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Factors Affecting the Primer for Deoxyribonucleic Acid Polymerase

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In previous reports from this Laboratory (Smellie, Keir & Davidson, 1959; Smellie *et al.* 1960; Gray *et al.* 1960), deoxyribonucleic acid polymerase of Ehrlich ascites-tumour cells has been described. The requirements for its action include Mg^{2+} ions, the 5'-triphosphates of deoxyadenosine, deoxycytidine, deoxyguanosine and thymidine, and primer deoxyribonucleic acid. The extent of synthesis of polydeoxyribonucleotide in a given time is dependent upon the amount of primer deoxyribonucleic acid present in the reaction mixture. This paper describes some of the experiments carried out to investigate the nature of the primer, and some of the treatments which can influence its priming capacity in the synthetic system.

EXPERIMENTAL

Abbreviations. Abbreviations used in this paper are: deoxyadenosine 5'-triphosphate, dATP; deoxycytidine 5'-triphosphate, dCTP; deoxyguanosine 5'-triphosphate, dGTP; thymidine 5'-triphosphate labelled with ^{32}P in the α-phosphate group, [^{32}P]TTP; thymidine 5'-monophosphate labelled with ^{32}P, [^{32}P]TMP; sodium salt of ethylenediaminetetra-acetic acid (pH 7·0), EDTA.

Biological material. The Landschutz-ascites carcinoma was maintained by serial transplantation in albino mice. The ascitic fluid was harvested by collection in an equal volume of 0·154M-NaCl 7 days after inoculation, and the following operations, differing slightly from the original procedure (Smellie *et al.* 1959), were carried out at 0–4°.

Preparation of soluble extract. Cells were sedimented by centrifuging at 500*g* for 5 min. and were washed twice by suspending in 0·154M-NaCl, followed by centrifuging at 500*g* for 5 min. The cells were disrupted osmotically in 13–15 vol. of water, pH 7·0, and the preparation was made either 0·03M with KCl and 0·01M with potassium phosphate buffer, pH 7·5, or 0·01M with potassium phosphate buffer, pH 7·5. The soluble extract was obtained by centrifuging the disrupted-cell suspension at 105000*g* for 60 min., the sediment being retained for the preparation of DNA. There was no difference in DNA-polymerase activity of the extracts obtained by these two procedures if allowance was made for differences in the concentration of K^+ ions. In most of the experiments to be described, the soluble extract was the source of polymerase activity, but in some studies fractions prepared from the soluble extract were used. In these cases, the polymerase preparation is denoted by the term 'polymerase fraction'.

Preparation of DNA. The method of Kay, Simmons & Dounce (1952) was followed for the preparation of DNA from the 105000*g* sediment mentioned above. The DNA was dissolved in water or in 0·01M-NaCl to give a concentration of 2 mg. of DNA/ml.

A portion (200 ml.) of the solution was incubated with 400 μg. of ribonuclease (crystalline, Nutritional Biochemicals Corp., Cleveland, Ohio) and 20 ml. of 1·0M-tris-HCl buffer, pH 7·6, for 2 hr. at 37°. An equal volume of water-saturated phenol was then added and the mixture was gently shaken for 60 min. at 20°. The supernatant aqueous layer which was obtained on centrifuging the mixture at 2000*g* for 20 min. was drawn off, leaving the phenol layer and a thin interfacial film of insoluble material, which was carefully removed. The phenol layer was treated twice more with an equal volume of water in the manner described above, the aqueous layers being collected. The pooled aqueous layers were extracted three times with equal volumes of ether to remove phenol. The viscous solution was then made 1·0M with respect to NaCl, and DNA was precipitated by addition of ethanol to 50% (v/v). The precipitate was washed three times with ethanol and three times with acetone, and was dried in air and stored at −10°. Before use it was dissolved in 0·01M-NaCl and dialysed for 24 hr. at 4° against four 4 l. changes of

0·01 M-NaCl, the final concentration being 1·3–2·0 mg. of DNA/ml. DNA thus isolated from the Landschutz ascites-tumour cells is referred to as Landschutz DNA.

