A Comparison of Associated Enzyme Activities in Various Deoxyribonucleic Acid Polymerases*

Lucy M. S. Chang
From the Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032
F. J. Bollum
From the Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40506

SUMMARY

A comparison of activities associated with Escherichia coli polymerase I, calf thymus 6 to 8 S DNA polymerase and calf thymus 3.4 S DNA polymerase demonstrates that neither exonuclease activity nor pyrophosphorolysis is essential for polymerization. The 3.4 S enzyme has no demonstrable 3’- to 5’-exonuclease activity and cannot carry out pyrophosphate exchange. Under these conditions phosphodiester bond formation is kinetically irreversible.

The overall reaction for “replicative” polymerization of deoxynucleoside triphosphates into DNA is commonly written:

\[ \text{dNTP} \text{ initiated template} \xrightarrow{\text{Enzyme}} \text{dNMP-initiated template} + \text{PPi} + 
\]

and implies a pyrophosphate exchange. The substitution of OH− for PPi in this formulation produces exonuclease activity (1). The relationship of the polymerase function to the pyrophosphate exchange and hydrolytic functions in Escherichia coli polymerase I has been studied extensively (1-5). The high molecular weight species of DNA polymerase from calf thymus also has a pyrophosphate exchange (6), but exonuclease activities are apparently absent. This report demonstrates that the low molecular weight DNA polymerase (also from calf thymus gland) exhibits neither detectable pyrophosphate exchange nor exonuclease activities. These results indicate that pyrophosphorolysis and hydrolysis are not necessary consequences of polymerization function of DNA polymerases and point to the 3.4 S DNA polymerase as a favorable subject for mechanism studies.

The difference between the two species of mammalian DNA polymerases in “associated” enzyme activities is also of special interest, since they are immunologically related (7).

This research was supported by United States Public Health Service Research Grant CA 08487 from the National Cancer Institute.

MATeRIALS AND METHODS

Enzymes—Calf thymus low molecular weight DNA polymerase was prepared from calf thymus chromatin using slight modifications of the procedure described for rabbit bone marrow low molecular weight DNA polymerase (8). The enzyme was further purified by affinity chromatography on denatured DNA cellulose (9). The enzyme preparation obtained is essentially homogeneous and the purification procedure is reported in a separate publication (10). The high molecular weight DNA polymerase was prepared from the soluble extract of calf thymus gland, essentially as previously described (11). A homogeneous preparation of E. coli DNA polymerase I (12) was generously supplied by Dr. L. A. Loeb, Institute for Cancer Research, Fox Chase, Philadelphia, Pa.

Chemicals—Deoxynucleoside triphosphates (dNTPs), polydeoxynucleotides, polydeoxyribonucleotides containing a radioactive 3′-terminal nucleotide, oligodeoxyribonucleotides and Dnase I-treated calf thymus DNA were prepared as described earlier (8, 13). Polydeoxyribonucleotide-oligodeoxynucleotide complexes and polydeoxyribonucleotide-polydeoxyribonucleotide complexes were prepared by physical mixing of the appropriate materials in 0.01 M KCl followed by heating to 60° and slow cooling. The use of initiated polydeoxyribonucleotide templates has been described in earlier work (13, 14). Radioactive dNTPs were purchased from Schwarz-Mann or Amersham-Searle, and [32P]pyrophosphate was purchased from New England Nuclear.

