Wong, K. L., and Kearns, D. R. (1974b), *Biopolymers 13*, 371-380.
Wong, K. L., Wong, Y. P., and Kearns, D. R. (1975), *Biopolymers 14*, 749-762.
Yarus, M., and Rashbaum, S. (1972), *Biochemistry 11*, 2043-2049.

Preparation and Properties of the Repeating Sequence Polymers $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$ and $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$†

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ABSTRACT: The repeating sequence polymer $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$ has been prepared using the chemically synthesized oligomers $d(A\text{-}G\text{-}C)_4$ and $d(C\text{-}T\text{-}G)_4$ and the DNA polymerase from *Micrococcus luteus*. The enzymatically synthesized polymer was used as template for preparation of $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$. Both deoxyribonucleotide polymers were characterized by nearest neighbor analyses, buoyant density measurements in cesium chloride and cesium sulfate, melting temperature, and circular dichroism (CD) spectra. The ribopolymers $r(A\text{-}I\text{-}C)_n \cdot r(I\text{-}C\text{-}U)_n$ and $r(A\text{-}G\text{-}C)_n \cdot r(G\text{-}C\text{-}U)_n$ were transcribed from $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$, and their CD spectra were compared with those of the respective deoxyribonucleotide polymers.

Model DNAs and RNAs have played important roles in the development of our present understanding of nucleic acid structure, function, and enzymology. Snythetic DNAs with repeating nucleotide sequences have been instrumental in elucidation of the genetic code (Khorana et al., 1966) and in determination of repressor binding properties (Riggs et al., 1972). Synthetic DNA properties, such as buoyant density, have been used to derive relationships that describe the corresponding properties of natural DNA (Gray et al., 1974). Another type of model DNA has been used to study the influence of nucleotide composition in one region of a duplex DNA on the properties of a remote but contiguous region (Burd et al., 1975). It is clear from all these studies that the properties of a DNA depend upon its nucleotide sequence. For a recent review of the influence of nucleotide sequence on DNA properties, see Wells and Wartell (1974). Here we report the synthesis of both the new repeating trinucleotide DNA $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$[1] and its analogue $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$. Both polymers have been characterized by nearest neighbor analysis, buoyant density in cesium chloride and cesium sulfate, melting temperature, circular dichroism (CD) spectra, and ability to serve as template for RNA polymerase. A preliminary report of some of this work has been presented previously (Ratliff et al., 1974).

Experimental Procedures

Chemicals and Reagents. Unlabeled deoxyribonucleoside mono- and triphosphates, ITP, and ^{14}C-labeled ribonucleoside triphosphates were obtained from Schwarz/Mann and unlabeled ribonucleoside triphosphates from P-L Biochemicals. The α-^{32}P labeled deoxyribonucleoside and ribonucleoside triphosphates came from New England Nuclear. The dITP and [α-^{32}P]dITP were prepared by nitrous acid deamination of dATP and [α-^{32}P]dATP, respectively (Inman and Baldwin, 1964). 2-Mesitylenesulfonyl chloride, 2,4,6-triisopropylbenzenesulfonyl chloride, and *N,N'*-dicyclohexylcarbodiimide were purchased from Aldrich Chemical Co. The sulfonyl chloride reagents were recrystallized from petroleum ether immediately before use. Hydracrylonitrile (Eastman) was fractionally distilled under vacuum. Pyridine used as solvent in the chemical syntheses was distilled successively from chlorosulfonic acid and solid potassium hydroxide, then dried over a molecular sieve (Linde, type 3A). Cesium chloride and cesium sulfate were obtained from Harshaw Chemical Co. Agarose for gel permeation chromatography (Bio-Rad Laboratories) was purchased from Calbiochem. Whatman DE32 cellulose was employed for ion-exchange chromatography of chemically synthesized oligonucleotides.

Enzymes. Micrococcal DNase and alkaline phosphatase were purchased from Worthington Biochemical Corp. Snake venom phosphodiesterase was obtained from Calbiochem. Spleen phsophodiesterase was prepared by the methods of Hilmoe (1960) and Richardson and Kornberg (1964). *Micrococcus luteus* DNA polymerase, prepared ac-

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[1] The abbreviation notation for complementary double-stranded polydeoxyribotrinucleotides is in one of the two general forms recommended by the IUPAC-IUB Combined Commission on Biochemical Nomenclature (1970) and further incorporates an alphabetization convention 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 (Solvent: Ethanol–1 *M* NH_4OAc, pH 7.5, 7:3, descending).

Compound	R_f	Compound	R_f
d-p-BzA-p-*i*BuG	0.55	d(pApGpC)	0.06
d(pApG)	0.13	d(ApGpC)	0.24
d-CNEt-p-AnC-p-T	0.90	d-p-AnC-p-T-p-*i*BuG	0.19
d-p-AnC-p-T	0.42	d(pCpTpG)	0.10
d-p-BzA-p-*i*BuG-p-AnC	0.43	d(CpTpG)	0.43

cording to the procedures of Zimmerman (1966) and Harwood et al. (1970), had a specific activity of 600 units/mg of protein [$d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ assay]. RNA polymerase, from *Escherichia coli* was purified by a modification of the procedure of Chamberlain and Berg (1962) and had a specific activity of 3000 units/mg of protein.

Analytical Procedures and Physical Measurements. Paper chromatography of chemical intermediates was done on Whatman No. 40 paper by the descending technique using the solvent system ethanol–1 *M* NH_4OAc (pH 7.5, 7:3). Chromatographic mobilities of various nucleotides and protected derivatives are given in Table I.

Paper electrophoretic separations were performed at pH 3.5 (0.05 *M* formate buffer) on Whatman 3MM strips in a Savant high-voltage tank-type apparatus which employs isoparaffinic coolant. After electrophoresis, dTp and dIp were separated by paper chromatography in a solvent system of isobutyric acid–concentrated NH_4OH–water (66:1:33); 2′(3′)-Ip and 2′(3′)-Up were separated similarly in a solvent composed of 1-propanol (20 ml)–0.1 *M* sodium phosphate (pH 6.8) containing 60% (w/v) ammonium sulfate (1 l.).

Size distributions of synthetic polynucleotides were determined by chromatography through calibrated Bio-Gel A-50m and A-5m columns (8 $cm^2 \times 82.4$ cm) and equilibrated with 50 m*M* triethylammonium bicarbonate (pH 8.5), as described previously (Hayes and Mitchell, 1969; Jang and Bartl, 1971).

Analytical buoyant density centrifugation was carried out essentially as described previously (Ratliff et al., 1973). All buoyant density measurements in cesium chloride included a marker of $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$, with a buoyant density of 1.672 g/ml relative to *E. coli* DNA (1.703 g/ml) (Wells and Blair, 1967). The buoyant density of $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ in cesium sulfate was determined using phage T-4 DNA (1.444 g/ml) (Wells and Larson, 1972) as marker. We found that $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$ could not be differentiated from T-4 DNA in cesium sulfate; therefore, $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ with a buoyant density of 1.425 g/ml (Wells and Larson, 1972) was used as a marker to determine the buoyant density of this polymer in cesium sulfate. Values of β used to calculate the buoyant density were 1.19×10^9 for cesium chloride (Ifft et al., 1961) and 7.13×10^8 for cesium sulfate (Erickson and Szybalski, 1964).

Absorption spectra and melting temperature determinations were obtained with a Cary Model 14 spectrophotometer equipped as previously described (Gray et al., 1973). The CD data were obtained with a Cary Model 61 circular dichrometer. Calibration of the circular dichrometer, instrument operation, on-line computer acquisition of data, and control of sample temperatures have been described (Gray et al., 1973). The CD data are presented as CD/mole of monomer: $\epsilon_L - \epsilon_R$. Digitized CD data are plotted in the figures to show directly the random error due to noise in each spectrum. The CD and absorption data obtained at elevated temperatures were corrected for volume expansion of the sample solutions, which was 3% at most. No correction was made for evaporation, which was no more than 3%.