A suspension of bacteriophage T2 (kindly supplied by Dr L. Crawford) in 0·145 M-NaCl–0·01 M-sodium phosphate buffer, pH 7, was treated with sodium dodecyl sulphate according to the method of Kay et al. (1952) for the preparation of DNA. At intermediate stages of the procedure, the crude DNA preparation was dissolved in 1 mM-NaCl rather than water as described by Kay et al. (1952) and the final product was dissolved in 1 mM-NaCl at a DNA concentration of 190 μg./ml.

DNA from bacteriophage ØX174 was a gift from Dr R. L. Sinsheimer.

Thermal denaturation of DNA. Except where otherwise stated, heat-treatment of solutions of DNA (approx. 1·3–2·0 mg./ml.) was carried out by immersing portions (1 ml.) contained in 15 ml. graduated centrifuge tubes in a water bath at 100° for 10 min., and rapidly cooling the solutions by shaking the tubes in an ice bath. This treatment is referred to as thermal denaturation, and DNA not submitted to the heating process is termed native DNA.

Limited treatment with deoxyribonuclease. Native Landschutz DNA (4 ml. of 2 mg./ml. in 0·01 M-NaCl) was incubated at 37° for 60 min. with 45 μmoles of MgCl₂, 40 μmg. of deoxyribonuclease (crystalline, Nutritional Biochemicals Corp.) and 400 μmoles of tris–HCl buffer, pH 7·5. The total volume was 10 ml. After incubation, a portion (5 ml.) was thermally denatured and the remaining portion (5 ml.) was cooled to 0°.

A control experiment was conducted with the same reaction conditions but in the absence of deoxyribonuclease.

Measurement of priming capacity. The ability of a DNA preparation to act as primer in the polymerase synthetic system was measured by the previously described assay (Gray et al. 1960) modified slightly in individual experiments as described in the Results section. This assay involves the measurement of the incorporation of [³²P]-TMP from [³²P]TTP into an acid-insoluble form.

Potassium ions stimulate the polymerase reaction with an optimum at 0·04 M whereas increase in Na⁺ ion concentration progressively inhibits the reaction; moreover, small amounts of EDTA stimulate the system by as much as tenfold, whereas higher concentrations (above 0·75 mM) inhibit extensively.

RESULTS

For routine assay of polymerase activity, thermally denatured DNA from the Landschutz-ascites-tumour cells was used as primer in the reaction. The superiority of thermally denatured DNA over native DNA in this respect is illustrated in Fig. 1. Under standard-assay conditions, 200 μg. of thermally denatured DNA/ml. of assay mixture produced a maximal synthetic effect, and at this concentration a sevenfold stimulation of incorporation of [³²P]TMP residues was obtained, as compared with experiments with equal amounts of native DNA as primer. Prolonged heating (65 min. at 100°) reduced the priming efficiency of the DNA, presumably by causing excessive degradation. DNA from bacteriophage ØX174 was most effective

of all. The maximal synthetic effect of thermally denatured Landschutz DNA at 200 μg./ml. of reaction mixture was attained by ØX174 DNA at a concentration of 16 μg./ml.

In the experiments shown in Fig. 1, 0·68 μm-mole of [³²P]TMP was incorporated in the presence of 4 μg. of bacteriophage ØX174 DNA. This is equivalent to the incorporation of about 0·9 μg. of total nucleotide residues into DNA (assuming that equal amounts of dATP, dCTP, dGTP and [³²P]TTP were utilized). Therefore, 25 % of the bacteriophage ØX174 DNA was replicated under the assay conditions. The corresponding figures for 4 and 50 μg. of thermally denatured Landschutz DNA were about 1 and 2 % respectively.

There were no detectable differences between the priming capacities of the Landschutz DNA preparations described in Fig. 1, and those of Landschutz DNA samples prepared by the detergent technique of Kay et al. (1952), without further purification, dissolved in water, pH 7·0, and treated as in Fig. 1.

