Enzymatic Synthesis of Deoxyribonucleic Acid

XII. A POLYMER OF DEOXYGUANYLATE AND DEOXYCYTIDYLATE

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The enzyme deoxyribonucleic acid polymerase, purified from Escherichia coli, catalyzes extensive formation of deoxyribonucleic acid by a mechanism that involves replication of a deoxyribonucleic acid primer. This replication requires the presence of the four deoxynucleoside triphosphates commonly found in deoxyribonucleic acid, and deoxyribonucleic acid itself (1). In the absence of one, two, or three of the deoxynucleoside triphosphates, a limited reaction occurs, which represents the addition of only one or very few deoxynucleotide residues to the deoxynucleoside end of the primer deoxyribonucleic acid molecule (2). Both the extensive and limited reactions occur without lag and are detectable only when deoxyribonucleic acid has been added.

Two reactions have now been discovered which are exceptions to this rule. They are observed after lag periods and occur in the absence of added DNA. As judged by viscometric, sedimentation, and spectrophotometric studies, the products, like DNA, are rigid, hydrogen-bonded macromolecules. In one case, already described (3), the product is a copolymer (dAT) composed exclusively of deoxyadenylate and deoxythymidylylate in exactly alternating sequence. In the second case, the subject of this report, the product contains only deoxyguanylate and deoxyctydylate arranged as hydrogen-bonded homopolymers (dGdC). When either primer, dAT or dGdC, is isolated and used as a primer for the enzymatic reaction, a shortened lag period occurs, which is related inversely to the amount of primer added (4), and extensive synthesis of an identical polymer results.

In this report we shall describe the synthesis and properties of dGdC polymer and an analogous polymer, dGdBC. The accompanying report describes studies on the kinetics and mechanism of synthesis of dAT and dGdC polymers (4).

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The abbreviations used are: dAT, a copolymer of deoxyadenylate and deoxythymidylylate; dAB, a copolymer of deoxyadenylate and 5-bromodeoxyuridylylate; dGdC, polymer consisting of homopolymers of deoxyguanylylate and deoxyctydylate; dGdBC, polymer consisting of homopolymers of deoxyguanylylate and 5-bromodeoxyctydylate; dIdC, polymer consisting of homopolymers of deoxyinosinate and deoxyctydylate; dIdBC, polymer consisting of homopolymers of deoxyinosinate and 5-bromodeoxyctydylate.

EXPERIMENTAL PROCEDURE

Materials

The polymerase fraction used to synthesize dGdC polymer was Fraction VII purified 2000-fold from E. coli (1) or a fraction (P-cell) further purified by chromatography on phosphocellulose (5). DNAase from E. coli (6) was a gift from Dr. I. R. Lehman. Other enzymes and deoxynucleoside triphosphates were obtained as cited previously (3).

Concentrations of the deoxyribonucleotide polymers are expressed as equivalents of nucleotide phosphorus.

Methods

Measurement of Synthesis of dGdC Polymer—Polymer was synthesized either with or without dGdC as primer. Reaction mixtures contained per ml: dGTP and dCTP, 0.75 to 1.5 μmoles of each; MgCl₂, 6 μmoles; potassium phosphate buffer, pH 7.4, 60 μmoles; and polymerase, 20 to 50 units. Primed reactions contained 10 to 30 μmoles of dGdC polymer.

The course of polymer synthesis was examined by measurement of (a) viscosity, (b) absorbancy, and (c) radioisotope incorporation, all as described previously for studies of the synthesis of dAT (3). In certain experiments, C¹⁴ and P³²-labeled substrates were used simultaneously. C¹⁴ and P³² were distinguished by measuring the radioactivity of the planchets before and after masking with cellophane tape. Two layers of cellophane absorbed >99% of the C¹⁴-radioactivity but only approximately 50% of the P³²-radioactivity.

