Interaction of DNA Polymerase I of *Escherichia coli* with Nucleotides. Antagonistic Effects of Single-Stranded Polynucleotide Homopolymers†

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Received November 28, 1984

ABSTRACT: Binding of deoxyribonucleoside 5′-triphosphates to DNA polymerase I of *Escherichia coli* was measured by using a microscale nonequilibrium dialysis method. It allowed rapid and economic measurement of dissociation constants, with negligible interfering side reactions. A stoichiometry of 1 mol of nucleoside 5′-triphosphate/mol of DNA polymerase was measured, and the occurrence of a single binding site was established, for which the nucleotides competed in the binary complex with the polymerase. Binding affinities decreased in the order dGTP ≳ dATP > dCTP ≃ dTTP. These results are in agreement with previous findings [Englund, P. T., Huberman, J. A., Jovin, T. M., & Kornberg, A. (1969) *J. Biol. Chem. 244*, 3038–3044] except that, in a few cases, values of dissociation constants were smaller by factors of 2–3. The cations Mg^{2+} and Mn^{2+}, as well as spermine, slightly enhanced complex stability at low levels and decreased it at high concentrations, while NaCl and Hg^{2+} had only destabilizing effects. Recognition between nucleoside 5′-triphosphates and nucleotide templates was studied by titration of the polymerase–[^{3}H]dGTP complex with polynucleotide homopolymers. Complementary poly(dC) did not affect binding of dGTP, and noncomplementary templates caused rejection of the nucleotide. Rejection of dGTP followed a saturation dependence with an equivalence of 110 ± 10 monomer units of polynucleotides bound per molecule of DNA polymerase. The results favor a model by which recognition arises chiefly from the stereogeometrical fit of complementary template and nucleoside 5′-triphosphate into a rigid binding site.

The mechanism of interaction between nucleoside 5′-triphosphates and DNA polymerase in the enzymatically productive complex with template and primer is still unsolved, despite extensive work in this field (Kornberg, 1980). Binding of nucleotides to DNA polymerase I of *Escherichia coli* has been studied by equilibrium dialysis, and dissociation constants derived have been thought to reflect the anchoring of the triphosphate moiety to the active site (Englund et al., 1969a). Selection among bases in the presence of template has been suggested to occur due to Watson–Crick base pairing between complementary bases, because the sugar and base components in binary enzyme–nucleotide complexes were of minor importance. What is lacking is a direct measurement of the effect of template on the binding of nucleoside 5′-triphosphates in order to prove this assumption.

Unfortunately, classical dialysis measurements involve periods during which an active template and primer inevitably promote polymerization, or become degraded (Englund et al., 1969b). We describe here a rapid dialysis method with which binding of nucleotides can be measured without appreciable interference by other enzymatic activities. Effects of homopolymer templates were examined in the absence of primer. The results indicate that binding of nucleoside 5′-triphosphates to DNA polymerase is strongly inhibited by noncomplementary templates.

Experimental Procedures

Materials

Escherichia coli DNA Polymerase I. The *E. coli* strain W3110 carrying the temperature-inducible prophage NM857 (pol A cI857 Qam 73 Sam7 attVred$^-$) was grown according to Kelley et al. (1977). DNA polymerase was purified to homogeneity from these cells [>95% purity by polyacrylamide gel electrophoresis according to system 1 described by Maurer (1968)] following the procedure of Kelley & Stump (1979). Its specific activity was 24 000 units/mg.

Nucleotides, Nucleic Acids, and Other Chemicals. Tritiated dATP, dGTP, and dTTP (10–50 Ci/mmol) were purchased from Amersham International (England). Unlabeled nucleotides, poly(dA), and poly d(AT) were from Boehringer/Mannheim, other synthetic oligo- and polynucleotides were from P-L Biochemicals, salmon testis DNA, spermine, and $HgCl_2$ were from Sigma, terminal deoxynucleotidyltransferase was from Bethesda Research Laboratories, and poly(ethylenimine)-impregnated cellulose sheets (Polygram Cel 300 PEI/UV_{254}) were from Machery Nagel (Düren). All other chemicals were of the highest purity available from Merck (Darmstadt).