For determination of melting temperatures (T_m), absorption, and circular dichroism (CD) spectra, all four polymers were dialyzed at 4° against several changes of a series of three solutions over a 4-day period: (a) 0.5 *M* NaCl, 0.01 *M* EDTA, and 0.005 *M* Na_2HPO_4 (0.01 *M* Na^+) adjusted to pH 7.0 with H_3PO_4; (b) 0.5 *M* NaCl and sodium phosphate buffer (0.01 *M* Na^+) (pH 7.0); and (c) sodium phosphate buffer (0.01 *M* Na^+) (pH 7.0). Most CD and absorption spectra were obtained with the polymers in this final medium. Prior to addition of ethanol to the $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ and $r(A\text{-}G\text{-}C)_n \cdot r(G\text{-}C\text{-}U)_n$ samples, these were either dialyzed or diluted into sodium phosphate buffer (5×10^{-4} to 1×10^{-3} *M* Na^+) (pH 7.0). Solutions containing ethanol were prepared by adding reagent-quality absolute ethanol (U.S. Industrial Chemicals Co.) to weighed aliquots of the polymer solutions to give the desired percent ethanol by weight. Solutions of $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ containing up to 20% NaCl by weight were prepared by adding solid NaCl to the polymer in 0.001 *M* Na^+ (phosphate buffer). Sample concentrations in aqueous solution were determined from measured optical densities of the stock solutions and molar extinction coefficients. Extinction coefficients, determined as for previous polymers (Gray et al., 1973), were 6630 l./(mol cm) at 256 nm for $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$, 5170 at 252 nm for $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$, 7270 at 257 nm for $r(A\text{-}G\text{-}C)_n \cdot r(G\text{-}C\text{-}U)_n$, and 5660 at 253 nm for $r(A\text{-}I\text{-}C)_n \cdot r(I\text{-}C\text{-}U)_n$. Polymer concentrations in diluted samples containing ethanol were calculated from the known stock concentrations, the measured weight percent ethanol concentrations, and the known densities of water–ethanol mixtures.

Nearest neighbor analyses of the enzymatically synthesized polymers were done according to the procedures of Josse et al. (1961) and Ratliff et al. (1968, 1973). The method of following the kinetics of transcription of $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$ by *E. coli* RNA polymerase was essentially that described by Morgan (1970).

Chemical Synthesis of Oligonucleotide Templates. Preparation and chemical polymerization of d(A-G-C) and d(C-T-G) sequences from protected deoxynucleoside 5′-monophosphates were modeled on published procedures of Narang et al. (1967a,b). Details of the method have been published perviously by Ratliff et al. (1973). The protected monomer *N*-anisoyl-$C^{3'}$-isobutyryldeoxycytidine 5′-monophosphate [d-p-AnC(*i*Bu)] was prepared by shaking a suspension of pyridinium *N*-anisoyldeoxycytidine 5′-monophosphate in anhydrous pyridine with isobutyric anhydride for 24 hr. The solution was concentrated in vacuo and the residue dried by repeated evaporations of added dry pyridine. The protected nucleotide was isolated in solid form by dropwise addition of its solution in dry pyridine to 70 volumes of dry ether. The 5′-phosphomonoester and end groups of the mono- and dinucleotides were protected by esterification with hydracrylonitrile and dicyclohexylcarbodiimide (Narang et al., 1967a).

Dinucleotide d-(cyanoethyl)-p-(*N*-benzoyl)A-p-($N,O^{3'}$)-di(isobutyryl)G was synthesized by shaking a solution of pyridinium d-CNEt-p-BzA (2.6 mmol), pyridinium d-p-di(*i*Bu)G (3.4 mmol), and dicyclohexylcarbodiimide (4.6 g, 20 mmol) in dry pyridine (10 ml) with dry pyridinium Dowex 50W-X4 resin (1 g) at room temperature for 3.5

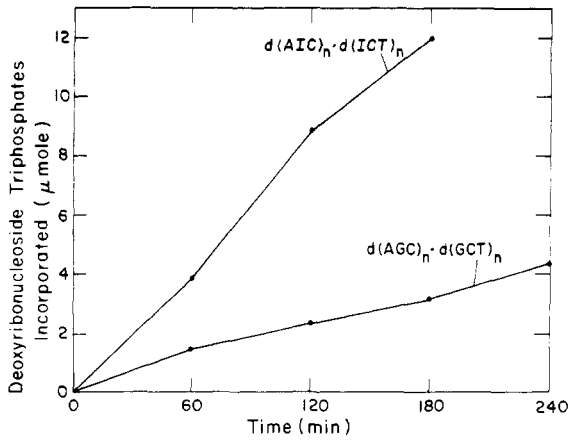

FIGURE 1: Kinetics of d(A-I-C)$_n$·d(I-C-T)$_n$ and d(A-G-C)$_n$·d(G-C-T)$_n$ syntheses from d(A-I-C)$_n$·d(I-C-T)$_n$ as template. The reaction mixtures were 0.5-ml aliquots taken from a large reaction which contained the following components in a total volume of 10 ml: 50 m*M* Tris-HCl buffer (pH 8.6), 10 m*M* $MgCl_2$, 1 m*M* 2-mercaptoethanol, 5 m*M* each of dATP and dTTP, 10 m*M* each of dCTP and dITP or dGTP, 0.39 mono-μmol of d(A-I-C)$_n$·d(I-C-T)$_n$, and 1000 units of *M. luteus* DNA polymerase. Each contained [α-^{32}P]dCTP as the labeled substrate. All reactions were at 37°, and the formation of acid-insoluble product and the isolation of unlabeled product were done as described in the Experimental Procedures.

days. The yield of d-p-BzA-p-*i*BuG isolated after work-up and ion-exchange chromatography on DEAE-cellulose (Ratliff et al., 1973) was 16740 A_{279} units [0.585 mmol, 22% based on $\epsilon(P)_{279}$ 14.3 × 10^3 l./(g-atom of P cm) in water].

Dinucleotide d-(cyanoethyl)-p-(*N*-anisoyl)C-p-(*O*^{3}'-acetyl)T was synthesized by shaking pyridinium d-CNEt-p-AnC (1.02 mmol), pyridinium d-p-T(Ac) (1.56 mmol), and dicyclohexylcarbodiimide (1.3 g, 5.7 mmol) in dry pyridine solution (5 ml) with dry pyridinium Dowex 50W-X4 resin (0.8 g) for 3 days. The yield of d-p-AnC-p-T isolated after work-up and ion-exchange chromatography was 8950 A_{275} units [0.350 mmol, 35%, based on $\epsilon(P)_{275}$ 12.8 × 10^3 l./(g-atom of P cm) measured at pH 7.0].

Trinucleotide d-[CNEt-p-BzA-p-*i*BuG-p-AnC(*i*Bu)] was synthesized by treating a mixture of d-(CNEt-p-BzA-p-*i*BuG) (0.565 mmol) and d-p-AnC(*i*Bu) (3.43 mmol) in dry pyridine solution (12 ml) with triisopropylbenzenesulfonyl chloride (2.59 g, 8.5 mmol) for 5 hr. The yield of d-p-BzA-p-*i*BuG-p-AnC isolated by ion-exchange chromatography after work-up was 4590 A_{282} units [0.106 mmol, 19%, based on $\epsilon(P)_{282}$ 14.4 × 10^3 l./(g-atom of P cm) measured in water].