Fig. 2 shows the ultraviolet-absorption spectra of Landschutz DNA before and after thermal denaturation in 0·167 mM-NaCl and at a DNA concentration of approx. 27 μg./ml. The heat-treatment caused a hyperchromicity of about 29 % at 260 mμ.
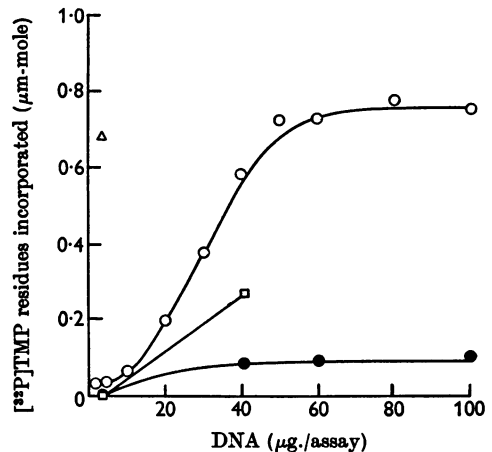

Fig. 1. Comparison of priming capacities of thermally denatured and native DNA in the polymerase assay. The reaction mixture contained these components, in a total volume of 0·25 ml.; 25 μmoles of tris–HCl buffer, pH 7·5; 1·0 μmole of MgCl₂; 0·12 μmole of EDTA; 1·0 μmole of potassium phosphate buffer, pH 7·5; 75 μm-moles each of dATP, dCTP, dGTP and [³²P]TTP (2·2 × 10⁶ counts/min./μmole); 0·3 mg. of polymerase fraction protein. The indicated amounts of DNA were added in solution in water. Incubation was at 37° for 60 min. ●, Native Landschutz DNA; ○, Landschutz DNA thermally denatured under standard-assay condition; □, Landschutz DNA thermally denatured at 100° for 65 min.; △, ØX174 DNA.

When the experiment was repeated with DNA at an identical concentration but in 0·154M-NaCl–0·01M-sodium citrate, pH 7·3, the spectra obtained were unaltered in shape. The spectrum of the un-heated sample was also unaltered in magnitude but the thermally denatured sample displayed a hyperchromic effect of 13%, i.e. 16% lower than the corresponding DNA solution heated in 0·167 mM-NaCl.

Almost identical results were obtained with Landschutz DNA prepared by the method of Kay et al. (1952) without further purification, and dissolved in water, pH 7, or in 0·154M-NaCl–0·01M-sodium citrate, pH 7·3.

Smellie et al. (1959) had shown that the primer DNA was no longer effective after exposure to deoxyribonuclease (50 µg./mg. of DNA) for 18 hr. However, further investigations showed that brief treatment of the native DNA with minute amounts of deoxyribonuclease (1 µmg./205 µg. of DNA) enhanced its priming capacity. If this was followed by thermal denaturation the preparation was 11 times as active as the native DNA (Table 1). The effect was optimum when deoxyribonuclease was used at a concentration of 8 µmg./ml. of reaction mixture and 1 µmg./100 µg. of DNA. Increase of the deoxyribonuclease concentration beyond this figure progressively reduced primer activity of the DNA until, at 150 µmg. of deoxyribonuclease/ml. of reaction mixture, it fell to the low value characteristic of the native Landschutz DNA (D. Bell & H. M. Keir, unpublished work).
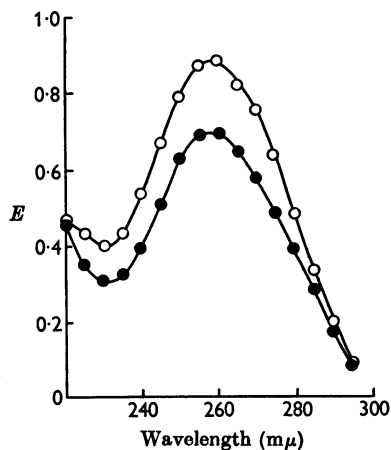

Fig. 2. Hyperchromicity of Landschutz DNA thermally denatured in 0·167 mM-NaCl. Landschutz DNA (0·1ml.; 1·62 mg./ml. in 0·01M-NaCl) was diluted to 6·0 ml. with water, pH 7·0. The solution was divided into two portions (3 ml.), one of which was heated for 10 min. at 100° and rapidly cooled. ●, Native DNA; ○, thermally denatured DNA.