DNA Polymerase Reactions—The reaction mixtures generally contained 0.1 mM polymer nucleotide (deoxyadenylate) and 0.01 mM oligonucleotide (thymidylate) for template, and 0.1 mM [3H]dTTP (40 to 60 cpm per pmole) as monomer. The 6 to 8 S calf thymus DNA polymerase reactions were carried out in the presence of template, [3H]dTTP, 0.5 mM MnCl₂, 1 mM 2-mercaptoethanol, 100 μg per ml of bovine serum albumin, and 0.02 M potassium phosphate, pH 7.2. The 3.4 S calf thymus DNA polymerase reactions were carried out under the same conditions.
except that 0.05 M ammidol buffer at pH 8.6 or Tris-Cl at pH 7.6 was used as buffer. The *E. coli* polymerase I reactions were carried out as described for calf thymus 6 to 8 S polymerase except that 8 mM MgCl$_2$ was used. Polymerase activity was measured by the increase of acid-insoluble material on GF/C discs (15). One unit of DNA polymerase is defined as 1 nmole of dTMP incorporated into acid-insoluble material per hour. Specific activity of the enzyme is defined as units of enzyme activity per mg of protein. The initiated template system used to determine the specific activity of various DNA polymerases is d(pA)$_{30}$-d(pT)$_{12}$. The specific activities of enzymes used with this template system are 167,000 for *E. coli* polymerase I, 5,000 for calf thymus 6 to 8 S enzyme, and 250,000 for calf thymus 3.4 S enzyme.

**Pyrophosphate Exchange Reactions**—Pyrophosphate exchange reactions with calf thymus 6 to 8 S polymerase were carried out at 35° in the presence of 200 µg per ml of DNase I-treated calf thymus DNA, 0.1 mM each of dATP, dCTP, dGTP, and dTTP, 8 mM MgCl$_2$, 1 mM 2-mercaptoethanol, 0.02 M potassium phosphate (pH 7.2), 100 µg per ml of bovine serum albumin, and various concentrations of [*32P]*sodium pyrophosphate. At various times of incubation, an aliquot of the reaction mixture was added to ice-cold 5% trichloroacetic acid, nucleotides were absorbed onto activated charcoal, the charcoal was washed, and the nucleotides were then eluted from the charcoal with 3 M acetic acid in pyridine. Quantitative elution of dNTP was obtained by this method. The amount of [*32P]*dTMP was determined by counting Cerenkov radiation of the acidic acetic acid-pyridine solution directly in the liquid scintillation counter.

Pyrophosphate exchange reactions with calf thymus 3.4 S polymerase were carried out under identical conditions except that nonradioactive sodium pyrophosphate and [*3H]*dTTP were used, and the progress of the reaction was followed by measuring acid-insoluble material. Pyrophosphate exchange reactions for the various enzymes were also carried out with initiated homopolymer templates under conditions described for polymerization reactions. The reaction conditions were the same as described for polymerization reactions. Reaction progress was followed by measuring the decrease of acid-insoluble material using the glass fiber disc method. When [*3H]*dTTP was present, radioactivities were determined by double isotope counting procedures. Since the 3'- to 5'-exonuclease activity in the calf thymus enzyme preparations was absent or at a very low level, these enzyme preparations were tested for the 5'-exonuclease activity at various pHs, in MgCl$_2$ or MnCl$_2$, and over a range of protein concentrations.

**Deoxynucleotide Triphosphate Degradation Reactions**—The dNTP degradation reactions of the calf thymus enzymes were carried out under conditions described for polymerization except that 0.5 mM [*3H]*dTTP (5 times the template nucleotide concentration; specific activity, 8000 cpm per nmole) was used, and the reactions were carried to completion (one complete round of replication of the available template nucleotide). Analysis of the products of the reaction with added markers (dTTP, dTDP, and dTMP) was carried out on Whatman No. 1 paper strips developed with isobutyric acid-NH$_3$-H$_2$O and liquid scintillation counting as above.

**RESULTS**

**Polymerization** All three enzymes used in this study seem to have similar requirements for polymerization. The essential reactants are the template: initiator system, divalent cation, and dNTPs. The enzymes act by extending the initiator chain by phosphodiester bond formation with complementary nucleotides.
Pyrophosphate exchange and DNA polymerase reactions were carried out as described under "Materials and Methods." In Experiment I, the specific activity of [32P]pyrophosphate was 3,000 to 12,000 cpm per nmole. Escherichia coli polymerase I reaction was done under conditions described for the 6 to 8 S enzyme from calf thymus. DNA polymerase units present in each reaction were 0.02, 12.7, and 1.0 for polymerase I, calf thymus 6 to 8 S, and calf thymus 3.4 S enzyme, respectively. In Experiment II, the specific activity of [32P]pyrophosphate was 254,000 cpm per nmole. The E. coli polymerase I reaction was done with conditions described for calf thymus 3.4 S enzyme, and 117 and 126 DNA polymerase units were present for polymerase I and calf thymus 3.4 S enzyme, respectively. The rate was calculated from the linear portion of the reaction. The ratio of polymerization to exchange was calculated from rates measured under identical conditions.