The reaction was stopped by the addition of NaCl to a final concentration of 0.4 M, and heating at 75° for 5 minutes. The solution was dialyzed overnight, first against 1000 volumes of 0.2 M NaCl and 0.02 M potassium phosphate buffer, pH 7.5, and then against 0.02 M NaCl or 0.02 M phosphate buffer or both, or against distilled water for 4 hours or more.

Hydrolysis of dGdC Polymer—Enzymatic hydrolysis to 3'-deoxyribonucleotides by micrococcal DNase and spleen phosphodiesterase was performed as described previously (7).

Chemical hydrolysis of P³²-labeled dGdC by diphenylamine in formic acid was carried out by the method of Burton and Peterson (8). An aliquot of polymer (approximately 1 μmole per ml, 1 × 10⁶ c.p.m. per ml) was incubated with twice its volume of freshly prepared 3% diphenylamine in formic acid for 16 hours at 30°. A 0.3-ml aliquot was mixed with 0.6 ml of water and then extracted twice with 3-ml portions of ether to remove the diphenylamine. Calf thymus DNA (0.1 ml, 2.5 μg per ml) was added as carrier to the aqueous phase, which was then treated...
with 0.3 ml of 7% perchloric acid. The precipitate was rinsed in 0.3 ml of 0.2 N NaOH and precipitated again with 0.3 ml of 3.5% perchloric acid. The combined supernatants and the precipitate, dissolved in 0.5 ml of 2 M NH₄OH, were each assayed for radioactivity.

Measurement of Transitions in Optical Density and Viscosity of Polymers—Ultraviolet absorption as a function of temperature was measured in a Zeiss PMQ II spectrophotometer as described by Inman and Baldwin (9). Ultraviolet absorption and viscosity as a function of pH were also measured; additions of alkali or acid were made to solutions stirred by a stream of nitrogen. pH was measured in quartz cuvettes with a Leeds-Northrup pH meter and in the reservoir of the viscometer with a Beckman 39183 Probe Combination Electrode. Measurements of viscosity were made at 37° in an Ostwald-type viscometer.

Chemical Determinations—Purine deoxypentose was measured by the method of Dische (10) and phosphate by the method of Chen, Toribara, and Warner (11) with the ashing procedure described by Ames and Dubin (12). Deoxycytidylate was calculated by difference between total phosphate and purine deoxypentose or by radioactivity when Pa*-dCTP had been used.

RESULTS

Unprimed Synthesis (de Nove) of dGdC Polymer

With dCTP, dGTP, Mg++, and polymerase in a reaction mixture but without added primer, the synthesis of dGdC polymer followed the same time course described for the synthesis of dAT (3). Measurements of the optical density, viscosity, and the incorporation of radioactivity into acid insoluble product demonstrated an extensive lag period, followed by a period of rapid synthesis (Fig. 1). Finally a period of degradation ensued when polymer was broken down by nucleases in the enzyme preparation to nonviscous, less hypochromic, acid-soluble products.

The length of the lag varied in the same way as the lag period in dAT synthesis. With different enzyme preparations, the lag period varied from less than 1 hour to 13 hours. With increasing amounts of enzyme, the lag period was shortened. In one experiment, maximal synthesis occurred at 8.0, 2.2, and 1.2 hours with 5, 10, and 20 units of enzyme, respectively. Up to 95% incorporation of labeled substrate was observed, although most frequently incorporation did not exceed 50%. In some instances reduced yields might be ascribed to destruction of the dGTP by dGTPase in the enzyme preparation (13). No synthesis of polymer was observed when polymerase, Mg++, dGTP, or dCTP was omitted.

Unprimed synthesis of dGdC polymer required concentrations of enzyme 5 to 10 times greater than that required for synthesis of dAT.

Primed Synthesis of dGdC Polymer

When the product of an unprimed synthesis was added to a reaction mixture containing dGTP, dCTP, Mg++, and DNA polymerase, the lag period was shortened (Fig. 2). The percentage utilization of substrate was the same in a primed synthesis as in an unprimed synthesis; the extent of net synthesis (ratio of polymer made to primer added) therefore depended on the ratio of substrate to primer used. Net synthesis greater than 100-fold was easily obtained.