The hyperchromicity and improved priming ability displayed by solutions of DNA thermally denatured in water or in dilute NaCl solutions (0·1–0·2 mM) was also given, as expected, by solutions of DNA in 0·01M-tris–HCl buffer, pH 7·0 (Tables 2 and 3). On the other hand, solutions of DNA of the same concentration, but in 0·01M-sodium phosphate buffer, pH 8·0, or 0·01M-sodium borate buffer, pH 9·0, possessed the high priming capacities before as well as after thermal denaturation when assayed in the polymerase system at pH 7·5 (Table 2). This phenomenon was not reflected in the extinction values of the solutions at 260 mµ, for a hyperchromic effect was still obtained after thermal denaturation (Table 3).

Table 1. *Effect of limited deoxyribonuclease action on priming capacity of Landschutz deoxyribonucleic acid*

The polymerase assay contained these components, in total volume 0·25 ml.: 10 µmoles of tris–HCl buffer, pH 7·5; 0·08 µmole of EDTA; 1 µmole of MgCl₂; 8 µmoles of KCl; 1 µmole of potassium phosphate buffer, pH 7·5; 18 µg. of DNA preparation; 50 µm-moles each of dATP, dCTP, dGTP and [³²P]TTP (4·85 × 10⁵ counts/min./µmole); 240 µg. of soluble extract protein. Incubation was at 37° for 60 min.

Treatment of DNA before assay	Priming capacity in polymerase assay (µm-moles of [³²P]TMP residues incorporated)
None	0·2
Limited deoxyribonuclease	0·32
Thermal denaturation	1·34
Limited deoxyribonuclease + thermal denaturation	2·15

Table 2. *Priming capacity of Landschutz deoxyribonucleic acid before and after thermal denaturation at different pH values*

The assay mixture contained these components, in total volume 0·25 ml.: 10 µmoles of tris–HCl buffer, pH 7·5; 0·75 µmole of MgCl₂; 0·08 µmole of EDTA; 50 µm-moles each of dATP, dCTP, dGTP and [³²P]TTP (6 × 10⁵ counts/min./µmole); 280 µg. of soluble extract protein; 65 µg. of DNA contained in 0·05 ml. of 0·01M buffer. the buffers being tris–HCl for pH 7·0, sodium phosphate for pH 8·0 and sodium borate for pH 9·0. Incubation was at 37° for 30 min. (These DNA solutions were diluted 1 in 40 with the appropriate 0·01M buffer for ultraviolet measurements as described in Table 3.)

pH	Priming capacity in polymerase assay (µm-moles of [³²P]TMP residues incorporated/mg. of protein/hr.)	
	Before thermal denaturation	After thermal denaturation
7·0	2·33	8·64
8·0	10·44	9·46
9·0	8·26	9·46

The extinctions at 260 mμ of solutions of Landschutz DNA in 0·15M-NaCl–0·015M-sodium citrate, pH 7·3, were measured before and after heat-treatment at 100° for 10 min., which was followed by either the usual rapid cooling or by slow cooling (the temperature being allowed to fall gradually from 100° to 20° over 3 hr.). Samples of the preparations were also taken for measurement of their priming capacities in the polymerase-assay system. The results (Table 4) indicate that the slow-cooling process produced a significantly greater hyperchromic effect and increase of primer activity than did rapid cooling.

In other experiments in which the thermal treatments were carried out at lower DNA concentrations and in 0·154M-NaCl–0·01M-sodium citrate, pH 7·3, hyperchromic effects of 11·4 and 13% were consistently obtained after the rapid- and slow-cooling processes respectively.