| Enzyme                  | Experiment I | Experiment II |
|-------------------------|--------------|---------------|
|                         | PPi          | Exchange      | Poly- | PPi  | Exchange      | Poly- |
|                         | molecule/hr  | merization: | meriza- | molecule/hr  | merization: | meriza- |
| E. coli polymerase I    | 1            | 0.69          | 1.2   | 1.2  | 29.8          | 3.34  |
| Calf thymus 6 to 8 S DNA polymerase | 2            | 0.90          | 13.6  | 5    | 1.23          | 6.2   |
| Calf thymus 3.4 S DNA polymerase | 1            | <0.01         | >100  | 1    | <0.001        | >100  |
|                         | 2            | <0.01         | >100  | 2    | <0.01         | >100  |
|                         | 5            | <0.01         | >100  | 5    | <0.01         | >100  |

Pyrophosphorolysis—The ability of polymerase I from E. coli and pyrophosphate liberation. All reactions proceeded to completion without degradation with the mammalian enzymes. E. coli polymerase I degrades the product. New chains are not formed by primary initiation events.

Pyrophosphate Exchange—The ability of E. coli polymerase I, calf thymus 6 to 8 S DNA polymerase, and calf thymus 3.4 S DNA polymerase to catalyze pyrophosphate exchange is compared in Table I. The data in this table show that the first two enzymes carry out an easily demonstrated exchange reaction, but no measurable exchange was found for the 3.4 S enzyme. The second experiment in Table I records the result obtained when the enzyme level was increased 50-fold and the specific activity of [32P]pyrophosphate was increased about 100-fold. Although almost 700,000 cpm were exchanged by E. coli polymerase I in a 10-min reaction, no charcoal-absorbable count significantly above the background was found for calf thymus 3.4 S polymerase after 1 hour of reaction.

Considerable experimentation has been done to verify and evaluate the significance of the negative results obtained with the 3.4 S polymerase. In addition to the 50-fold increase in enzyme concentration and 100-fold increase in specific activity of the [32P]pyrophosphate in the reaction noted above, complete pyrophosphate concentration curves, studies at pH 7.2, 7.6, and 8.6, and exchange with synthetic templates in Mg++, and Mn++, were carried out. All attempts to observe an exchange reaction using the 3.4 S polymerase were negative. The 3.4 S enzyme was tested directly for pyrophosphatase and dNTP degrading activities and none were found. We conclude that the 3.4 S DNA polymerase does not catalyze a pyrophosphate exchange.

Pyrophosphorolysis—The ability of polymerase I from E. coli and 6 to 8 S polymerase from calf thymus to catalyze pyrophosphorolytic degradation of DNA in the absence of dNTPs has been demonstrated (3, 6). This observation was confirmed in this study (Table II) as part of the controls in the pyrophosphate exchange experiments described in Table I. No pyrophosphorolysis was found with 3.4 S polymerase, although both polymerase I from E. coli (Table II) and calf thymus 6 to 8 S polymerase were active (1, 3, 6).

Using template-initiator systems containing 3'-labeled termini, we also examined the nature of the products of degradation in the presence of pyrophosphate. The results listed in Table III show that with a matched template, polymerase I produced [3H]dCTP, [3H]dTMP, and some [3H]dGTP (possibly from nucleoside diphosphate kinase contamination). With the mismatched template polymerase I produced a large amount of [3H]dCMP and no [3H]dCTP. These findings are in agreement with those reported by Brutling and Kornberg (5). Calf thymus 6 to 8 S polymerase produces only [3H]dCTP with the matched template. It produced only trace amounts of [3H]dCMP with the mismatched template. The 3.4 S polymerase produced no hydrolytic or pyrophosphorolytic products.