Purity of nucleoside 5′-triphosphates and polynucleotides with respect to contaminating nucleotides was assessed by chromatography on poly(ethylenimine)–cellulose sheets as described later. Molecular weights of homopolymers were 3.5 × 10^5 for poly(dA), 2.0 × 10^5 for poly(dC), 7 × 10^5 for poly(dG), and 2.8 × 10^5 for poly(dT).

Polynucleotide $(dT)_{1000}$-[^{3}H]$(dT)_{3.5}$ was synthesized from poly(dT) and [^{3}H]dTTP (3 Ci/mmol) according to Wang et al. (1974), and salmon testis DNA was activated according to Loeb (1969).

Methods

DNA Polymerase Assay. The assay for DNA polymerase was carried out in 150 μL volumes containing 50 mM 3-(*N*-morpholino)propanesulfonic acid buffer, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 3 mM ethylenediaminetetraacetic acid (EDTA),[1] 3 mM 2-mercaptoethanol, 33 μM dATP, 33 μM

† This work was financially supported by Deutsche Forschungsgemeinschaft Grant Ho 416/11.

0006-2960/85/0424-3618$01.50/0

dCTP, 33 μM dGTP, 3 μM [^{3}H]dTTP (1 Ci/mmol), 30 μg of activated salmon testis DNA, 80 μg of bovine serum albumin, and DNA polymerase. After a 30-min incubation at 37 °C, 2 mL of 10% (v/v) of saturated cold TCA was added, and the precipitate was collected on Whatman GF/C filters, washed first with TCA and then with 70% (v/v) aqueous ethanol, then dried, and counted with 30% efficiency in a toluene-based scintillation cocktail (Nuclear Chicago Isocap 300). One unit of DNA polymerase activity is defined as the amount of enzyme catalyzing the incorporation of 10 nmol of dTTP in 30 min at 37 °C.

Thin-Layer Chromatography on Poly(ethylenimine)-Cellulose Sheets. To determine the composition of mixtures of radiolabeled nucleotides by thin-layer chromatography, 1–2 μL of the solutions to be tested was spotted on to chromatograms, as were 1-μL amounts of solutions containing reference nucleotides (4 mM each), which were run in parallel. The chromatogram was developed in 0.75 M potassium phosphate buffer, pH 3.5, at room temperature. Nucleotide spots were localized under UV light, cut out, and counted in a toluene-based scintillation cocktail. Slices of the chromatogram were counted with an efficiency of 30%.

Incorporation of ^{3}H-Labeled Nucleotides into Polynucleotides. To 1 μL of reaction solution from the dialysis cell was added 100 μL of DNA-carrier solution containing 50 mM EDTA, 1 M sodium acetate, pH 4.0, and 2 A_{260} units/mL salmon testis DNA. This step was followed by precipitation with 2 mL of cold TCA and collection on GF/C filters as described for the DNA polymerase assay.

Nonequilibrium Dialysis. Binding of nucleotides to DNA polymerase I was measured by the method of Colowick & Womack (1969) adapted here for application on a microscale. The system consisted of an upper chamber (100 μL volume, 6 mm in diameter) and a lower chamber (1.8 μL volume, 1.5 mm in diameter), separated by a dialysis membrane. The upper cell contained, in 40–50 μL of buffer, DNA polymerase and varying amounts of nucleoside 5′-triphosphates or other ligands, which were added from the top by using a calibrated syringe (1-or 10-μL capacity). The reaction mixture was stirred at constant speed with a small magnetic bar. Small molecules passed by dialysis into the lower compartment via a Visking dialysis membrane (1.5 mm in diameter, exclusion limit M_r 10000–20000). The lower compartment was flushed with buffer (same as in the upper compartment) at a constant rate of 3 drops/min (LKB VarioPerpex pump). Fractions of 3 drops were collected directly in a suitable scintillation cocktail for counting in a Nuclear Chicago Isocap 300.