Both unprimed and primed synthesis of dGdC were catalyzed less well by more purified polymerase preparations. Thus, 10 units of a DEAE fraction (VII) incorporated 88 μmoles of deoxycytidylate into polymer in a primed synthesis in a 3-hour period; under the same conditions, 30 units of a fraction (P-cell) purified one step further showed no synthesis at all (<0.1 μmole), nor was synthesis by the P-cell fraction observed when heated DEAE fraction was added. Thirty units of the P-cell fraction did not inhibit synthesis catalyzed by 10 units of DEAE fraction. In addition, different preparations of dGdC have varied in their efficiency as primers. With the same P-cell enzyme fraction, one preparation of primer led to the incorporation of only 38 μmole of deoxyguanylate in 30 minutes as compared to 510 μmole with another primer. The "melting curve" of these two primers were the same. A tentative interpretation of these data is that inefficient primers are rendered more active by some heat labile factor(s) present in the crude enzyme fractions. Current studies are probing the relationship between enzyme purification and priming efficiency.

The incorporation of labeled substrates in primed dGdC synthesis presents several interesting features which may be rationalized more readily after a consideration of the structure of dGdC polymer.

Characterization of dGdC Polymer

Composition—Analysis of 16 dGdC preparations showed 7 with equivalent amounts of deoxguanylate and deoxycytidylate.

Fig. 1. Course of synthesis of dGdC polymer as measured by viscometry, spectrophotometry, and radioisotope incorporation in a single experiment. The reaction mixture contained, in a volume of 1 ml: potassium phosphate buffer at pH 7.4, 60 μmole; MgCl₂, 6 μmole; CTP, 1.5 μmole; 8 X 10⁶ c.p.m. per μmole; dAT, 1.5 μmole, 1.2 X 10⁶ c.p.m. per μmole; and polymerase, 47 units. The reaction was carried out in a viscometer at 37°, and the course of the reaction was observed by frequent viscosity measurements. At intervals, 20-μl aliquots were taken for measurement of optical density and acid-precipitable radioactivity. In particular, the "melting curves" were examined to determine whether the amount of the minor component (cf. Figs. 3 and 8) might correlate with primer efficiency.
whereas the remainder had deoxyguanylate in excess, with molar percentages ranging from 56 to 81 (average = 65). No samples have been found with deoxycytidylate in significant excess. Unlike dGdC polymer, dAT copolymer contains equal amounts of its component bases, adenine and thymine (3).

**Sequence of Nucleotides**—dAT is a copolymer in which deoxyadenylate and deoxythymidylate occur in alternating sequence (3). By contrast, dGdC consists of homopolymers of deoxyguanylate and deoxycytidylate, respectively, with no covalent linkages (≤0.5%) between deoxyguanylate and deoxycytidylate. This has been shown in two ways:

The first method is the nearest-neighbor analysis previously described (7). This entails the synthesis of dGdC in two separate primed reactions. In one the substrates were dCTP and P32-dGTP; in the other reaction, they were P32-dCTP and dGTP. The resulting polymers were isolated and enzymatically hydrolyzed to 3'-deoxyribonucleotides. In this way the P32 originated at the 5'carbon of the substrate was released in the form of the Pa ester at the 3'carbon of the adjacent deoxyribonucleotide. Table I shows that deoxyguanylate was always adjacent to deoxycytidylate and deoxythymidylate was always adjacent to deoxythymidylate. At pH 9.2, which favors the incorporation of dGTP (cf. Table IV), the same nucleotide sequence was observed as at pH 7.4.