3' to 5' Exonuclease—All three DNA polymerases were tested for 3' to 5'-hydrolytic activity using single-stranded 3' terminus-labeled polydeoxynucleotides. Although the 3' to 5'-nuclease activity was easily demonstrated with polymerase I, no significant level of this activity could be detected with the 6 to 8 S and 3.4 S calf thymus DNA polymerases.

The 3' to 5'-exonuclease activity described as "proofreading exonuclease" in E. coli polymerase I proceeds from the 3' terminus toward the 5' end (5). The results obtained with E. coli polymerase I and calf thymus DNA polymerases are exhibited in Fig. 1. In contrast to the easily detectable level of activity observed with polymerase I, the 3.4 S calf thymus polymerase had no proofreading exonuclease, and the activity seen with the 6 to 8 S enzyme was really too low to be interpreted as an important "associated" activity. In the pyrophosphorolysis experiments described in Table II, the complete reactions were the same as described for the pyrophosphate exchange reaction for the calf thymus 3.4 S polymerase under "Materials and Methods." The enzyme units present were 0.02 for polymerase I and 4.0 for calf thymus 3.4 S polymerase.

It may be noted that polymerization is occurring with the mismatched template in the 3.4 S polymerase reactions. This finding was examined using a chemically defined initiator, d(pT)pd(HpC), in poly(dA) replication, and alkaline sucrose gradient analysis of the product. The results show that this initiator with a single mismatched base is used for polymerization and is incorporated into product. Upon extending the mismatched sequence to two or three bases, little reaction is observed.
periment (Table III), where enzyme concentrations were higher and when the products were analyzed, calf thymus 6 to 8 S enzyme produced no hydrolytic product ([3H]dTMP) when a matched template was used and produced a very low level of hydrolytic product ([3H]dCMP) when the template with a mismatched base was used.

Deoxynucleoside Triphosphate Degradation—The observation that dNTPs are converted to dNMPs in a template-dependent reaction was first described by Deutscher and Kornberg for E. coli polymerase I (3). The same reaction has also been extensively studied for T4 DNA polymerase (17). This reaction is assumed to be a combination of hydrolytic and polymerization reactions. When the calf thymus enzymes were tested for dNTP degradation, none was observed, as might be expected for enzymes not having 3'-to-5'-exonuclease activity. A typical result for calf thymus 3.4 S enzyme is shown in Fig. 2. The trace amount of [3H]dTMP present (note that the figure is on a 4 cycle log scale) in the substrate was unchanged during the 160-min incubation. Similar results were obtained with the 6 to 8 S polymerase.

**Table III**

Pyrophosphorolysis and hydrolysis with matched and mismatched templates

Conditions of the reactions and analyses of the products were the same as described under “Materials and Methods.” Incubation was for 30 min at 35°C. DNA polymerase units present were 117 for each polymerase I reaction, 125 for each calf thymus 6 to 8 S enzyme reaction, and 126 for each calf thymus 3.4 S reaction. Numbers in the body of the table are actual counts per minute above background.

| Enzyme                  | Template     | Products   |
|------------------------|--------------|------------|
|                        |              | polymer    | dNTP | dNDP | dNMP |
| E. coli polymerase I   | d(pA)₆₀₀ d(pT)₄₆₄ [³H]dTMP | 4884       | 1008 | 84   | 1020 |
|                        | d(pA)₆₀₀ d(pT)₄₆₄ [³H]dCMP | 231        | 0     | 0    | 6462 |
| Calf thymus 6-8 S     | d(pA)₆₀₀ d(pT)₄₆₄ [³H]dTMP | 6642       | 1956 | 0    | 0    |
| polymerase             | d(pA)₆₀₀ d(pT)₄₆₄ [³H]dCMP | 5984       | 0     | 0    | 54   |
| Calf thymus 3.4 S     | d(pA)₆₀₀ d(pT)₄₆₄ [³H]dTMP | 8342       | 0     | 0    | 0    |
| polymerase             | d(pA)₆₀₀ d(pT)₄₆₄ [³H]dCMP | 6325       | 0     | 0    | 0    |