Table 3. *Hyperchromicity of Landschutz deoxyribonucleic acid thermally denatured at different* pH *values*

Landschutz DNA (1·8 ml.; approx. 1·35 mg./ml. in 0·01M-NaCl) was added to 0·2 ml. of 0·1M-tris–HCl buffer, pH 7·0, or 0·1M-sodium phosphate buffer, pH 8·0, or 0·1M-sodium borate buffer, pH 9·0, and the extinction coefficients were measured after dilution by 1 in 40 with the appropriate 0·01M buffer. Thermally denatured samples were diluted for ultraviolet measurements after heat-treatment and cooling.

| | Extinction coefficient at 260 mμ | | |
| | Before thermal denaturation | After thermal denaturation | Hyperchromicity (%) |
pH			
7·0	0·855	1·080	26·3
8·0	0·865	1·080	25·2
9·0	0·875	1·090	24·8

A DNA preparation from bacteriophage T2 was capable of acting as primer for the polymerase system before as well as after thermal denaturation, and to the same extent (Fig. 3). Under the assay conditions 5 and 30 μg. of bacteriophage T2 DNA were replicated to the extent of 10 and 7% respectively. In a separate experiment (Table 5) it
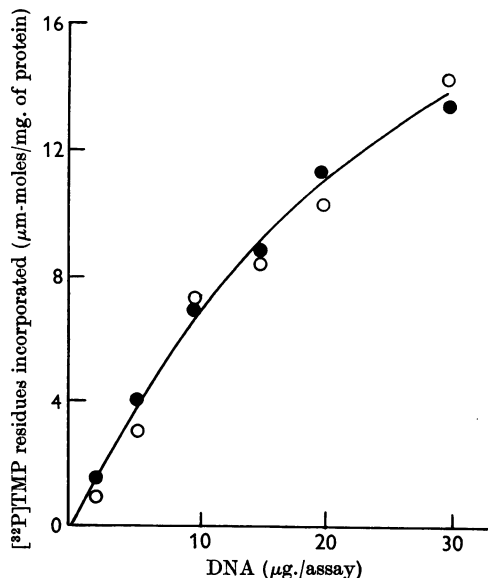

Fig. 3. Priming capacity of bacteriophage T2 DNA. The assay mixture contained these components, in total volume 0·25 ml.: 10 μmoles of tris–HCl buffer, pH 7·5; 1·0 μmole of MgCl$_2$; 0·08 μmole of EDTA; 8·5 μmoles of KCl; the indicated amounts of bacteriophage T2 DNA in 0·15 ml. of 0·01M-KCl; 50 μm-moles each of dATP, dCTP, dGTP and [^{32}P]TTP (7·5 × 10^5 counts/min./μmole); 110 μg. of soluble extract protein. Incubation was at 37° for 60 min. O, Native bacteriophage T2 DNA; ●, thermally denatured bacteriophage T2 DNA.

Table 4. *Effects of slow and rapid cooling of Landschutz deoxyribonucleic acid solutions after thermal denaturation*

Portions (1·0 ml.) of Landschutz DNA solution in 0·15M-NaCl–0·015M-sodium citrate were heated for 10 min. at 100°, followed by rapid cooling or slow cooling (see text). Samples (0·5 ml.) were diluted 1 in 20 with 0·15M-NaCl–0·015M-sodium citrate for measurements of extinction; also samples (0·5 ml.) were diluted 1 in 4 with the 0·15M-NaCl–0·015M-sodium citrate solution for use in the polymerase assay. The assay components in 0·25 ml. of reaction mixture were: 10 μmoles of tris–HCl buffer, pH 7·5; 0·625 μmole of MgCl$_2$; 0·08 μmole of EDTA; approx. 15 μg. of DNA (treated as indicated and contained in 0·1 ml. of 0·15M-NaCl–0·015M-sodium citrate); 50 μm-moles each of dATP, dGTP, dCTP and [^{32}P]TTP (1·4 × 10^6 counts/min./μmole); 0·48 mg. of soluble extract protein. Incubation was at 37° for 60 min.