Trinucleotide d-[CNEt-p-AnC-p-T-p-di(*i*Bu)G] was synthesized by treating a dry pyridine solution (10 ml) which contained pyridinium d-(CNEt-p-AnC-p-T) (0.319 mmol) and pyridinium d-p-di(*i*Bu)G (3.43 mmol) with triisopropylbenzenesulfonyl chloride (2.76 g, 9.2 mmol) for 6 hr. The yield of d-p-AnC-p-T-p-*i*BuG isolated by ion-exchange chromatography was 4810 A_{260} units [0.162 mmol, 51%, based on $\epsilon(P)_{260}$ 9910 l./(g-atom of P cm) in water].

Characterization of Trimers. For analysis, the chemically synthesized trinucleotides d(pApGpC) and d(pCpTpG) were first dephosphorylated with *E. coli* alkaline phosphatase and then degraded further with either snake venom or spleen phosphodiesterase. Snake venom diesterase digestion of d(ApGpC) resulted in the release of d-pC, d-pG, and d-Ado in the molar ratio 0.90:0.95:1.00; spleen diesterase digestion liberated d-Ap, d-Gp, and d-Cyd in the ratio 1.00:0.90:0.96. Venom diesterase digestion of d(CpTpG) produced d-pG, d-pT, and d-Cyd in the molar ratio 0.90:0.94:1.00, while spleen diesterase digestion gave d-Cp, d-Tp, and d-Guo in the ratio 1.00:0.97:0.95.

Table II: Nearest Neighbor Analyses of d(A-I-C)$_n$·d(I-C-T)$_n$.[a]

α-^{32}P-Labeled Triphosphate	Radioactivity in Deoxyribonucleoside 3'-Phosphate							
	dCp		dAp		dIp		dTp	
	cpm	%	cpm	%	cpm	%	cpm	%
dATP	179500	98.4	800	0.4	900	0.5	1200	0.7
dCTP	200	0.2	300	0.2	128500	99.2	600	0.4
dITP	100	0.3	18400	49.2	0	0.0	18900	50.5
dTTP	484200	99.6	200	0.0	500	0.1	1400	0.3

[a] For the analyses of d(A-I-C)$_n$·d(I-C-T)$_n$ and d(A-G-C)$_n$·d(G-C-T)$_n$ (see Table III), four 0.5-ml aliquots were removed from each of the large reaction mixtures (see Figure 1), and one of the deoxyribonucleoside triphosphates labeled with ^{32}P in the α-phosphate was added to each. The reactions were incubated at 37° for 3 hr and terminated by placing in a boiling water bath for 10 min. The samples were dialyzed against 0.01 *M* sodium pyrophosphate and then distilled water to remove unreacted triphosphates. The polymers were degraded to their 3'-mononucleotides using 8 m*M* Tris-HCl buffer (pH 8.6), 4 m*M* $CaCl_2$, and 1800 units of micrococcal DNase. After overnight digestion at 37°, the pH of each solution was adjusted to 7.0 with 0.1 *N* HCl, and 0.5 unit of calf spleen phosphodiesterase was added initially and at the end of the first and second hours. The total time of incubation was 3 hr at 37°. The solutions were evaporated to dryness, taken up in 50 μl of a mixture which contained the four 3'-dNMPs, and electrophoresed on Whatman 3MM paper at 4000 V for 3 hr in 0.05 *M* ammonium formate buffer (pH 3.5) (Markham and Smith, 1952). The radioactive spots were located with a radiochromatogram scanner. All counts per minute (cpm) have been rounded off to the nearest 100.

Polymerization of the trihexylammonium salts of both the trimers d-p-BzA-p-*i*BuG-p-AnC and d-p-AnC-p-T-p-*i*BuG, as well as subsequent fractionation of the mixtures of oligomeric products, was carried out by procedures described by Ratliff et al. (1973).

Enzymatic Synthesis of d(A-I-C)$_n$·d(I-C-T)$_n$. The reaction mixture (10 ml) contained 50 m*M* Tris-HCl buffer (pH 8.6), 5 m*M* $MgCl_2$, 1.0 m*M* 2-mercaptoethanol, 1.7 m*M* each of dATP and dTTP, 3.3 m*M* each of dCTP and dITP, 50 mono-nmol each of the chemically synthesized oligodeoxyribonucleotides d(A-G-C)$_4$ and d(C-T-G)$_4$, and 300 units of *M. luteus* DNA polymerase. After a 0.2-ml sample was removed, [α-^{32}P]dATP was added to it. Both reaction mixtures were incubated at 15° for 24 hr, then transferred to a 37° bath for 3 hr. Radioisotope incorporation into acid-insoluble material (Hayes et al., 1966) indicated that 40% of the triphosphates had been utilized in 3 hr. A total of 3.29 mono-μmol of d(A-I-C)$_n$·d(I-C-T)$_n$ was isolated after extraction of the reaction mixture with chloroform–isoamyl alcohol and successive dialysis against 0.01 *M* sodium pyrophosphate, 0.015 *M* NaCl–0.0015 *M* sodium citrate (pH 7.0), and finally distilled water. Nearest neighbor analyses of the [^{32}P]dAMP-containing polymer indicated that 99% of the label was located in dCp. Less than 1% was detected in dTp. The isolated d(A-I-C)$_n$·d(I-C-T)$_n$ was fractionated by chromatography through a Bio-Gel A-50m column, and polymer with an average size of 900 base pairs was used for preparation of more of the same polymer, as well as for the synthesis of d(A-G-C)$_n$·d(G-C-T)$_n$.

Transcription of d(A-I-C)$_n$·d(I-C-T)$_n$ to Give r(A-I-C)$_n$·r(I-C-U)$_n$ and r(A-G-C)$_n$·r(G-C-U)$_n$. Two 5-ml reac-

Table III: Nearest Neighbor Analyses of d(A-G-C)$_n$·d(G-C-T)$_n$.[a]

α-^{32}P-Labeled Triphosphate	Radioactivity in Deoxyribonucleoside 3'-Phosphate							
	dCp		dAp		dGp		dTp	
	cpm	%	cpm	%	cpm	%	cpm	%
dATP	391900	98.3	400	0.1	800	0.2	5600	1.4
dCTP	300	0.2	300	0.2	159000	99.5	200	0.1
dGTP	400	0.2	115300	49.3	500	0.2	117500	50.3
dTTP	417300	99.5	700	0.2	600	0.1	800	0.2

[a] See footnote in Table II for details.

Table IV: Nearest Neighbor Analyses of r(A-I-C)$_n$·r(I-C-U)$_n$.[a]

α-^{32}P-Labeled Triphosphate	Radioactivity in Ribonucleoside 2'(3')-Phosphate							
	Cp		Ap		Ip		Up	
	cpm	%	cpm	%	cpm	%	cpm	%
ATP	239000	97.9	1900	0.8	1100	0.4	2100	0.9
CTP	700	0.2	1900	0.4	352370	99.0	900	0.3
UTP	353300	98.8	400	0.1	2100	0.6	1700	0.5

[a] Three or four 0.2-ml aliquots were removed from the two 5-ml transcription reactions containing 3.87 mono-μmol of d(A-I-C)$_n$·d(I-C-T)$_n$, 1.7 m*M* each of rATP and rUTP, 3.3 m*M* each of rCTP and rITP or rGTP, 40 m*M* Tris-HCl buffer (pH 8.0), 4 m*M* $MgCl_2$, 1 m*M* $MnCl_2$, 12 m*M* 2-mercaptoethanol, and 4000 units of RNA polymerase. α-^{32}P-Labeled rATP, rCTP, rGTP, and rUTP were each added in turn to label the RNA. Incubation was at 37° for 3 hr. The ^{32}P-labeled reactions were dialyzed against 0.01 *M* sodium pyrophosphate (pH 7.0) and distilled water. The samples were made 0.3 *N* in KOH, incubated at 37° for 18 hr, neutralized with HCl, and evaporated to dryness. Each sample was taken up in 50 μl of a solution containing each of the four ribonucleoside 2'(3')-monophosphates, and the 2'(3')-ribonucleotides were separated by paper electrophoresis using the procedure described in Table II.

tions were prepared containing 3.87 mono-μmol of d(A-I-C)$_n$·d(I-C-T)$_n$, 1.7 m*M* each of rATP and rUTP, 3.3 m*M* each of rCTP and rITP or rGTP, 40 m*M* Tris-HCl buffer (pH 8.0), 4 m*M* $MgCl_2$, 1.0 m*M* $MnCl_2$, 12 m*M* 2-mercaptoethanol, and 4000 units of RNA polymerase. Incubation was at 37° for 3 hr. The two ribopolymers were isolated as previously described (Gray et al., 1973). The yield of r(A-I-C)$_n$·r(I-C-U)$_n$ was 3.17 mono-μmol, having an average size of 135 base pairs as determined by gel-filtration chromatography. The yield of r(A-G-C)$_n$·r(G-C-U)$_n$ was 1.0 mono-μmol, having an average size of 160 base pairs.