**Fig. 1.** 3'- to 5'-Exonuclease. In Frames A and B 117 polymerase units of Escherichia coli polymerase I were used. In Frames C and D 63 units of calf thymus 6 to 8 S polymerase were used. In Frames E and F 126 polymerase units of calf thymus 3.4 S polymerases were used. A, C, and E are reactions using the matched template (d(pA)₆₀₀ d(pT)₄₆₄ [³H]dTMP) and B, D, and F are reactions using mismatched template (d(pA)₆₀₀ d(pT)₄₆₄ [³H]dCMP). Other conditions for the reactions were as described under ‘Materials and Methods.’ O — O, ³H radioactivity remaining in the template in the absence of [³C]dTTP; Δ — Δ, radioactivity [³H] remaining in the template in the presence of [³C]dTTP; ▲ — ▲, nanomoles of [³C]dTMP incorporated.
3402

NUCLEOTIDE TURNOVER—34 S POLYMERASE

Fig. 2. Degradation of deoxynucleoside triphosphate by calf thymus 3.4 S polymerase. The conditions for the reaction and the analyses of the products were as described under "Materials and Methods." 126 units of polymerase present. Frame A shows the distribution of products after 1 min of incubation. Frame B is after 40 min of incubation and Frame C is after 160 min of incubation. The numbers 1, 2, 3, and 4 designate the positions of polymer product, dTTP, dTDP, and dTMP, respectively.

δ' - to 3'-Exonuclease—This activity was assayed as described under "Materials and Methods." Hydrolysis of the 5' terminus-labeled polydeoxyadenylate complexed with polythymidylate was found with E. coli polymerase I, but not with 3.4 S or 6 to 8 S calf thymus DNA polymerases.

Effects of Pyrophosphate and Orthophosphate—Although the 3.4 S polymerase did not have either pyrophosphate exchange or pyrophosphorolysis activity, its polymerization rate was inhibited by pyrophosphate. To examine the nature of PPi inhibition of calf thymus DNA polymerases, we studied the effects of PPi on the polymerization rates of the calf thymus enzymes at various concentrations of dTTP. These results are shown in Fig. 3. At 0.215 mM PPi or less inhibition of the 6 to 8 S polymerase was noncompetitive with respect to dTTP (Fig. 3A). At higher concentrations of PPi (above 0.286 mM) the inhibition was mixed and eventually became competitive at concentrations of 0.358 mM and 0.429 mM. The 3.4 S DNA polymerase was inhibited by PPi at concentrations of PPi less than 0.15 mM and the inhibition was competitive with respect to dTTP (Fig. 3B). Since the optimum Mn++ ion concentration (0.25 to 0.5 mM) was about the same for both calf thymus enzymes under our reaction conditions, the competitive inhibition of the 3.4 S enzyme by such low pyrophosphate concentrations could not be due to depletion of Mn++ ions (present at 0.6 mM). The inhibition of the 6 to 8 S calf thymus enzyme at such concentrations (less than 0.215 mM PPi) appears to be noncompetitive. The data clearly indicate that at higher concentrations of PPi (above 0.215 mM) the kinetics of inhibition becomes competitive between PPi and dNTP. Since PPi is competing with dNTP for Mn++ ions only at higher concentrations, the competitive inhibition seen with the 3.4 S enzyme at less than 0.15 mM PPi cannot be attributed simply to competition for Mn++ ions.

Equilibrium dialysis studies with E. coli polymerase I showed that pyrophosphate does not compete with dNTP (18) and kinetic studies indicated that PPi competes with OH− in the pyrophosphate exchange reaction catalyzed by E. coli polymerase I (3). The results of our experiments, together with those reported for polymerase I, allow the simple conclusion that the action of PPi on calf thymus 3.4 S enzyme is different from its action on polymerase I (3, 18) and calf thymus 6 to 8 S enzyme. The pyrophosphate exchange activity levels of these enzymes also suggest that the action of PPi is different for each enzyme.