Titration was performed at room temperature by addition of 0.1–1.0-μL amounts of ligands of known concentration and specific radioactivity. Between additions, three to four fractions (3–4 min) were collected. about this much time was needed to approach the steady-state rate of the appearance of the radioactivity in the effluent. At the end of the experiment, a high concentration of unlabeled nucleotide was added, and collection of fractions continued for several minutes. This last portion of the time dependence was required in order to correct for losses of radioactivity during dialysis (Güntner & Holler, 1979). Duration of the complete experiment was 60 min for a titration experiment or approximately 4 min for a single ligand addition. Single additions were particularly useful where side reactions might obscure the results of the longer titrations. Before the start of the experiment, DNA polymerase was equilibrated in the upper compartment by dialysis overnight against circulating buffer in the lower compartment. At the end of the experiment, the precise volume of the reaction mixture was measured and the volume before the first addition of ligands recalculated. Initial concentrations of DNA polymerase were of the order of 5–100 μM depending on the affinity for the particular ligand.

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; TCA, trichloroacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

Table I: Dissociation Constants of Binary DNA Polymerase I–Nucleotide Complexes[a]

nucleotide	dissociation constant (μM) measured	reference[b]
[^{3}H]dATP	8 ± 2	
via [^{3}H]dGTP[c]	8 ± 2	
via [^{3}H]dGTP[d]	16 ± 6	33 ± 8
dCTP via [^{3}H]dGTP	47 ± 5	147 ± 59
[^{3}H]dGTP	9.1 ± 1.1	
[^{3}H]dGTP[d]	9.9 ± 1.8	12 ± 3
[^{3}H]dTTP	60 ± 5	81 ± 29
via [^{3}H]dGTP	51 ± 10	
[^{3}H]dTTP[e]	105 ± 20	
dAMP via [^{3}H]dGTP	200–300	

[a] In Tris-HCl buffer (50 mM, pH 7.5 at 23 ± 2 °C), 7 mM $MgCl_2$, and 10 mM 2-mercaptoethanol. [b] Englund et al. (1969a). [c] Competition assay with [^{3}H]dGTP. [d] In potassium phosphate buffer (50 mM, pH 7.5 at 23 ± 2 °C), 7 mM $MgCl_2$, and 10 mM 2-mercaptoethanol. [e] In the presence of 2.5 mM $HgCl_2$.

Titration data were evaluated as described (Güntner & Holler, 1979). For each addition of ligand during titration, the value of the free diffusible radioactivity in the upper chamber was corrected for the amount lost by diffusion into the effluent buffer.

Binding of a given nucleotide to DNA polymerase was followed either by measuring the amount of radioactivity that became nondiffusible after binding to DNA polymerase (Güntner & Holler, 1979) or by determining the amount of radioactivity that became diffusible because it was released from the complex during the course of competition with an unlabeled ligand under study (Englund et al., 1969a). In the first case, concentrations of free and bound ligands were used to determine numbers of binding sites and values of dissociation constants according to Scatchard (1949). In the second case, values of dissociation constants were calculated according to the formula derived by Englund et al. (1969a) which assumes a single binding site. In control experiments, it was verified that added polynucleotides alone had no effect on the diffusion of nucleotides.

Results

Binary Complexes of DNA Polymerase I with Nucleoside 5′-Triphosphates. Examples of titration of DNA polymerase with [^{3}H]dGTP and of displacement titration with dATP in the presence of [^{3}H]dGTP are shown in parts A and B of Figure 1, respectively. Data for binding to the polymerase, as plotted according to Scatchard, are shown in the inset in Figure 1A. The data points can be approximated by a straight line, as expected for a single binding site. Displacement of [^{3}H]dGTP in the presence of increasing concentrations of dATP is shown in the inset in Figure 1B.

