_m of the three polymers containing deoxyadenosine and deoxythmidine were included in the calculation by the nearest neighbor method of buoyant density of $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ in cesium chloride and cesium sulfate, good agreement was obtained between the observed value (1.666 g/ml) and the calculated value (1.666 g/ml) in cesium chloride but not in cesium sulfate (obsd 1.420, calcd 1.430). The agreement between observed and calculated buoyant density values of $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ in cesium chloride but not in cesium sulfate could be expected, since the melting temperature and cesium chloride buoyant density are mutually related to base composition.

Although $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$, when transcribed by the RNA polymerase from *E. coli*, provides previously unavailable sequences of RNA, selective transcription of one of the strands has not been observed. Attempts to separate the strands of $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$ by centrifugation in alkaline cesium chloride have so far failed.

Morgan (1970) has made an extensive study of transcription of several synthetic DNAs. In the case of transcription of $d(A\text{-}A\text{-}C)_n \cdot d(G\text{-}T\text{-}T)_n$, which is somewhat analogous to $d(A\text{-}A\text{-}T)_n \cdot d(A\text{-}T\text{-}T)_n$, he found that synthesis of the U-rich strand was preferred. We have also observed this preference under conditions where the concentrations of deoxyribonucleotide template and enzyme were much less than in the experimental results reported here. Morgan (1970) found that synthesis of RNAs containing the nonsense codons UAG or UAA terminated abruptly after about 15 min. In the RNA which contained the ochre codon UAA, the product was actually $r(G\text{-}U\text{-}A\text{-}A)_n$ in which each r(U-A-A) was sepaarated by a G. Our results show that $r(A\text{-}A\text{-}U)_n$ synthesis does not give the plateau kinetics seen by Morgan for $r(G\text{-}U\text{-}A\text{-}A)_n$ synthesis. Thus, the presence of repeating UAA is not sufficient to cause plateau kinetics. Morgan also observed that synthesis of poly[$r(U\text{-}G\text{-}A)_n$] containing the repeating nonsense codons UGA did not show plateau kinetics.

References

Bollum, F. J., Groeniger, E., and Yoneda, M. (1964), *Proc. Nat. Acad. Sci. U. S. 51*, 853.

Burd, J. F., and Wells, R. D. (1970), *J. Mol. Biol. 53*, 435.

Cantor, C. R., and Tinoco, I., Jr. (1965), *J. Mol. Biol. 13*, 65.

Chamberlin, M., and Berg, P. (1962), *Proc. Nat. Acad. Sci. U. S. 48*, 81.

Chamberlin, M., and Berg, P. (1964), *J. Mol. Biol. 8*, 297.

Erikson, R. L., and Szybalski, W. (1964), *Virology 22*, 111.

Frank-Kamenetskii, M. D. (1971), *Biopolymers 10*, 2623.

Grant, R. C., Harwood, S. J., and Wells, R. D. (1968), *J. Amer. Chem. Soc. 90*, 4474.

Gray, D. M., Ratliff, R. L., and Williams, D. L. (1973), *Biopolymers 12*, 1233.

Gray, D. M., and Tinoco, I., Jr. (1970), *Biopolymers 9*, 223.

Hayes, F. N., Lilly, E. H., Ratliff, R. L., Smith, D. A., and Williams, D. L. (1970), *Biopolymers 9*, 1105.

Hayes, F. N., and Mitchell, V. E. (1969), *J. Chromatogr. 39*, 139.

Hayes, F. N., Mitchell, V. E., Ratliff, R. L., Schwartz, A. W., and Williams, D. L. (1966), *Biochemistry 5*, 3625.

Hilmoe, R. J. (1960), *J. Biol. Chem. 235*, 2117.

Ifft, J. B., Voet, D. H., and Vinograd, J. (1961), *J. Phys. Chem. 65*, 1138.

Inman, R. B., and Baldwin, R. L. (1964), *J. Mol. Biol. 8*, 452.

IUPAC–IUB Combined Commission on Biochemical Nomenclature (1970), *Biochemistry 9*, 4022.