It is also interesting to note that orthophosphate is an extremely effective inhibitor for the calf thymus 3.4 S enzyme. E. coli polymerase I and the calf thymus 6 to 8 S enzyme are not affected at comparable concentrations of Pi. The effects of Pi on E. coli polymerase I, calf thymus 6 to 8 S DNA polymerase, and calf thymus 3.4 S polymerase are compared in Table IV. Our unpublished results show that potassium phosphate at neutral pH is the most suitable buffer for general use in calf thymus 6 to 8 S enzyme reactions. Although Tris-Cl, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes), and cacodylate buffers at comparable pH can be used in poly dA replication, they are somewhat inhibitory with DNA, poly dG, and poly dT templates. Table IV shows that calf thymus 6 to 8 S enzyme activity is not inhibited by Pi at pH 7.0 in the presence of 25 mM Pi. When Tris-Cl at pH 7.6 was used as buffer, the calf thymus 6 to 8 S enzyme was more sensitive to Pi, yet E. coli polymerase I was not inhibited by Pi under the same conditions. As would be expected, the effect of Pi in a DNA polymerase reaction is a complex function of the divalent cation, the type of buffer used, and the pH of the reaction. The strong inhibition of the 3.4 S enzyme probably cannot be explained by the competition for Mn++ to dNTP and Pi and the result amplifies the differences that may be found in these DNA polymerases.

DISCUSSION

The enzymatic properties and associated enzyme activities of E. coli polymerase I have been studied extensively (1-5, 16, 17). Since the characteristics of this enzyme are well known it has
PYROPHOSPHATE INHIBITION

Fig. 3. Pyrophosphate inhibition of calf thymus 6 to 8 S and 3.4 S DNA polymerases. The reactions were carried out as described for polymerization under "Materials and Methods" in the presence of 0.6 mM MnCl₂, varying \(^{3}H\)dTPP, and sodium pyrophosphate concentrations. The results are expressed on double reciprocal plots. A: ○---○, 0 µM PP; △---△, 1,143 µM PP; □---□, 215 µM PP; ■---■, 286 µM PP; O---O, 325 µM PP; O---O, 429 µM PP. B: ○---○, 0 µM PP; △---△, 43 µM PP; □---□, 86 µM PP; O---O, 129 µM PP.

frequently been used as a model for all other DNA-synthesizing enzymes. With the exception of the 5'- to 3'-exonuclease function of polymerase I the associated enzyme activities appear to be present in all prokaryotic DNA polymerases studied (19). The highly purified enzyme preparations we obtained from calf thymus gland have allowed us to carry out a comparison of the enzyme activities of these mammalian DNA polymerases with E. coli polymerase I. The results clearly indicate that the mammalian DNA polymerases we have examined differ from E. coli polymerase I both in the kinds and the levels of associated enzyme activities present. A summary of the results of this comparison is shown in Table V.

The absence of the 5'- and 3'-exonuclease function of DNA polymerase has been demonstrated in a number of prokaryotic and eukaryotic enzymes (19). Results presented in this study as well as our unpublished data on several purified mammalian DNA polymerases showed the absence of this activity. This particular exonuclease activity appears to be present only in certain prokaryotic DNA polymerases.

Both E. coli polymerase I and the calf thymus 6 to 8 S enzyme carry out pyrophosphate exchange and pyrophosphorolysis reactions. In contrast to E. coli polymerase I the 6 to 8 S enzyme did not exhibit a significant level of 3'- to 5'-exonuclease activity with matched templates nor did it degrade dNTP. Since it was possible to demonstrate the presence of a trace level of 3' to 5'-exonuclease activity in the 6 to 8 S calf thymus enzyme when a mismatched template was used, a final interpretation of this finding must be postponed. The 6 to 8 S enzyme preparation used in this study is not homogeneous and it may be possible that the 3'- to 5'-exonuclease activity seen with the mismatched template is simply caused by a contaminating nuclease. On the other hand, it may be possible that the conformation of the template-enzyme complex determines the magnitude of the 3'- to 5'-exonuclease function. We have noted, for example, that the calf thymus 6 to 8 S enzyme does seem to stabilize the interaction between the initiator chain and the template strand (20).