Results

In attempts to prepare the repeating trinucleotide polymer d(A-G-C)$_n$·d(G-C-T)$_n$ with the DNA polymerases from either *E. coli* or *M. luteus* and a mixture of the chemically synthesized oligomers d(A-G-C)$_4$ and d(C-T-G)$_4$ as template, we observed only a five-fold synthesis over the initial template concentration. When the isolated polymer of d(A-G-C)$_n$·d(G-C-T)$_n$ was used as template for preparation of more of the same polymer, no net synthesis was observed. On the other hand, polymer d(A-I-C)$_n$·d(I-C-T)$_n$ was prepared in good yield using the chemically prepared oligomers d(A-G-C)$_4$ and d(C-T-G)$_4$ as primer and *M. luteus* DNA polymerase. This polymer then served as template to direct the synthesis of either more d(A-I-C)$_n$·d(I-C-T)$_n$ or d(A-G-C)$_n$·d(G-C-T)$_n$ catalyzed by the same enzyme. The kinetics of polymer-directed synthesis of d(A-I-C)$_n$·d(I-C-T)$_n$ and d(A-G-C)$_n$·d(G-C-T)$_n$ are shown in Figure 1, where total incorporation of deoxyribonucleoside triphosphate is determined from the percent incorporation into acid-insoluble material of the [α-^{32}P]dCTP. From the unlabeled reactions described in the legend to Figure 1, 3.77 mono-μmol of d(A-G-C)$_n$·d(G-C-T)$_n$ and 16.44 mono-μmol of d(A-I-C)$_n$·d(I-C-T)$_n$ were isolated, as determined by absorption measurements.

Fractionation of d(A-I-C)$_n$·d(I-C-T)$_n$ by chromatography through a calibrated A-50m column showed that 55% of the polymer had a size range of 3000–5000 base pairs, 20% had a size range of 2000–2800 base pairs, and 25% had a size range of 170–1700 base pairs. The size range of d(A-G-C)$_n$·d(G-C-T)$_n$ was 400–4800 base pairs, with 60% of the polymer having a size range of 2400–4800 base pairs.

The results of nearest neighbor analyses of both deoxyribonucleotide polymers are summarized in Tables II and III. The analysis of d(A-I-C)$_n$·d(I-C-T)$_n$ is that expected from the presumed sequence. That for d(A-G-C)$_n$·d(G-C-T)$_n$ does not rule out possible contamination by a small amount of d(A-T)$_n$·d(A-T)$_n$ arising de novo. However, the procedure of Morgan et al. (1974) for assay of contaminating d(A-T)$_n$·d(A-T)$_n$ shows that neither polymer contained d(A-T)$_n$·d(A-T)$_n$, since no [^{14}C]UTP was incorporated during a 60-min incubation period.

Nearest neighbor analyses of the products of transcription of d(A-I-C)$_n$·d(I-C-T)$_n$ by *E. coli* RNA polymerase (Tables IV and V) indicate that both the r(A-I-C)$_n$·r(I-C-U)$_n$ and r(A-G-C)$_n$·r(G-C-U)$_n$ sequences are faithfully transcribed. The kinetics of transcription of ribopolymers containing inosine are shown in Figure 2 and those for transcription of ribopolymers containing guanosine in Figure 3. In both figures, the kinetics of transcription of one strand of the polydeoxynucleotide template alone and of both strands simultaneously are shown. When transcription was limited to only one strand of the DNA template (by omission of one nucleoside triphosphate), less than a onefold transcription of the template was observed. When both strands were transcribed simultaneously, ribonucleoside triphosphate incorporation equivalent to a onefold net synthesis took place in 2 hr.

A scan of a buoyant density experiment in which d(A-T)$_n$·d(A-T)$_n$ and d(A-I-C)$_n$·d(I-C-T)$_n$ were centrifuged to equilibrium in neutral cesium chloride is shown in Figure 4. Using a value of 1.672 g/ml for d(A-T)$_n$·d(A-T)$_n$, the buoyant density of d(A-I-C)$_n$·d(I-C-T)$_n$ was calculated to be 1.716 g/ml. The buoyant density of d(A-G-C)$_n$·d(G-C-T)$_n$ using the same DNA marker was determined to be 1.718 g/ml. The buoyant densities of the two synthetic polymers in neutral cesium sulfate were found to be 1.444 g/ml for d(A-I-C)$_n$·d(I-C-T)$_n$, compared to 1.425 for d(A-

Table V: Nearest Neighbor Analyses of $r(A\text{-}G\text{-}C)_n \cdot r(G\text{-}C\text{-}U)_n$.[a]

α-^{32}P-Labeled Triphosphate	Radioactivity in Ribonucleoside 2'(3')-Phosphate							
	Cp		Ap		Gp		Up	
	cpm	%	cpm	%	cpm	%	cpm	%
ATP	218300	99.4	1100	0.5	100	0.1	200	0.1
CTP	700	0.2	900	0.2	375600	99.4	800	0.2
GTP	1700	0.6	139400	50.3	300	0.1	135900	49.0
UTP	370300	98.9	1000	0.3	500	0.1	2600	0.7

[a] See footnote in Table IV for details.

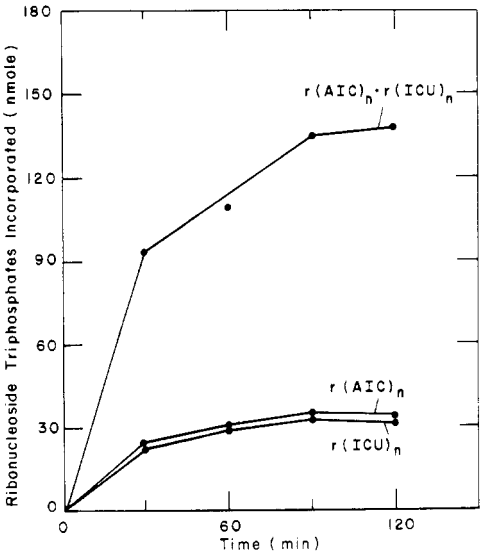

FIGURE 2: Kinetics of transcription of $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$ by the RNA polymerase from *E. coli*. Each reaction mixture contained 40 m*M* Tris-HCl buffer (pH 8.0), 4 m*M* $MgCl_2$, 1 m*M* $MnCl_2$, 12 m*M* 2-mercaptoethanol, 100 μ*M* of each ribonucleoside triphosphate for the transcription of one strand and 85 μ*M* each of rATP and rUTP and 165 μ*M* each of rCTP and rITP for the transcription of both strands, 180 mono-nmol of $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$ as template, and 200 units of RNA polymerase in a final volume of 1 ml. The reactions were incubated at 37°, and the incorporation of [^{14}C]rCTP into acid-insoluble material was determined. The total amount of ribonucleotide triphosphates incorporated was determined from the percent of labeled triphosphate incorporated into acid-insoluble material.