Titration was performed with [^{3}H]dATP, [^{3}H]dGTP, and [^{3}H]dTTP. From the Scatchard plots, values of 1.0 ± 0.1 were obtained for the number of binding sites. The dissociation constants of the enzyme–nucleotide complexes are listed in Table I and are compared with those obtained from the competition/displacement method. Values for both methods were the same within experimental error. The similar results confirm those of Englund et al. (1969a), which indicate a single

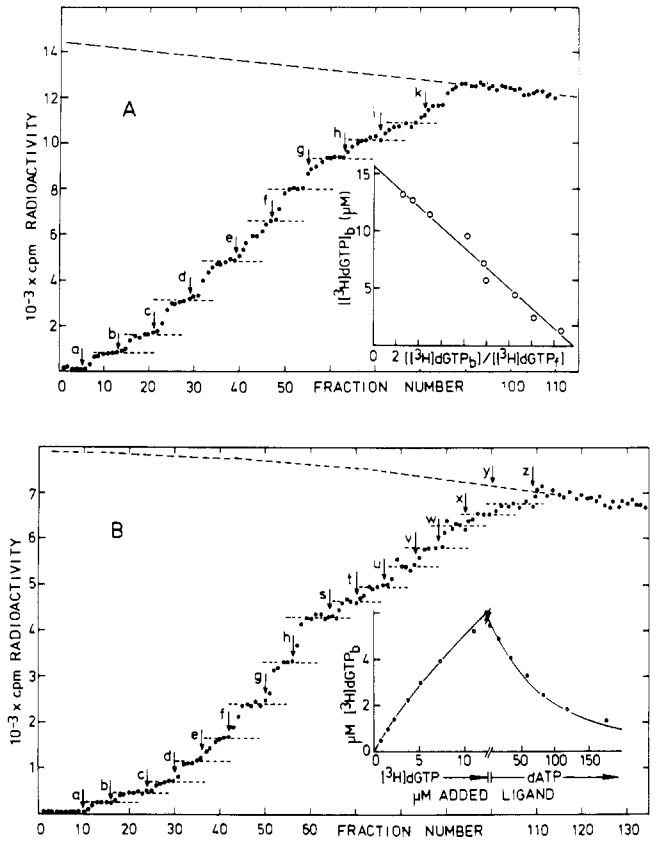

FIGURE 1: Titration of DNA polymerase I with nucleoside 5′-triphosphates measured by nonequilibrium dialysis. (Panel A) Titration with [^{3}H]dGTP of 19 μM DNA polymerase in 50 mM Tris-HCl at pH 7.5. At positions of arrows a–i, [^{3}H]dGTP was added to the upper chamber of the dialysis apparatus to give final concentrations of 4.1–63.4 μM. Diffusible radioactivity in the effluent of the lower chamber was collected in the fractions indicated. At the position of arrow k, dGTP was added to give a final concentration of 6.8 mM, in order to render all available radioactivity free to diffuse. Steady-state levels as indicated, in relation to total free, diffusible radioactivity (broken line at the top of the figure), were used to calculate concentrations of [^{3}H]dGTP that was bound to the polymerase and of unbound [^{3}H]dGTP. These values were plotted according to Scatchard in the figure inset. (Panel B) Titration first with [^{3}H]dGTP (0.78–12.7 μM indicated by arrows in positions a–h) followed by titration with dATP (6.06–120 μM, arrowed s–y) of 13 μM DNA polymerase. In position z, complete radioactivity was made diffusible by addition of 5.6 mM dGTP. Concentrations of bound [^{3}H]dGTP were calculated as under panel A and were plotted in the figure inset as functions of total added [^{3}H]dGTP and dATP, respectively. The points for dATP were used for calculation of dissociation constants of the polymerase–dATP complex by employing the formula published by Englund et al. (1969a). All calculations were performed after proper correction for the loss of diffusible radioactivity (Güntner & Holler, 1979) and for dilutions (not shown).

binding site for all nucleoside 5′-triphosphates on the DNA polymerase, at least in the case of binary complexes.

As judged from the relative magnitudes of dissociation constants, purine nucleotides bind with higher affinities than pyrimidine nucleotides. Affinity decreases in the order dGTP $\gtrsim$ dATP > dCTP $\simeq$ dTTP. Values of dissociation constants differ at most by a factor of 6. The triphosphate moiety is indispensable for tight binding (Englund et al., 1969a) as dAMP shows only low affinity.