The second method involves chemical rather than enzymatic hydrolysis, and although less precise, is quick and simple. It is based upon acidic depurination with subsequent scission of the polynucleotide chain at the depurinated sites (8). dGdC polymer was synthesized in a primed reaction with dGTP and P32-dCTP. The product was then hydrolyzed by diphenylamine in formic acid according to Burton and Petersen (see "Methods"). Unhydrolyzed polynucleotide was precipitated by perchloric acid and the radioactivity in the supernatant and precipitate was determined. DNA isolated from E. coli, grown in a medium containing P32-inorganic phosphate, was also subjected to the same treatment to serve as a control. Under conditions which resulted in complete acid-solubilization of the E. coli DNA, no significant cleavage of pyrimidine-labeled dGdC into radioactive, acid-soluble fragments was observed (Table I). This indicates that deoxyguanylate was not interspersed in deoxythymidylate chains often enough to result in acid-soluble oligonucleotides of deoxythymidylate after scission at depurinated sites.

**Physical Properties**—The sedimentation coefficient of a dGdC preparation made in an unprimed synthesis was 19S, the reduced viscosity 23 (g/100 ml)-1. From these data, a molecular weight of 4 x 106 was estimated, with the equation of Scheraga and Mandelkern (14). These data are very similar to those previously reported for natural and enzymatically synthesized DNA (16), and for dAT polymer (3), indicating that dGdC is similar in size and shape to DNA and dAT.

**Holm Cell Transitions**—Further evidence for similarity of structure of dGdC to that of dAT and DNA was obtained by studies of optical density and viscosity transitions induced by heating or alkaline titration. Heating of dGdC resulted in a sharp "melting curve" with a midpoint (Tm) at 83° (Fig. 3); at the same ionic strength, 0.015, the Tm for dAT was 41°. The difference of 42° between the Tm values for dGdC and dAT agrees with the value predicted from the data of Marmur and Doty (16) which relate Tm to the guanine and cytosine content. On quick cooling of melted dGdC, the optical density returned to its value before heating (≤2% difference). The reversibility of the "melting curve" of dGdC after quick cooling of the melted polymer (Fig. 3) recalls the behavior of dAT (3) and is quite unlike that of DNA (17). The absence of hysteresis in the melting curves of these polymers might be attributed to the exact matching of base pairs between the strands of alternating A-T
**TABLE II**

_Determination of nucleotide sequence in dGdC polymer by chemical hydrolysis_

dGdC polymer was synthesized in a primed reaction from P<sup>32</sup>-dCTP and unlabeled dGTP and then hydrolyzed by the method of Burton and Petersen (see "Methods"). Uniformly labeled P<sup>32</sup>-E. coli DNA was treated in the same manner as the dGdC polymer.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Distribution of label</th>
<th>Hydrolysis time</th>
<th>Distribution of radioactivity in:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>supernatant</td>
</tr>
<tr>
<td>dGdC</td>
<td>*pC-</td>
<td>hours</td>
<td>c.p.m.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>82</td>
</tr>
<tr>
<td>DNA (E. coli)</td>
<td>Uniform</td>
<td>0</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>6234</td>
</tr>
</tbody>
</table>

sequence in the dAT copolymer or of the uniform dG and dC homopolymers in the case of dGdC. However, lack of hysteresis has also been observed for enzymatically synthesized DNA primed with wheat germ or bacteriophage DNA. Since enzymatically synthesized DNA has a complex nucleotide sequence which appears to be like that of the primer DNA (7), some additional or alternative factors must determine whether reversible melting can occur.

At very low ionic strength (2.6 × 10<sup>-4</sup>), the reversibility of the dGdC melting curve was no longer observed, even when the temperature of the heated dGdC was slowly decreased, over a period of several hours, in the temperature range of the transition.

The melting curve of dGdC in Fig. 3 shows, as a minor component, a rise in optical density below the temperature of the major transition. This minor component, although variable in extent, was observed in all dGdC samples studied and comprised as much as 20% of the total hyperchromic change (cf. Fig. 8). An attempt to separate these components by density-gradient sedimentation was not successful.