Treatment of DNA before polymerase assay	Extinction coefficient at 260 mμ	Hyperchromicity (%)	Priming capacity in polymerase assay (μm-moles of [^{32}P]TMP residues incorporated)
None	0·778	—	0·43
Heat, rapid cooling	0·870	11·8	0·67
Heat, slow cooling	0·904	16·2	0·71

Table 5. *Comparison of bacteriophage* T2 *deoxyribonucleic acid and Landschutz deoxyribonucleic acid as primers in the polymerase system*

The assay mixture contained these components, in total volume 0·25 ml.: 10 μmoles of tris–HCl buffer, pH 7·5; 1 μmole of $MgCl_2$; 0·08 μmole of EDTA; 9 μmoles of KCl; 1 μmole of potassium phosphate buffer, pH 7·5; 30 μg. of DNA contained in 0·15 ml. of 1 mM-NaCl; 50 μm-moles each of dATP, dCTP, dGTP and [^{32}P]TTP (0·79 × 10^6 counts/min./μmole); 110 μg. of soluble extract protein. Incubation was at 37° for 60 min.

Primer	[^{32}P]TMP residues incorporated (μm-moles/mg. of protein/hr.)
Native Landschutz DNA	1·8
Thermally denatured Landschutz DNA	11·45
Native bacteriophage T2 DNA	17·45
Thermally denatured bacteriophage T2 DNA	17·55

was observed that native or thermally denatured bacteriophage T2 DNA primed the reaction more efficiently than thermally denatured Landschutz DNA. The latter was replicated to the extent of 5·5 % and the former to the extent of 8·5 %.

The ultraviolet-absorption spectra of bacteriophage T2 DNA, before and after thermal denaturation in 0·167 mM-NaCl at a DNA concentration of 32 μg./ml., were similar in shape to those shown in Fig. 2 for Landschutz DNA. The ratio of the extinction at 250 mμ to that at 260 mμ was slightly lower, and the ratio of extinction at 275 mμ to that at 260 mμ slightly higher than the corresponding ratios obtained with Landschutz DNA. The hyperchromic effect observed after heating was 18 %. A repeat of this experiment with bacteriophage T2 DNA solutions (32 μg./ml.) in 0·154 M-NaCl–0·01 M-sodium citrate showed a 12 % hyperchromic effect after thermal denaturation.

DISCUSSION

Aqueous solutions of DNA undergo a striking physical transition when heated. The transition ('denaturation') involves collapse of the double helical configuration (Watson & Crick, 1953) of the DNA, and takes place within a very narrow temperature range, usually 80–100°, according to the ionic strength of the medium and the number of guanine–cytosine hydrogen-bonded pairs in the double helix. Guanine–cytosine pairs confer greater stability on the molecule than do adenine–thymine pairs (Rice & Doty, 1957; Marmur & Doty, 1959). The denaturation is regarded as replacement of the highly ordered double helix by randomly coiled polydeoxyribonucleotide chains, which display characteristics to be expected of

single-stranded material (Doty, Marmur, Eigner & Schildkraut, 1960).

Thermal denaturation is accompanied by a hyperchromic effect at about 260 mμ (Thomas, 1954; Lawley, 1956). With aqueous solutions of calf-thymus DNA, 0·195 M with respect to Na$^+$ ions, Doty, Boedtker, Fresco, Haselkorn & Litt (1959) showed that an increase in temperature from 25° to 95° resulted in a hyperchromicity of 42 % at 259 mμ; return of the temperature to 25° yielded a final hyperchromicity of 10 %. Marmur & Lane (1960) submitted certain biologically active bacterial DNA preparations to thermal denaturation. If the cooling rate were carefully controlled, there was a partial recovery of biological activity, indicating that specific reconstitution of complementary strands had taken place to form perfectly registered helical regions. Doty *et al.* (1960) reached similar conclusions by physicochemical methods.