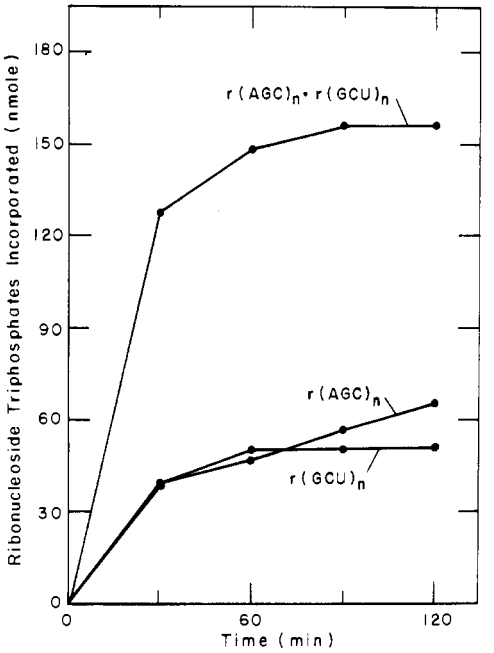

FIGURE 3: The conditions were the same as those described in Figure 2 except that rGTP has been substituted for rITP in each of the reaction mixtures.

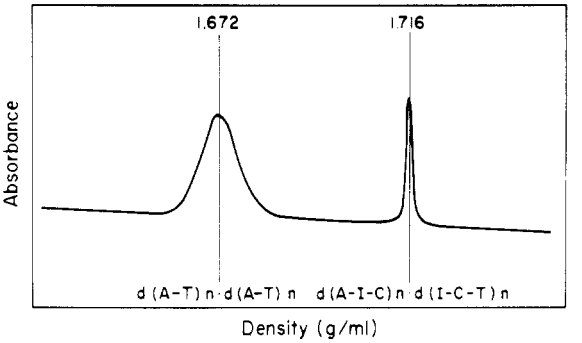

FIGURE 4: Cesium chloride buoyant density centrifugation of $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$. The solvent for cesium chloride was 0.017 *M* Tris (pH 8.0) and 0.003 *M* EDTA. Centrifugation was at 44000 rpm at 25° for 26 hr. A density marker of $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ was added.

$T)_n \cdot d(A\text{-}T)_n$, and 1.436 g/ml for $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$, compared to T-4 DNA (1.444 g/ml).

Melting profiles of the polymers are shown in Figure 5. Each shows a single hyperchromic increase, with melting temperatures of 36.5, 37.0, 74.5, and 83.0° for $r(A\text{-}I\text{-}C)_n \cdot r(I\text{-}C\text{-}U)_n$, $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$, $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$, and $r(A\text{-}G\text{-}C)_n \cdot r(G\text{-}C\text{-}U)_n$, respectively. After heat-denaturation and cooling of the samples for 15 min in a cell holder that was quenched to 20°, three of the four samples [$d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ and the two RNAs] annealed, losing 85% of the hyperchromicities and regaining the features of their native CD spectra. However, $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$ was an exception; it annealed only very slowly under the same conditions (20°, 0.01 *M* Na^+, phosphate buffer, pH 7.0).

The absorption and CD spectra of the four native polymers at 20° are shown in Figures 6 and 7; spectra of the polymers heat-denatured at 90° are shown in Figures 8 and 9. Differences in the absorption properties of hypoxanthine and guanine result in dramatic differences in the spectral properties of native double-stranded polymers that contain these bases. Differences due to hypoxanthine and guanine are evident even in the absorption and CD spectra of the denatured polymers, while the spectra of the two denatured guanine-containing polymers become similar to one another, as do the spectra of the two denatured hypoxanthine-containing polymers. Note that the CD spectra of both RNAs (Figure 7) are much greater in magnitude than are the spectra of the corresponding native DNAs (Figure 6).

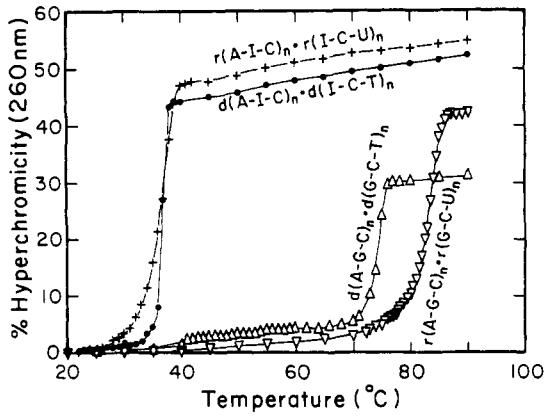

FIGURE 5: Melting profiles of the four polymers in 0.01 *M* Na^+ (phosphate buffer, pH 7.0). The percent hyperchromicity values at each temperature (T) were evaluated as 100 [OD(T) − OD(20°)]/OD(20°), where OD is the optical density at 260 nm of the sample at the indicated temperature. OD(T) values were corrected for the decrease in concentration due to volume expansion.

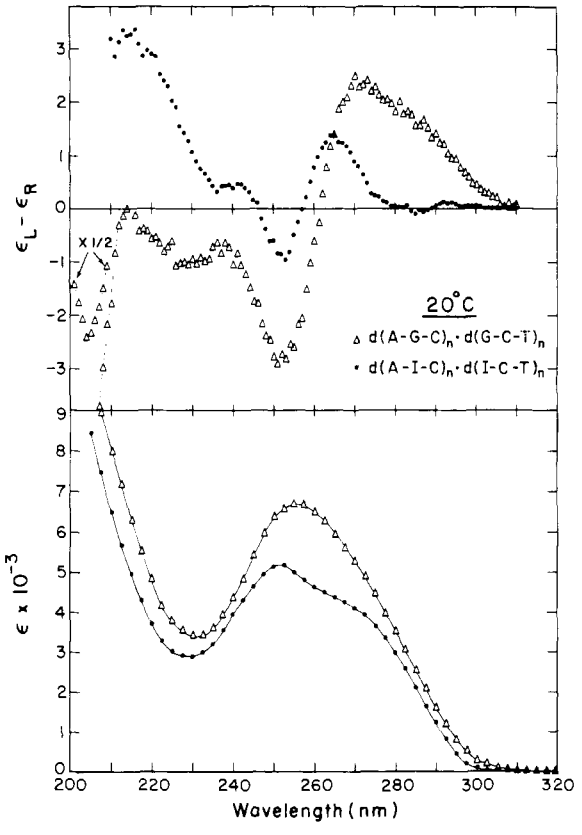

FIGURE 6: Circular dichroism (upper panel) and absorption (lower panel) spectra at 20° of d(A-G-C)$_n$·d(G-C-T)$_n$ (-Δ-) and d(A-I-C)$_n$·d(I-C-T)$_n$ (-●-) in 0.01 *M* Na^+ (phosphate buffer, pH 7.0).

This is generally the case when comparing CD spectra of RNAs and DNAs in aqueous solutions.