Alkaline titration of dGdC also led to a sharp hyperchromic change (Fig. 4). On back-titration, the hyperchromic transition was largely reversible. A viscosity transition was also demonstrated on alkaline titration (Fig. 5). The midpoints for the optical density and viscosity transitions, respectively, occurred at pH 11.4 and 11.1, values which lie within experimental error for the conditions of these experiments. By contrast with the hyperchromic transition, which was largely reversible, the viscosity transition was irreversible, indicating failure to resume the original structure.

**Spectra**—The spectrum of dGdC varied with pH in a manner predictable from the behavior of its component nucleotides (Fig. 6). The shape of the absorption curve of the single-stranded (random coil) configuration of dGdC (i.e. dG + dC) at pH 13 was approximately the sum of spectra of deoxyguanylate and deoxycytidylate, but the spectrum of the helical configuration

![Fig. 3. Optical density transitions of dGdC and dAT polymers due to heating. The original optical densities of dGdC and dAT were 0.481 and 0.573, respectively. The solvent contained 0.001 M Na<sub>2</sub>EDTA, 0.005 M NaH<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, and 0.0025 M Na<sub>2</sub>HPO<sub>4</sub> (pH 6.5, ionic strength approximately 0.015). dGdC was cooled quickly before the second heating by running cold tap water through the thermal spacer of the "melting" apparatus. Other details are cited in "Methods." We thank Dr. R. B. Inman for permission to reproduce the data on dAT obtained by him.](http://www.jbc.org/)
cytidylate chains require dGTP. However, the synthesis of dAT copolymer is impossible without both of the component nucleotides. dGdC fails to prime the incorporation of dATP or dTTP (<0.5%).

At neutral pH, deoxyguanylate was incorporated approximately twice as rapidly as deoxycytidylate; at pH 9.2 the discrepancy was increased to ninefold by reduction in the rate of incorporation of deoxycytidylate (Table IV). The results were observed with several DNA polymerase preparations from E. coli; the polymerase induced by T2 infection (5) showed even greater preference for incorporation of deoxyguanylate at both alkaline and neutral pH's (Table IV).

Inman and Baldwin (18) have recently separated the component strands of dGdC and analogous homopolymers by density-gradient sedimentation at alkaline pH. In preliminary experiments, polydeoxycytidylate has proved to be an effective primer for synthesis of dGdC whereas polydeoxyguanylate fails to prime even limited polymerization of deoxycytidylate residues. Similarly, when dGdC is used as a primer for RNA polymerase (19), the incorporation of guanylate is greatly favored over incorporation of cytidylate; polydeoxycytidylate primes the synthesis of polyguanylate, but polydeoxyguanylate does not prime the synthesis of polyctydylate (20). It seems likely that complexities of polydeoxyguanylate structure, as yet not understood, account for its failure as a primer, especially at alkaline pH (cf. "Discussion").

Susceptibility to Enzymatic Hydrolysis—Observations of the action of three different nucleases from E. coli showed that dGdC was much less sensitive than dAT or DNA. (a) DNA endonuclease degraded dGdC at approximately 5% of the rate at which dAT was hydrolyzed (21). (b) DNA diesterase (6) degraded dGdC at 10% of the rate at which dAT was attacked and less than 0.2% of the rate of cleavage of heated Ps2-DNA from E. coli. (c) An exonuclease persisting in the purified polymerase fractions (22) degraded dGdC less than 30% as rapidly as dAT.

Primed Synthesis of dGdC Polymer

A polymer was synthesized with dGdC as primer and 5-bromo-dCTP as a substitute for dCTP. The nondialyzable product, representing 100-fold net synthesis, had a reduced viscosity of 5.4 (g/100 ml)^{1}. This preparation contained 73% deoxyguanylate (based on deoxypentose and phosphate). The optical density values have been corrected for volumetric changes due to addition of acid or alkali. Other details are given in "Methods."