Samples were taken from the reaction mixture as controls and assayed by chromatography on poly(ethylenimine)–cellulose sheets for their nucleotide composition. It was found that triphosphates were hydrolyzed to diphosphates and monophosphates to an extent proportional to the amount of protein. With one preparation of DNA polymerase (30 μM), half of the original triphosphate was hydrolyzed in approximately 90 min. As found by Englund et al. (1969a), degradation was inhibited in the presence of $HgCl_2$ (in the above case, 3% hydrolysis in 90 min at 0.4 mM $HgCl_2$).

Effects of Ionic Strength and Cations. The amount of [^{3}H]dGTP bound to DNA polymerase was measured as a function of concentrations of NaCl, $MgCl_2$, $MnCl_2$, spermine hydrochloride, and $HgCl_2$ (Figure 2). In principle, binding did not require addition of any cation. In the case of NaCl, effects of which are presumed to reflect only ionic strength, the binary enzyme–nucleotide complex was destabilized, with half the maximum amount of complex present at 200 mM NaCl. In this regard it was surprising that replacement of Tris-HCl (50 mM, pH 7.5) by potassium phosphate (50 mM, pH 7.5) did not result in a higher value for the dissociation constant (Table I).

The effects of the other cations were specific inasmuch as they showed a slight enhancement of complex formation with maxima obtained with 0.04 mM spermine, 0.1 mM Mn^{2+}, and 3 mM Mg^{2+}. Destabilizing effects on the complex occurred in the concentration range of 0.04–8 mM for spermine, 0.1–10 mM for Mn^{2+}, and 0–0.6 mM for Hg^{2+}, and these cannot be accounted for by ionic strength effects.

Effects of Synthetic Polydeoxyribonucleotide Homopolymers. Effects of synthetic polydeoxyribonucleotide homopolymers, of $(dG)_{12-18}$, and of dAMP on the stability of the [^{3}H]dGTP–DNA polymerase complex were measured by keeping the concentration of the radioactively labeled nucleotide constant (in the order of 8–10 μM, the value of the dissociation constant of the complex) and changing the concentration of the added homotemplate. Results as shown in Figure 3, are as follows. (1) Addition of polynucleotides and of dAMP caused an increase in diffusible radioactivity, equivalent respectively to polynucleotide- and dAMP-dependent dissociation of the dGTP–DNA polymerase complex. (2) Displacement of [^{3}H]dGTP from the complex occurred only in the presence of noncomplementary homopolymers [poly(dA), poly(dG), and poly(dT)] but not in the presence of poly(dC), which is complementary. Analogous results were obtained with [^{3}H]dATP and [^{3}H]dTTP instead of [^{3}H]dGTP, although they were less clear-cut because of obscuring side reactions (see below). The size of the homopolymer is of importance since $(dG)_{12-18}$ exhibits a small effect on complex stability (Figure 3), whereas the monophosphate dAMP was more potent although it is even smaller (Figure 3). (3) The degree of [^{3}H]dGTP displacement depended on the concentrations of both homopolymer and DNA polymerase. At higher concentrations of protein, more polynucleotide was required in order to achieve the same extent of displacement. The dependence on protein concentration was linear. This was accounted for in Figure 3 by plotting the ratio of polymer (in terms of monomers) to enzyme concentration. The same degree of dependence was then found for all noncomplementary polymers. (4) This dependence was linear within experimental error for low concentrations of polymers and approached zero concentration of [^{3}H]dGTP–DNA polymerase complex at high concentrations. A titration curve, as the one in Figure 3, would be linear at low, varied concentrations of ligand if the titrated protein was present at a concentration higher than the value of the dissociation constant for the complex with the ligand. In practice, the point of equivalence between protein and ligand (here, polynucleotides) is given by the intersection of lines through that linear portion and through the portion of the titration curve representing saturation (complete displacement of [^{3}H]dGTP). In our case, we de-