Using identical reaction conditions, oligothymidylylase with a chain length of 7 is sufficient for initiation of poly(dA) replication catalyzed by calf thymus 6 to 8 S enzyme, while oligothymidylylase with chain length 9 cannot initiate poly(dA) replication catalyzed by E. coli polymerase I. By lowering the reaction temperature from 35°C to 25°C to stabilize the interaction between the tem-

### Table IV

| Enzyme                | Buffer    | \(P_i\) | Reaction rate |
|-----------------------|-----------|---------|---------------|
| Calf thymus 6 to 8 S  | KP (pH 7.0) | 4.2     | 4.62          |
|                       | 8.3       |         | 6.06          |
|                       | 16.7      |         | 5.72          |
|                       | 25.0      |         | 5.70          |
|                       | 33.3      |         | 2.68          |
|                       | 50.0      |         | 0.72          |
| Tris-Cl (pH 7.6)      | 0.0       |         | 3.60          |
|                       | 1.56      |         | 3.58          |
|                       | 3.13      |         | 2.88          |
|                       | 6.25      |         | 1.54          |
|                       | 12.50     |         | 0.58          |
|                       | 25.00     |         | 0.12          |
| E. coli polymerase I  | Tris-Cl (pH 7.6) | 0.0     | 7.17          |
|                       |           | 6.25    | 6.98          |
|                       |           | 12.50   | 7.26          |
|                       |           | 25.00   | 7.02          |
|                       |           | 30.00   | 6.48          |
| Calf thymus 3.4 S     | Ammediol  (pH 8.6) | 0.0     | 9.38          |
|                       |           | 0.065   | 6.62          |
|                       |           | 0.13    | 3.34          |
|                       |           | 0.26    | 0.70          |
|                       |           | 0.56    | 0.55          |
|                       |           | 2.08    | 0.47          |
|                       |           | 8.52    | 0.31          |

* No additional buffer was used in these reactions.

Reactions were carried out using the polymerization conditions described under "Materials and Methods" for poly(dA) templates except that various amounts of \(P_i\) were added. The pH of KP solutions were adjusted to the pH of the reaction before use.

### Table V

| Summary of DNA polymerase-associated activities |
|-----------------------------------------------|
| Enzyme activity                              | E. coli polymerase I | Calf thymus 6 to 8 S | Calf thymus 3.4 S |
|------------------------------------------------|----------------------|---------------------|------------------|
| Polymerization                                | +                    | +                   | +                |
| Pyrophosphate exchange                        | +                    | +                   | -                |
| Pyrophosphorolysis                            | +                    | +                   | -                |
| 3'- to 5'-exonuclease                         | +                    | +                   | -                |
| dNTP degradation                              | +                    | -                   | -                |
| 5'- to 3'-exonuclease                         | -                    | -                   | -                |

4 Using identical reaction conditions, oligothymidylylase with a chain length of 7 is sufficient for initiation of poly(dA) replication catalyzed by calf thymus 6 to 8 S enzyme, while oligothymidylylase with chain length 9 cannot initiate poly(dA) replication catalyzed by E. coli polymerase I. By lowering the reaction temperature from 35°C to 25°C to stabilize the interaction between the tem-
and this stabilizing effect of the enzyme may be a reflection of conformation near the active center of the enzyme. In any event, it should be emphasized that the ratio of exonucleolytic activity to polymerizing activity observed with the 6 to 8 S enzyme is several orders of magnitude lower than that of E. coli polymerase I.

The absence of all associated activities in the calf thymus 3.4 S enzyme is the most unusual finding in this investigation. Although it does seem obvious that pyrophosphorolysis is merely reversal of the polymerization reaction of DNA polymerase (5, 6), the negative findings with the 3.4 S polymerase suggest that this may not be generally true. The different effects of PPi on the various DNA polymerases also suggest that all features of the reaction pathways of polymerization and pyrophosphorolysis may not be identical in these enzymes. The competition between PPi and dNTP on the calf thymus 3.4 S enzyme and the lack of a pyrophosphorolysis reaction suggest that binding of PPi at the dNTP site on the enzyme surface does not allow pyrophosphorolysis to take place. The noncompetitive effect of PPi with dNTP on calf thymus 6 to 8 S enzyme suggests the possibility of a secondary site on this enzyme that does bind PPi. The presence of a PPi binding site around the catalytic center might effect a higher concentration of PPi there and may also be required for the apparent reversal of the polymerization reaction (pyrophosphate exchange and pyrophosphorolysis).