An interesting property of dGdBC was the absence of a sharp, hyperchromic transition on heating, even at ionic strength 2.6 x 10^{-3} (Fig. 8). A melting curve for dGdC at the same ionic strength is shown for comparison in Fig. 8: the T_m of the major hyperchromic transition under these conditions was 66°. At temperatures above 70°, dGdC showed irreversible, time-

Fig. 4. Optical density transitions of dGdC and dGdBC polymers due to alkaline titration at 26°. The initial optical densities of dGdC and dGdBC at 270 mμ were, respectively, 0.344 and 0.346. The solvent contained 0.2 M NaCl and 1.5 x 10^{-4} M potassium phosphate. The optical density values have been corrected for volumetric changes due to addition of acid or alkali. Other details are given in "Methods."

Fig. 5. Viscosity transitions of dGdC and dGdBC polymers due to alkaline titration at 37°. The solvent was the same as that in Fig. 4. Other details are described in "Methods."
dependent hyperchromic changes attributed to degradation. dGdBC by contrast showed no sharp hyperchromic transition but only a gradual, irreversible increase in optical density between 70 and 96°, also attributed to degradation.

To distinguish between (a) absence of secondary helical structure and (b) secondary helical structure of relatively increased temperature stability, the behavior of dGdBC on alkaline titration was studied. Like dGdC, dGdBC underwent a sharp, reversible, hyperchromic transition (Fig. 4) on alkaline titration with a concomitant sharp, irreversible drop in viscosity (Fig. 5). The respective midpoints for these transitions occurred at pH 11.9 and 12.4. dGdBC therefore appears to consist of hydrogen-bonded strands which are unusually stable to separation by heat.

**Discussion**

In describing the enzymatic synthesis of dAT copolymer (3), we speculated about the probable synthesis of a similar polymer containing only deoxyguanylate and deoxycytidylate. The conditions for the synthesis of such a polymer were found, as reported here, but the homopolymeric structure of dGdC was not anticipated. The reason for the copolymeric structure of dAT on the one hand, and the homopolymeric structure of dGdC on the other hand, is of importance as far as the interpretation of the data obtained with each of these polymers is concerned. The present work is concerned with the interpretation of the data obtained with dGdC.

**Table III**

Enzymatic replication of dGdC compared with dAT

<table>
<thead>
<tr>
<th>Primer</th>
<th>Substrates</th>
<th>Incorporation of labeled deoxyribonucleotide (pmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dGdC</td>
<td>P32-dGTP + dCTP</td>
<td>1230</td>
</tr>
<tr>
<td></td>
<td>P32-dGDP*</td>
<td>1350</td>
</tr>
<tr>
<td></td>
<td>P32-dCTP + dGTP</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>P32-dCTP</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>P32-dATP</td>
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</tr>
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<td>P32-dCTP</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>dAT</td>
<td>P32-dATP + dTTP</td>
<td>293</td>
</tr>
<tr>
<td></td>
<td>P32-dATP</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>P32-dTTTP + dAT</td>
<td>372</td>
</tr>
<tr>
<td></td>
<td>P32-dTTTP</td>
<td>7.8</td>
</tr>
</tbody>
</table>

* In another experiment favoring more extensive synthesis (140 mmoles of P32-dGTP and 120 mmoles of dGdC primer), 12 mmoles of deoxyguanylate were polymerized, representing a 20% increase in this residue over that supplied in the primer.

**Fig. 7.** Diagrammatic comparison of replication of dGdC and dAT polymers.
dGdC on the other hand is still unknown. The regularity of structure, which dAT and dGdC have in common, must be related to the mechanism of synthesis de novo catalyzed by polymerase. An understanding of this mechanism might enable us to synthesize related copolymers and homopolymers and provide some insight into the evolution of DNA structure.