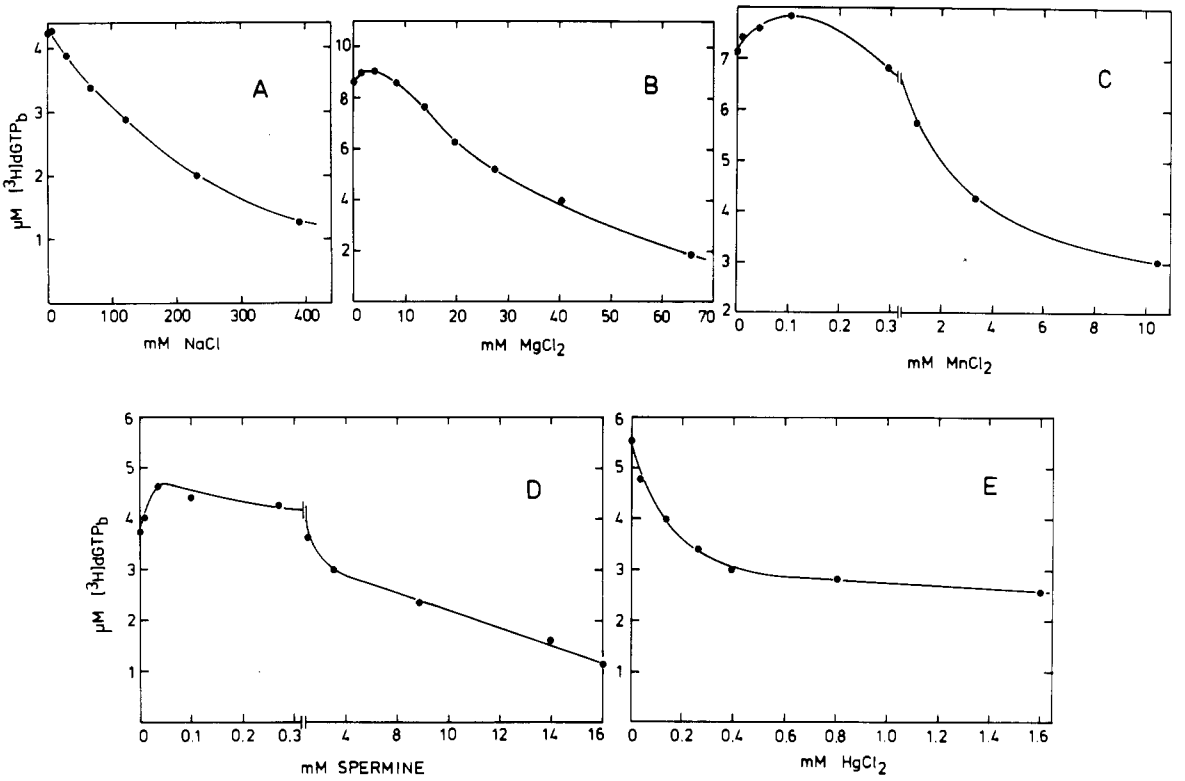

FIGURE 2: Titration of DNA polymerase I–[^{3}H]dGTP complex with various cations. The concentration of [^{3}H]dGTP that remained bound to the polymerase is shown as a function of concentration of total added cation. (Panel A) NaCl in the presence of 10 μM polymerase and 11 μM [^{3}H]dGTP. (Panel B) $MgCl_2$ in the presence of 21 μM polymerase and 13.1 μM [^{3}H]dGTP. (Panel C) $MnCl_2$ in the presence of 23 μM polymerase and 11 μM [^{3}H]dGTP. (Panel D) Spermine hydrochloride in the presence of 10.1 μM polymerase and 9.5 μM [^{3}H]dGTP. (Panel E) $HgCl_2$ in the presence of 21.4 μM polymerase, 7 mM $MgCl_2$, and 9 μM [^{3}H]dGTP.