It seems therefore that thermal denaturation of DNA could produce a macromolecule which would serve efficiently as a primer in our polymerase-assay system, since it would present to the enzyme a template on which new polydeoxyribonucleotide could be synthesized to give a product of the Watson–Crick type. Bollum (1959) showed that thermally denatured DNA was substantially superior to native DNA in primer activity in a calf-thymus-polymerase system, and Lehman (1959) obtained a twofold increase in primer activity with thermally denatured calf-thymus DNA in the purified polymerase system from *Escherichia coli*. Our Landschutz DNA primer with the ascites-enzyme system possesses similar properties (Fig. 1), although the thermally denatured primer does not, even after prolonged heating, attain the priming capacity of bacteriophage ØX 174 DNA, which is known to be single-stranded (Sinsheimer, 1959).

The question whether thermal denaturation of DNA is associated with lowering of molecular weight has not been fully resolved, although there are indications that there is no dramatic reduction after heating (Shooter & Butler, 1956; Shooter, Pain & Butler, 1956; Rice & Doty, 1957); but the situation is complex and depends on other factors, including ionic strength and DNA concentration (Thomas, 1954; Shooter & Butler, 1956; Cavalieri, Rosoff & Rosenberg, 1956; Inman & Jordan, 1960a, b). Thermal denaturation of bacterial DNA brings about a molecular-weight reduction by a factor of two (Doty *et al.* 1960).

The improved priming capacity of Landschutz DNA effected by limited treatment with deoxyribonuclease (Table 1) is interpreted as the consequence of increased availability of 3′-hydroxy terminal groups (Laskowski, 1961) and, in conjunction with thermal denaturation, of exposure of

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further stretches of single-stranded template to the polymerase system. Limited deoxyribonuclease action produces random splitting of phosphodiester bonds in individual DNA strands without substantial reduction of molecular weight but with a lowering of viscosity (Dekker & Schachman, 1954; Schumaker, Richards & Schachman, 1956; Thomas, 1956).

Highly purified polymerase preparations from *E. coli* can catalyse extensive synthesis of DNA from substrate triphosphates with native calf-thymus DNA as primer (Bessman, Lehman, Simms & Kornberg, 1958). These polymerase fractions contain nucleases (Lehman, Bessman, Simms & Kornberg, 1958), which could well account for the success of the native DNA primer in the system. Lehman (1959) pointed out that treatment of primer DNA with minute amounts of pancreatic deoxyribonuclease (1 μg./200 mg. of DNA) increases the rate of the polymerase reaction two- to three-fold. A DNA-synthesizing system from cultured lymphoblasts from a mouse was stimulated fivefold when deoxyribonuclease was included in the assay mixture at a concentration of 16 μg./ml. (Mantsavinos & Canellakis, 1959); increases of the deoxyribonuclease concentration up to 300 μg./ml. resulted in complete inhibition of the reaction. Sarkar (1961) found that pretreatment of native calf-thymus DNA with deoxyribonuclease gave a product capable of serving as primer in the polymerase systems from calf-thymus and regenerating rat liver.

The hyperchromic effect which accompanied thermal denaturation of Landschutz DNA in 0·167 mM-sodium chloride (Fig. 1) was substantially lowered when the experiment was done with DNA solutions in 0·184 M-Na$^+$ ions, presumably because of decreased electrostatic repulsion between complementary strands. Thermal denaturation for primer production was therefore carried out at a DNA concentration of 0·13–0·2 g./100 ml. in water or in 0·01 M-sodium chloride, so that a greater denaturation effect would be obtained than in more concentrated salt solutions.

Exposure of DNA solutions to mildly alkaline buffers may reduce bond strength in one or more of the known hydrogen-bond types, without concomitant bond rupture, so that at pH 8 and 9 the Landschutz DNA exhibits high primer activity before and after heating, but still produces the expected hyperchromicity after heating (Tables 2 and 3). In pH 7 buffer, however, heat is required to induce a high priming capacity. Denaturation of DNA at pH 11–12 is known to occur (Ehrlich & Doty, 1958), and we observed that exposure of Landschutz DNA to 0·01 M–0·10 M-potassium hydroxide solutions for 2–5 hr. at 37° increased its priming capacity.