The CD spectrum of native r(A-G-C)$_n$·r(G-C-U)$_n$ shown in the top panel of Figure 7 has features like those of the spectra of other synthetic RNAs we have measured, r(A-C)$_n$·r(G-U)$_n$ (Gray and Ratliff, 1975) and r(G-C)$_n$·r(G-C)$_n$ (Gray et al., 1971), with a long wavelength negative band, a large positive band near 260 nm, and two negative bands at shorter wavelengths, including a large band near 210 nm. These features are similar to those of double-stranded natural RNAs (Gratzer and Richards, 1971;

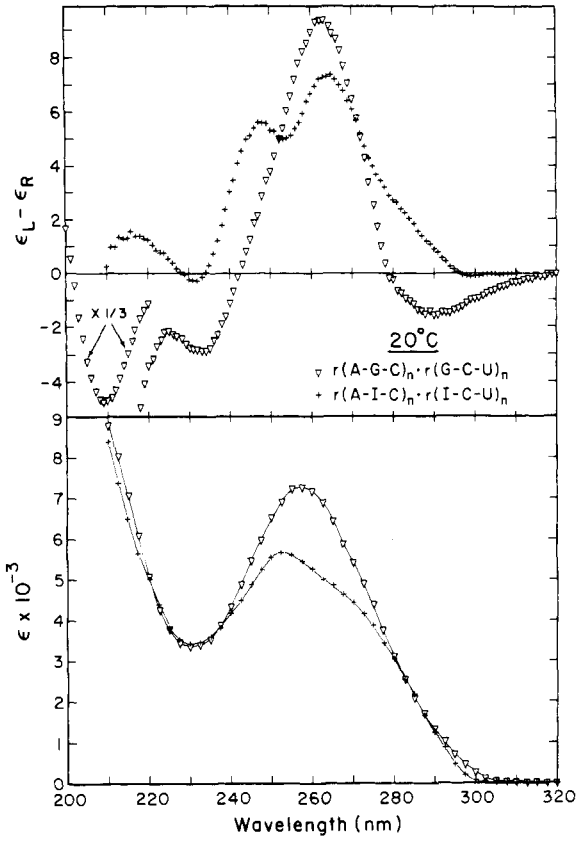

FIGURE 7: Circular dichroism and absorption spectra at 20° of r(A-G-C)$_n$·r(G-C-U)$_n$ (-▽-) and r(A-I-C)$_n$·r(I-C-U)$_n$ (-+-) in 0.01 *M* Na^+ (phosphate buffer, pH 7.0).

Wells and Yang, 1974), but they are not universal for all synthetic RNAs. It has been suggested that the occurrence of a major long wavelength negative band in the spectra of double-stranded RNAs may be due to G·C base pairs (Gray et al., 1971). Thus, we interpret the absence of a large negative band at long wavelengths in the r(A-I-C)$_n$·r(I-C-U)$_n$ spectrum (top panel of Figure 7) to be due to the absorption properties of hypoxanthine, not due to any unusual conformation of the I-containing polymer compared with the G-containing polymer. We also note that the CD spectrum of r(A-I-C)$_n$·r(I-C-U)$_n$ is similar to a previously published spectrum of r(C-I)$_n$·r(C-I)$_n$ (Mitsui et al., 1970), a fact that makes it likely that the two I-containing ribopolymers are in a similar conformation. We see no reason to suggest that the conformation of these polymers is unusual.

Our CD spectrum of d(A-I-C)$_n$·d(I-C-T)$_n$ also has some similarity to the spectrum of d(C-I)$_n$·d(C-I)$_n$ (Mitsui et al., 1970) in the magnitude of the two large positive bands at about 265 and 215 nm. The d(A-I-C)$_n$·d(I-C-T)$_n$ spectrum has much smaller bands in the region above 275 nm than do most DNAs, although the spectrum does not become negative in this region as does the d(C-I)$_n$·d(C-I)$_n$ spectrum. Arnott et al. (1974) have shown that DNAs containing alternating purine–pyrimidine sequences can exist in a novel D conformation (45° rotation per residue and 3.03 Å rise per residue) that is different from the classical B conformation (36° rotation per residue and 3.38 Å rise per residue; the DNA A, B, and C conformations have been reviewed by Arnott, 1970). We do not know whether differences in the CD spectra of d(C-I)$_n$·d(C-I)$_n$ and d(A-I-C)$_n$·d(I-C-T)$_n$ are due partly to differences in solution conformations of these polymers or are due solely to differences in base se-

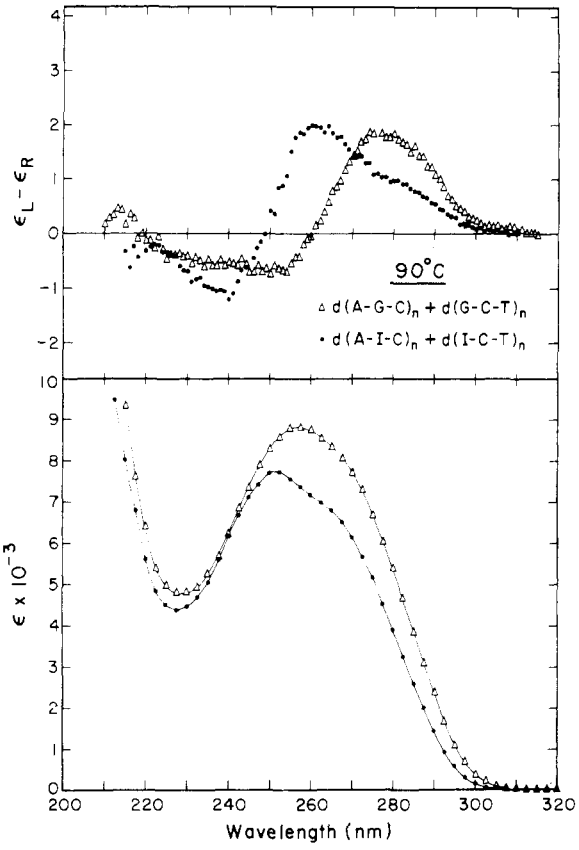

FIGURE 8: Circular dichroism and absorption spectra at 90° of d(A-G-C)$_n$·d(G-C-T)$_n$ (-△-) and d(A-I-C)$_n$·d(I-C-T)$_n$ (-●-) in 0.01 *M* Na^+ (phosphate buffer, pH 7.0).

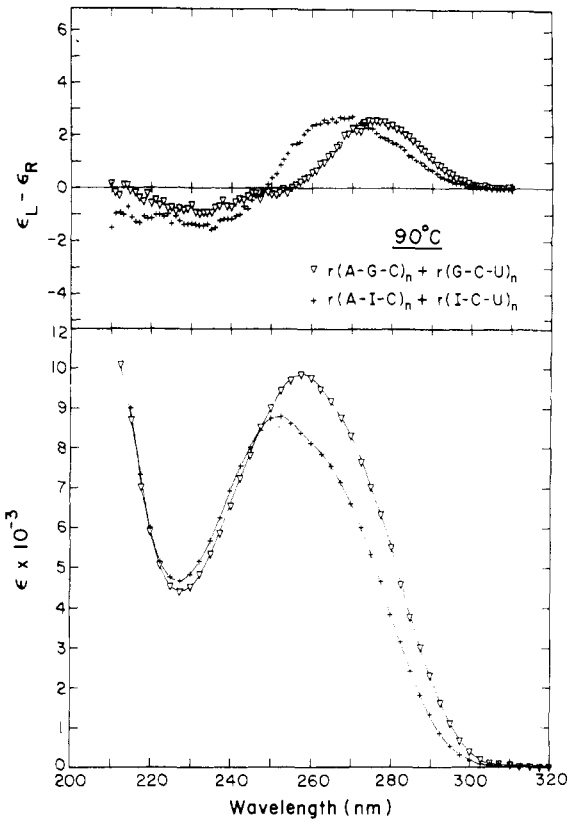

FIGURE 9: Circular dichroism and absorption spectra at 90° of r(A-G-C)$_n$·r(G-C-U)$_n$ (-▽-) and r(A-I-C)$_n$·r(I-C-U)$_n$ (-+-) in 0.01 *M* Na^+ (phosphate buffer, pH 7.0).

quence. The differences between the spectral properties of the two repeating triplet DNAs, d(A-I-C)$_n$·d(I-C-T)$_n$ and d(A-G-C)$_n$·d(G-C-T)$_n$, are most simply attributed to differences in absorption properties of the guanine and hypoxanthine bases.