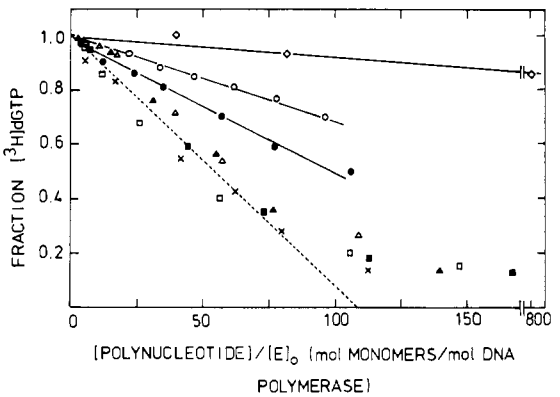

FIGURE 3: Titration of DNA polymerase I–[^{3}H]dGTP complex with polynucleotide homopolymers and with dAMP. The ratio of [^{3}H]-dGTP that remained bound to the polymerase is shown as a function of total concentration (in monomer units) of polynucleotides (and respectively dAMP) divided by the concentration of DNA polymerase. (×) Poly(dA) in the presence of 22 μM polymerase and 10 μM [^{3}H]dGTP, (◇) poly(dC) in the presence of 6.8 μM polymerase and 10 μM [^{3}H]dGTP [data points refer to repeated experiments with single additions of poly(dC)], (△) poly(dG) in the presence of 8 μM polymerase, 15 μM [^{3}H]dGTP, and 50 mM potassium phosphate buffer, pH 7.5, (▲) poly(dG) in the presence of 8.5 μM polymerase, 15 μM [^{3}H]dGTP, and 50 mM potassium phosphate, (□) poly(dT) in the presence of 16.1 μM polymerase and 10 μM [^{3}H]dGTP, (■) poly(dT) in the presence of 20 μM polymerase and 10 μM [^{3}H]dGTP, (○) $(dG)_{12-18}$ in the presence of 9.5 μM polymerase and 35 μM [^{3}H]dGTP, and (•) dAMP in the presence of 29 μM polymerase and 10 μM [^{3}H]dGTP. Unless otherwise indicated, all reaction solutions contained Tris-HCl, buffer, pH 7.5, 7 mM $MgCl_2$, and 10 mM 2-mercaptoethanol.

termined an equivalence of 110 ± 10 mol of polynucleotide monomers/mol of DNA polymerase and an upper limit of 8 μM for the dissociation constant.

Control experiments carried out in the case of the displacement measurements showed that with [^{3}H]dATP and [^{3}H]dTTP as diffusible ligands and poly(dT) and poly(dA), respectively, as added complementary polynucleotides, assessment of results was hindered by side reactions. In the first case, addition of poly(dT) to the enzyme–[^{3}H]dATP complex caused rapid hydrolysis of the triphosphate. In the second case, addition of poly(dA) to the enzyme–[^{3}H]dTTP complex led to incorporation of radioactivity into trichloroacetic acid precipitable material, which subsequently disappeared during formation of [^{3}H]dTMP.

The degree of side reactions was low when [^{3}H]dGTP was used as diffusible ligand in the presence of homopolymers. For example, in the presence of 1.1 mM poly(dC) only 8% of total (10.5 μM [^{3}H]dGTP) was incorporated into acid-insoluble material after 140 min (27 μM DNA polymerase), and the amount of hydrolysis was approximately that observed in the absence of homopolymer. This small degree of side reactions cannot explain the effects on diffusibility observed in Figure 3.

Discussion

Method. Binding of nucleoside 5′-triphosphates to DNA polymerase I has been previously measured by an equilibrium dialysis method (Englund et al., 1969a). A single binding site on the enzyme was reported, and this accommodated all four deoxyribonucleoside 5′-triphosphates. Values of dissociation constants are listed in Table I for comparison and are in general agreement, though we observed somewhat lower values. This can be attributed to our using shorter dialysis times, during which a minimum of hydrolysis of the triphosphates occurs. Another source of the discrepancies could be the previous inclusion of $HgCl_2$, which suppressed the hydrolysis (Englund et al., 1969a), but which we found to be

inhibitory (Table I and Figure 2E).

Nucleoside 5′-Triphosphate Binding Sites. The dialysis binding experiments of Englund et al. (1969a) were followed by experiments in which electron paramagnetic resonance and water proton relaxation rates were measured (Slater et al., 1972) and later by kinetic investigations of DNA polymerization in the presence of polynucleotide homopolymers as template-primers (Travaglini et al., 1975). It was then suggested, on the basis of both observed enhancement of longitudinal nuclear magnetic relaxation rates of water protons in the presence of Mn^{2+} ions and nonlinear reaction kinetics, that nucleoside 5′-triphosphates could bind to multiple sites in the active center of the polymerase. Under our experimental conditions, in confirmation of the results of Englund et al. (1969a), we obtained linear Scatchard plots and observed linear competition between nucleoside 5′-triphosphates in the formation of binary complexes with DNA polymerase. This does not exclude, however, occurrence of multiple sites in the presence of templates and primers.