The data presented indicate that dGdC is a hydrogen-bonded DNA-like macromolecule. The evidence also indicates that the structure of dGdC is not so simple or uniform as the structure of dAT has appeared to be: dGdC has varied in composition from 50 to 80 moles per cent deoxyguanylate. The configuration of the excess polydeoxyguanylate is unknown, nor need it be assumed that all the polydeoxyctydylate is hydrogen-bonded to polydeoxycytidylate. The minor component in the melting curves of dGdC provides a further indication of the complexity of its structure. Structural variations must also be responsible for the wide discrepancies in the efficiency of different dGdC preparations as primer. In this connection, the ability of dGdC to prime far more effectively for deoxyguanylate than deoxcytidylate polymerization is significant. So are the preliminary results that show isolated polydeoxyctydylate to be a good primer and polydeoxyguanylate practicely inert for both DNA and RNA polymerases (cf. above, and (20)). These findings suggest that a considerable fraction of the dGdC structure may under some conditions be organized as polydeoxyguanylate.

In addition to dAT and dGdC, several other analogous polymers have been prepared, including dGdBC as described above. Inman and Baldwin have prepared dABU, dIdC, and dIdBC, and have separated the constituent strands of the latter two polymers by density gradient sedimentation at alkaline pH (9, 18).

The availability of these polydeoxynucleotides of restricted base composition and known base sequence is proving useful in the study of the physical chemistry of DNA-like polymers (9, 18) and the study of the specificity and action of deoxyribonucleases (21).

**Table IV**

**Influence of pH and enzyme source on deoxyguanylate and deoxycytidylate incorporation in primed dGdC synthesis**

Reaction mixtures with E. coli polymerase contained in 0.3 ml: potassium phosphate buffer at pH 7.4, or "Tris" buffer, pH 8.6, 20 μmoles; MgCl₂, 2 μmoles; P₂₀-dCTP, 1.4 X 10⁴ p.m. per μmole, 10 μmoles; Cₓ-dGTP, dGdC polymer, 12 μmoles; and 0.04 unit of polymerase (P-cell fraction). Reaction mixtures with polymerase from T₂-infected E. coli contained 0.2 unit and, in addition, 3 amoles of β-mercaptoethanol and 2 μmoles of Na₃EDTA. Incubation was at 37° for 30 minutes and the incorporation of radioactivity into acid-insoluble material was determined as described previously (1).

<table>
<thead>
<tr>
<th>Source of polymerase</th>
<th>pH</th>
<th>Deoxyguanylate</th>
<th>Deoxycytidylate</th>
<th>Ratio: deoxyguanylate/deoxycytidylate</th>
</tr>
</thead>
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<tr>
<td>E. coli</td>
<td>7.5</td>
<td>601</td>
<td>255</td>
<td>2.4</td>
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<td>E. coli</td>
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<td>25</td>
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<tr>
<td>T₂-infected E. coli</td>
<td>8.6</td>
<td>546</td>
<td>45</td>
<td>12.2</td>
</tr>
</tbody>
</table>

**Fig. 8.** Optical density changes of dGdC and dGdBC polymers due to heating. The initial optical densities at 260 mg of dGdC and dGdBC, respectively, were 0.325 and 0.280. The solvent contained 4.25 X 10⁻⁴ m Na₃H₂PO₄, 8.5 X 10⁻⁴ m Na₃H₂PO₄, and 1.7 X 10⁻⁴ m Na₃EDTA (μ = 0.00265).

**SUMMARY**

1. The synthesis de novo of a polymer of deoxyguanylate and deoxycytidylate (dGdC polymer) from the respective deoxynucleotide triphosphates is catalyzed by DNA polymerase. The reaction proceeds in the absence of added primer after an extensive lag period. dGdC polymer isolated from this reaction primes the enzymatic synthesis of an identical polymer.

2. Physical measurements show that dGdC polymer is a hydrogen-bonded structure with a molecular weight of several million. Unlike the copolymer of deoxyadenylate + deoxythymidylate (dAT) synthesized by DNA polymerase under similar conditions, the dGdC polymer consists of homopolymers of deoxyguanylate and deoxycytidylate.

3. With 5-bromodeoxycytidine triphosphate as substrate in place of deoxycytidine triphosphate, a polydeoxyguanylate-polydeoxybromocytidylate polymer is produced; helix-coil transition studies reveal this polymer to be unusually stable to heating.

**REFERENCES**