We have also investigated the effect of ethanol upon the four polymer spectra. Previous work has shown that natural and synthetic DNAs can undergo two transitions upon addition of ethanol. At intermediate ethanol concentrations, the positive bands above 260 nm decrease in magnitude; this change has been attributed by Girod et al. (1973) to a conformational change from the B conformation to one nearer the C conformation (38.6° rotation per residue and 3.32 Å rise per residue). Above about 60% ethanol, the spectra of DNAs can obtain the characteristics of RNA spectra, likely due to a conformational change of the DNAs to the DNA A conformation (32.7° rotation per residue and 2.56 Å rise per residue). The CD spectra of RNAs are not as sensitive to the addition of ethanol (Gray and Ratliff, 1975; Ivanov et al., 1973, 1974). In Figure 10 are shown the CD spectra of d(A-G-C)$_n$·d(G-C-T)$_n$ at increasing ethanol concentrations. Up to 60% ethanol by weight, the positive bands above 260 nm decrease. Then, between 60 and 70% ethanol, the CD spectrum acquires a large positive band, which is a characteristic of the r(A-G-C)$_n$·r(G-C-U)$_n$ spectrum. The r(A-G-C)$_n$·r(G-C-U)$_n$ is altered in a more minor way by ethanol (compare the spectra in Figures 7 and 10). The negative band at 210 nm in the spectrum of d(A-G-C)$_n$·d(G-C-T)$_n$ in ethanol does not become as large as the 210-nm band in the RNA spectrum, unlike the 210-nm band in the spectrum of d(A-C)$_n$·d(G-T)$_n$ in ethanol which does match the corresponding RNA spectral band (Gray and Ratliff, 1975). Nevertheless, it seems clear that the d(A-G-C)$_n$·d(G-C-T)$_n$ polymer does acquire an A conformation in the presence of ethanol. This observation of a transition to an A conformation for a synthetic DNA of 66% (G + C) is in agreement with the results of infrared dichroism studies of films of natural DNAs by Brahms et al. (1973). These authors found that, for natural DNAs, there was a positive correlation of G + C content with the ability to acquire the A conformation at low relative humidities.

Finally, we tested the possibility that d(A-G-C)$_n$·d(G-C-T)$_n$ would undergo a salt-induced conformational change, as has been observed for d(C-G)$_n$·d(C-G)$_n$. Pohl and Jovin (1972) found that the CD spectrum of d(C-G)$_n$·d(C-G)$_n$ in solution is dramatically changed by addition of 20% by weight of solid NaCl. On the other hand, most natural DNAs show decreased magnitudes of their long wavelength bands at high salt concentrations (Ivanov et al., 1973), in a manner reminscent of the changes seen at intermediate ethanol concentrations. As seen in Figure 11, the effect of high concentrations of NaCl on the spectrum of d(A-G-C)$_n$·d(G-C-T)$_n$ is similar to the effect of up to 60% ethanol. Thus, the d(A-G-C)$_n$·d(G-C-T)$_n$ responds more like natural DNAs than does d(C-G)$_n$·d(C-G)$_n$ to high salt concentrations.

Discussion

Model systems have contributed substantially to our understanding of the complex physical properties and enzymology of DNA and RNA. Preparation of model DNAs of this type begins with laborious chemical synthesis of complementary oligonucleotides containing the desired repeating sequences. Incubation of these complementary oligonu-

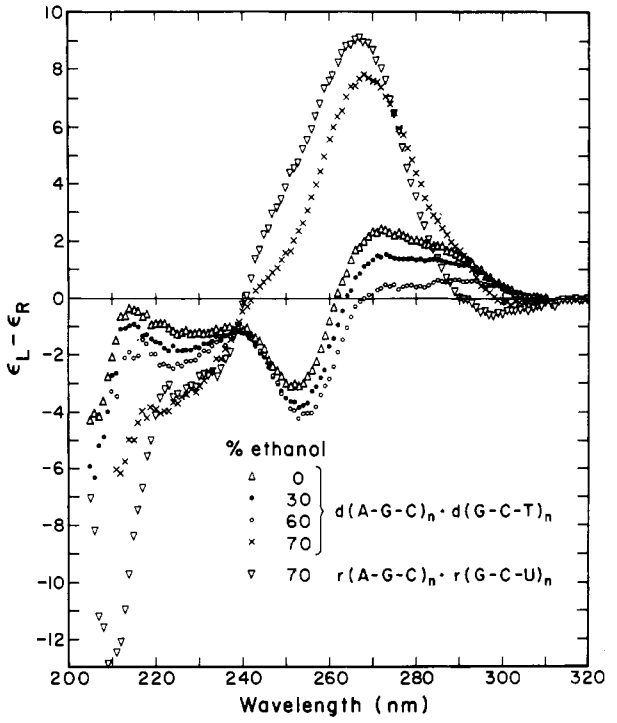

FIGURE 10: Circular dichroism spectra at 20° of $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ at increasing ethanol concentrations and of $r(A\text{-}G\text{-}C)_n \cdot r(G\text{-}C\text{-}U)_n$ at 70% ethanol. The final concentrations of Na^+ (phosphate buffer) were 5.0×10^{-4}, 3.3×10^{-4}, 1.8×10^{-4}, and 1.3×10^{-4} M, respectively, at ethanol concentrations of 0, 30, 60, and 70% by weight.

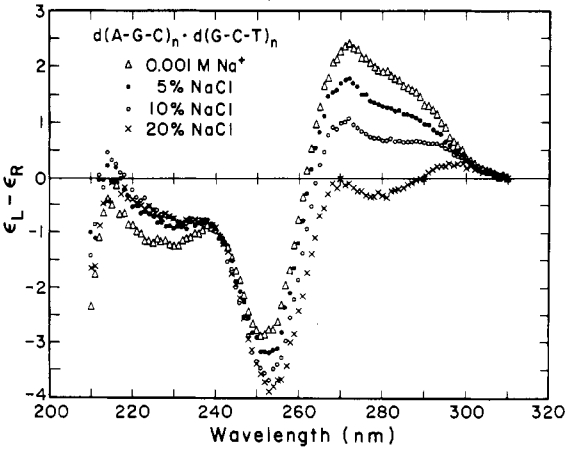

FIGURE 11: Circular dichroism spectra at 20° of $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ at increasing sodium chloride concentrations up to 20% by weight. Solid sodium chloride was added to a sample that originally contained 10^{-3} M Na^+ (phosphate buffer, pH 7.0).

cleotides with DNA polymerase and the appropriate deoxyribonucleoside triphosphates yields high molecular weight repeating sequence DNA (Wells and Wartell, 1974). Initial attempts to prepare $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ using the chemically prepared oligomers $d(A\text{-}G\text{-}C)_4$ and $d(C\text{-}T\text{-}G)_4$ as template gave only yields of the desired product. When use of the isolated product as template for preparation of additional $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ was attempted, no net synthesis resulted. Similarly, Grant et al. (1972) have observed that $d(C\text{-}G)_n \cdot d(C\text{-}G)_n$ is a much poorer template for $d(C\text{-}G)_n \cdot d(C\text{-}G)_n$ synthesis than is a $d(C\text{-}I)_n \cdot d(C\text{-}I)_n$ template. They also observed that $d(C\text{-}I)_n \cdot d(C\text{-}I)_n$ was much more effective in directing $d(C\text{-}I)_n \cdot d(C\text{-}I)_n$ synthesis than $d(C\text{-}G)_n \cdot d(C\text{-}G)_n$ synthesis.