Salt Effects. The effect of increasing ionic strength was to decrease the stability of the DNA polymerase–dGTP complex. This is in agreement with previous suggestions that an electrostatic interaction between the triphosphate moiety and the active center of the polymerase is the main driving force for complex formation (Englund et al., 1969a) and that shielding by counterions should lead to the observed destabilization. On the same basis, it had been expected that dissociation constants obtained in the presence of potassium phosphate buffer should be approximately 2-fold higher than for Tris-HCl buffer. Since this was not observed (Table I) a specific buffer effect has to be assumed. Specific effects of Mg^{2+}, Mn^{2+}, and spermine(4+) were expected inasmuch as these cations bind to nucleoside phosphates (Martell & Schwarzenbach, 1956; Holler, 1973) and, in the case of Mg^{2+} and Mn^{2+}, to DNA polymerase I (Slater et al., 1972). The presence of any of these cations was not essential for binding.

Effects of Deoxyribonucleoside 5′-Phosphate Homopolymers. Our results established that recognition of complementary nucleoside 5′-triphosphates in the presence of template was a consequence of anticooperativity between noncomplementary bases. This conclusion rests on the assumption that observed displacement of noncomplementary [^{3}H]dGTP from the complex with the polymerase, after addition of homopolymer, is not simply a result of competition for a common binding site. If that had been the case, complementary poly(dC) should have displaced [^{3}H]dGTP as well. In a previous report on the inhibition by noncomplementary nucleoside 5′-triphosphates, the difference of several orders of magnitude between values of Michaelis–Menten constants (complementary cases) and inhibition constants (noncomplementary cases) was attributed to base pairing between the complementary bases of the triphosphate substrate and the template which was missing in the noncomplementary case (Travaglini et al., 1975). However, our findings that there is no enhancement of binding affinity for the complementary nucleoside 5′-triphosphate in the presence of template seems to rule out this mechanism, at least for the case when the primer is absent. We favor a mechanism in which the noncomplementary triphosphate is rejected from binding because of the rigidity of the active site in the DNA polymerase–template complex. The template would bind adjacent to the triphosphate site on the enzyme. A conformational change need not be involved, since rigidity of the surrounding active center and of the template moieties would sterically prevent an approach of the noncomplementary triphosphate. The mechanism is probably more complicated in the complete system containing primer as well. Inasmuch as values of Michaelis–Menten constants for nucleoside 5′-triphosphates are smaller than values of dissociation constants of binary complexes (Table I; Travaglini et al., 1975), Watson–Crick base pairing might then contribute to binding.

Displacement of [^{3}H]dGTP in the presence of noncomplementary polymers showed a unique concentration dependence in the cases of poly(dA), poly(dG), and poly(dT) (Figure 3). This apparent lack of base specificity was due to the fact that the concentrations of DNA polymerase were higher than values of the dissociation constant for binding of the polynucleotides to the protein. Similar tight binding has been observed in centrifugation experiments (Englund et al., 1969b).

Our displacement studies with polynucleotides (Figure 3) have revealed that an equivalence of 110 ± 10 monomer units of polynucleotide per molecule of DNA polymerase is required in order to achieve complete saturation. The short $(dG)_{12-18}$ was ineffective in displacement. Although shorter stretches of nucleotide polymers have been reported to span the polymer binding site of DNA polymerase, values similar to ours for the number of monomer units (100–200) were calculated for binding of the protein to poly(dT) during centrifugation (Englund et al., 1969b).

ACKNOWLEDGMENTS

We are indebted to Dr. P. Orlean for improving the manuscript.

Registry No. dATP, 1927-31-7; dCTP, 2056-98-6; dGTP, 2564-35-4; dTTP, 365-08-2; Mg, 7439-95-4; Mn, 7439-96-5; spermine, 71-44-3; DNA polymerase, 9012-90-2.

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