Jang, C.-G., and Bartl, P. (1971), *Biopolymers 10*, 481.

Josse, J., Kaiser, A. D., and Kronberg, A. (1961), *J. Biol. Chem. 236*, 864.

Kato, K., Goncalves, J. M., Houts, G. E., and Bollum, F. J. (1967), *J. Biol. Chem. 242*, 2870.

Khorana, H. G., and Vizsolyi, J. P. (1961), *J. Amer. Chem. Soc. 83*, 675.

Markham, R., and Smith, J. D. (1952), *Biochem. J. 52*, 552.

Morgan, A. R. (1970), *J. Mol. Biol. 52*, 441.

Morgan, A. R., and Paetkau, V. (1972), *Can. J. Biochem. 50*, 210.

Morgan, A. R., and Wells, R. D. (1968), *J. Mol. Biol. 37*, 63.

Narang, S. A., Jacob, T. M., and Khorana, H. G. (1967), *J. Amer. Chem. Soc. 89*, 2167.

Ohtsuka, E., and Khorana, H. G. (1967), *J. Amer. Chem. Soc. 89*, 2195.

Ohtsuka, E., Moon, M. W., and Khorana, H. G. (1965), *J. Amer. Chem. Soc. 87*, 2956.

Paetkau, V. (1969), *Nature (London) 224*, 370.

Radding, C. M., Josse, J., and Kornberg, A. (1962), *J. Biol. Chem. 237*, 2869.

Ralph, R. K., and Khorana, H. G. (1961), *J. Amer. Chem. Soc. 83*, 2926.

Ratliff, R. L., and Hayes, F. N. (1967), *Biochim. Biophys. Acta 134*, 203.

Ratliff, R. L., Hoard, D. E., Ott, D. G., and Hayes, F. N. (1967), *Biochemistry 6*, 851.

Ratliff, R. L., Schwartz, A. W., Kerr, V. N., Williams, D. L., Ott, D. G., and Hayes, F. N. (1968), *Biochemistry 7*, 412.

Ratliff, R. L., Smith, D. A., and Trujillo, T. T. (1964), Los Alamos Scientific Laboratory Report LA-3132-MS, p 236.

Richardson, C. C., and Kornberg, A. (1964), *J. Biol. Chem. 239*, 242.

Richardson, C. C., Schildkraut, C. L., Aposhian, H. V., and Kornberg, A. (1964), *J. Biol. Chem. 239*, 222.

Riley, M., Maling, B., and Chamberlin, M. J. (1966), *J. Mol. Biol. 20*, 359.

Schachman, H. K., Adler, J., Radding, C. M., Lehman, I. R., and Kornberg, A. (1960), *J. Biol. Chem. 235*, 3242.

Schaller, H., and Khorana, H. G. (1963), *J. Amer. Chem. Soc. 85*, 3841.

Smith, D. A., Martinez, A. M., and Ratliff, R. L. (1970), *Anal. Biochem. 38*, 85.

Vinograd, J., Morris, J., Davidson, N., and Dove, W. F., Jr. (1963), *Proc. Nat. Acad. Sci. U. S. 49*, 12.

Weimann, G., Schaller, H., and Khorana, H. G. (1963), *J. Amer. Chem. Soc. 85*, 3835.

Wells, R. D., and Blair, J. E. (1967), *J. Mol. Biol. 27*, 273.

Wells, R. D., Büchi, H., Kossel, H., Ohtsuka, E., and Khorana, H. G. (1967a), *J. Mol. Biol. 27*, 265.

Wells, R. D., Jacob, T. M., Narang, S. A., and Khorana, H. G. (1967b), *J. Mol. Biol. 27*, 237.

Wells, R. D., Larson, J. E., Grant, R. C., Shortle, B. E., and Cantor, C. R. (1970), *J. Mol. Biol. 54*, 465.

Wells, R. D., Ohtsuka, E., and Khorana, H. G. (1965), *J. Mol. Biol. 14*, 221.