The strand separation and recombination ob-

served after heating and slow cooling of bacterial DNA in the presence of 0·195 M-Na$^+$ ions could not be demonstrated with calf-thymus DNA (Doty *et al.* 1960; Sarkar & Dounce, 1961) by measurements of extinction in ultraviolet light. Our results with Landschutz DNA (Table 4) confirm these findings for mammalian DNA by showing that reconstitution cannot be detected either by a fall in extinction at 260 mμ or by a decrease in the priming ability of the DNA. The small observed increases in extinction and priming capacity can be attributed to more extensive degradation of the DNA (Rice & Doty, 1957).

It was surprising that bacteriophage T2 DNA had high priming capacity both before and after thermal denaturation. However, the hyperchromicity of 18 % after thermal denaturation in 0·167 mM-sodium chloride compared with 29 % for Landschutz DNA (Fig. 2) suggests that the bacteriophage T2 DNA was partially denatured in the course of extraction from the bacteriophage. The relative instability of bacteriophage T2 DNA in this context might be ascribed to its high adenine and thymine content (Wyatt & Cohen, 1953). Other factors which might contribute to a weakened hydrogen-bonded system are the presence in this DNA of a small amount of 6-methylaminopurine (Dunn & Smith, 1958) and of 5-hydroxymethylcytosine instead of the more common pyrimidine, cytosine (Wyatt & Cohen, 1953), most of it substituted in the 5-hydroxymethyl position with glucosyl residues (Sinsheimer, 1960; Lehman & Pratt, 1960). Bollum (1959) found that thermally denatured bacteriophage T2 DNA (10 min. at 99° in water, pH 6·8) supported polydeoxyribonucleotide synthesis at a rate five times as great as that when the native DNA was used as primer in the polymerase system from calf-thymus gland. He also apparently found heating of bacteriophage T2 DNA for 2 min. at 99° to be adequate for production of satisfactory primer (Bollum, 1960).

SUMMARY

1. Experiments have been carried out to assess the priming ability of some deoxyribonucleic acid preparations in the deoxyribonucleic acid-polymerase system from Landschutz ascites-tumour cells.

2. Thermally denatured deoxyribonucleic acid from Landschutz ascites-tumour cells had a priming capacity seven times that of native deoxyribonucleic acid. Prolonged thermal treatment, however, reduced the priming capacity.

3. The single-stranded deoxyribonucleic acid from bacteriophage ØX 174 was greatly superior to thermally denatured Landschutz-tumour deoxyribonucleic acid in priming capacity.

4. Limited treatment of Landschutz-tumour deoxyribonucleic acid with pancreatic deoxyribonuclease improved its priming capacity by about 60%, but subsequent thermal denaturation gave a primer 11 times as efficient as the native deoxyribonucleic acid.

5. Solutions of Landschutz-tumour deoxyribonucleic acid in 0·01M buffer, pH 8 or pH 9, were capable of priming the polymerase reaction at pH 7·5 efficiently before as well as after thermal treatment. When the solution was prepared in 0·01M buffer, pH 7, thermal treatment was required to produce an active primer.

6. Thermal denaturation of deoxyribonucleic acid from Landschutz-tumour cells was accompanied by a hyperchromic effect at 260 mμ, the magnitude of which was dependent upon the salt concentration of the medium. Hyperchromicity after thermal treatment was also displayed by the deoxyribonucleic acid solutions in 0·01M buffer, pH 8 and pH 9.

7. Attempts to demonstrate a return of thermally denatured deoxyribonucleic acid to the native state by slowly cooling the heated solution in 0·15M-sodium chloride–0·015M-sodium citrate indicated that there was no reversal of denaturation as revealed by measurements of priming capacity and extinction.

8. A preparation of deoxyribonucleic acid from bacteriophage T2, although exhibiting hyperchromicity after thermal treatment, was capable of priming the polymerase reaction before as well as after heating, and to the same extent.

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