In our attempts to synthesize the various polydeoxyribonucleotides containing repeating trinucleotide sequences using complementary short-chain oligodeoxyribonucleotides as templates, we have found that the best yields were obtained with high concentrations of deoxyribonucleoside triphosphates (3–5 mM) and low temperature (15°) using either *E. coli* or *M. luteus* DNA polymerase. For the synthesis of more of the same DNAs with repeating trinucleotide sequences using previously prepared DNAas as template, the *M. luteus* DNA polymerase gave better yields of polymer than the *E. coli* DNA polymerase when the template had 100 base pairs or less (Ratliff et al., 1973). If an attempt was made to employ as template unfractionated polydeoxyribonucleotides which were themselves products of a previous enzymatic synthesis and consequently of high molecular weight, overall yields with either enzyme were less. The product was also usually significantly contaminated with $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$.

The new DNA polymers synthesized in this work were characterized by nearest neighbor analyses, buoyant density measurements in cesium chloride and cesium sulfate, melting temperature, and CD spectra and by transcriptional studies. All of these measurements support the sequences we have assigned to these polymers. The synthesis of $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ and the determination of its buoyant density in cesium chloride and cesium sulfate have allowed us to derive general equations that express the buoyant density of a polymer in terms of its first-neighbor base frequencies (Gray et al., 1974). It is interesting to compare the buoyant densities of $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$ and $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ with the buoyant densities of $d(C\text{-}I)_n \cdot d(C\text{-}I)_n$ and $d(C\text{-}G)_n \cdot d(C\text{-}G)_n$. The polymers which contain guanine have lower buoyant densities in cesium sulfate but higher buoyant densities in cesium chloride than their analogues containing hypoxanthine. The buoyant densities of $d(C\text{-}G)_n \cdot d(C\text{-}G)_n$ and $d(C\text{-}I)_n \cdot d(C\text{-}I)_n$ are 1.448 and 1.453 g/ml, respectively, in cesium sulfate and 1.741 and 1.735 g/ml, respectively, in cesium chloride (see Wells and Wartell, 1974). The buoyant densities of $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ and $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$ are 1.436 and 1.444 g/ml, respectively, in cesium sulfate and 1.718 and 1.716 g/ml, respectively, in cesium chloride. A buoyant density of 1.718 g/ml for $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ in cesium chloride is what one would expect on the basis of 67% G + C content (Schildkraut et al., 1962; Gray et al., 1974).

The CD spectrum of native $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ is a crucial addition to the library of CD spectra of synthetic DNA sequences that are needed to predict and analyze CD spectra of other double-stranded DNAs by first-neighbor approximations (Gray and Tinoco, 1970; Allen et al., 1972). The CD spectrum of $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ cannot itself be approximated by a first-neighbor combination of known CD spectra of other synthetic DNA sequences. The features of the $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ CD spectrum are distinct from those of other synthetic DNA sequences and should be useful in interpreting the CD spectra of other natural and synthetic DNA sequences. It has already been observed that the $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ CD spectrum is quite similar to that of the (G + C)-rich satellite II of the hermit crab, *Pagurus pollicaris* (D. M. Gray and D. M. Skinner, unpublished data).

In transcription studies with $d(A\text{-}I\text{-}C)_n \cdot d(I\text{-}C\text{-}T)_n$ as template, simultaneous transcription of both strands to produce either $r(A\text{-}G\text{-}C)_n \cdot r(G\text{-}C\text{-}U)_n$ or $r(A\text{-}I\text{-}C)_n \cdot r(I\text{-}C\text{-}U)_n$

gave a onefold synthesis. When only one strand of the polymer was transcribed by limiting the input ribonucleoside triphosphates, a less than onefold transcription of each strand was observed. Attempts to transcribe $d(A\text{-}G\text{-}C)_n \cdot d(G\text{-}C\text{-}T)_n$ yielded very little RNA product. We have likewise found that $d(C\text{-}G)_n \cdot d(C\text{-}G)_n$ is a very poor template for $r(C\text{-}G)_n \cdot r(C\text{-}G)_n$ synthesis, whereas use of $d(C\text{-}I)_n \cdot d(C\text{-}I)_n$ gives high yields of $r(C\text{-}G)_n \cdot r(C\text{-}G)_n$.

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References

Allen, F. S., Gray, D. M., Roberts, G. P., and Tinoco, I., Jr. (1972), *Biopolymers 11,* 853.

Arnott, S. (1970), *Prog. Biophys. Mol. Biol. 21,* 265–319.

Arnott, S., Chandrasekaren, R., Hukins, D. W. L., Smith, P. J. C., and Watts, L. (1974), *J. Mol. Biol. 88,* 523.

Brahms, J., Pilet, J., Lan, T.-T. P., and Hill, L. R. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 3352.

Burd, J. F., Wartell, R. M., Dogson, J. B., and Wells, R. D. (1975), *J. Biol. Chem.* (in press).

Chamberlain, M., and Berg, P. (1962), *Proc. Natl. Acad. Sci. U.S.A. 48,* 81.

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

Girod, J. C., Johnson, W. C., Jr., Huntington, S. K., and Maestre, M. F. (1973), *Biochemistry 12,* 5092.

Grant, R. C., Kodama, M., and Wells, R. D. (1972), *Biochemistry 11,* 805.

Gratzer, W. B., and Richards, E. G. (1971), *Biopolymers 10,* 2607.

Gray, D. M., Gray, C. W., Ratliff, R. L., and Smith, D. A. (1974), *Biopolymers 13,* 2265.

Gray, D. M., and Ratliff, R. L., (1975), *Biopolymers 14,* 487.

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.

Gray, D. M., Tinoco, I., Jr., and Chamberlain, M. (1971), *Biopolymers 11,* 1235.

Harwood, S. J., Schendel, P. F., and Wells, R. D. (1970), *J. Biol. Chem. 245,* 5614.

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.

Ivanov, V. I., Minchenkova, L. E., Minyat, E. E., Frank-Kamenetskii, M. D., and Schyolkina, A. K. (1974), *J. Mol. Biol. 87,* 817.

Ivanov, V. I., Minchenkova, L. E., Schyolkina, A. K., and Poletayev, A. I. (1973), *Biopolymers 12,* 89.

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

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

Khorana, H. G., Buchi, H., Ghosh, H., Gupta, N., Jacob, T. M., Kossel, H., Morgan, R., Narang, S. A., Ohtsuka, E., and Wells, R. D. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31,* 39.

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

Mitsui, Y., Langridge, R., Shortle, B. E., Cantor, C. R., Grant, R. C., Kodama, M., and Wells, R. D. (1970), *Nature (London) 228,* 1166.

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

Morgan, A. R., Coulter, M. B., Flintoff, W. F., and Paetkau, V. H. (1974), *Biochemistry 13,* 1596.

Narang, S. A., Jacob, T. M., and Khorana, H. G. (1967a), *J. Am. Chem. Soc. 89,* 2158.

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

Pohl, F. M., and Jovin, T. M. (1972), *J. Mol. Biol. 67,* 375.

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., Hayes, F. N., and Hoard, D. E. (1974), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 33,* 1424.

Ratliff, R. L., Williams, D. L., Hayes, F. N., Martinez, E. L., Jr., and Smith, D. A. (1973), *Biochemistry 12,* 5005.

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

Riggs, A. D., Lin, S., and Wells, R. D. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 761.

Schildkraut, C. L., Marmur, J., and Doty, P. (1962), *J. Mol. Biol. 4,* 430.

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

Wells, R. D., and Larson, J. E. (1972), *J. Biol. Chem. 247,* 3405.

Wells, R. D., and Wartell, R. (1974), Biochemistry Series One, MTP International Review of Science, Vol 6, Burton, K., Ed., Baltimore, Md., University Park Press.

Wells, R. D., and Yang, J. T. (1974), *Biochemistry 13,* 1317.

Zimmerman, B. K. (1966), *J. Biol. Chem. 241,* 2035.