If the enzymes studied are involved in DNA metabolism in living cells then it is of interest to consider possible biological implications of associated activities. For example, the 3' to 5' exonuclease found associated with E. coli polymerase I has been called a proofreading exonuclease (5). This term implies that base pairing errors occurring during polymerization are efficiently removed before further polymerization takes place. This type of activity has also been demonstrated in T4-induced polymerase and the fact that this polymerase does produce some errors may be deduced from the observation that noncomplementary dNTPs are converted to dNMPs in homopolymer directed reactions (17). A relation between 3' to 5' exonuclease activity and "antimutator" activity has also been observed in the T4 system (5, 21). It will be of interest to examine the mechanisms that control the error frequency in biological systems that contain DNA polymerases devoid of proofreading exonuclease activity.

The absence of pyrophosphate exchange and pyrophosphorolysis associated with the 3.4 S enzyme is somewhat disconcerting to chemical instincts and conveys no obvious biological advantage. At the present moment we can only assume that there must be some beneficial effect of the apparent irreversibility of DNA synthesis catalyzed by this chromatin-bound species of DNA polymerase.

REFERENCES

1. KORNBERG, A. (1969) Science 163, 1410-1418
2. KELLY, R. B., ATKINSON, M. R., HUBERMAN, J. A., AND KORNBERG, A. (1969) Nature 224, 495-501
3. DEUTSCHER, M. P., AND KORNBERG, A. (1969) J. Biol. Chem. 244, 3019-3028
4. ENGELUND, P. T., DEUTSCHER, M. P., JOVIN, T. M., KELLY, R. B., COZZARELLI, N. R., AND KORNBERG, A. (1968) Cold Spring Harbor Symp. Quant. Biol. 33, 1-0
5. BOLLUM, F. J., AND KORNBERG, A. (1972) J. Biol. Chem. 247, 241-248
6. BOLLUM, F. J. (1960) J. Biol. Chem. 235, 2399-2403
7. CHANG, L. M. S., AND BOLLUM, F. J. (1972) Science 175, 1116-1117
8. CHANG, L. M. S., AND BOLLUM, F. J. (1972) Biochemistry 11, 1264-1272
9. ALBERTS, B., AND HEERICK, G. (1971) Methods Enzymol. 21, 198-217
10. CHANG, L. M. S. (1973) J. Biol. Chem. 248, in press
11. ONEDA, M., AND BOLLUM, F. J. (1965) J. Biol. Chem. 240, 3983-3991
12. JOVIN, T. M., ENGELUND, P. T., AND BERTSCH, L. L. (1969) J. Biol. Chem. 244, 2906-3008
13. CHANG, L. M. S., AND BOLLUM, F. J. (1971) Biochemistry 10, 536-542
14. BOLLUM, F. J. (1967) in Genetic Elements (SHUGAR, D., ed), pp. 296300, Harper and Row, New York
15. BOLLUM, F. J. (1966) in Procedures in Nucleic Acid Research (CANTONI, G, AND DAVIES, D., ed), pp. 296-300, Harper and Row, New York
16. MARTIN, J. B., AND DvY, D. M. (1949) Anal. Biochem. 21, 965-967
17. HERSHFIELD, M. S., AND NOSAL, N. G. (1972) J. Biol. Chem. 247, 3303-3404
18. ENGELUND, P. T., HUBERMAN, J. A., JOVIN, T. M., AND KORNBERG, A. (1969) J. Biol. Chem. 244, 3038-3044
19. COULIAM, M. (1971) Ann Rev. Biochem. 40, 855-898
20. CHANG, L. M. S., CASSANI, G. R., AND BOLLUM, F. J. (1972) J. Biol. Chem. 247, 7718-7723
21. MEYER, N., POLAND, R. L., AND BESSMAN, M. J. (1972) J. Biol. Chem. 247, 7110-7122
A Comparison of Associated Enzyme Activities in Various Deoxyribonucleic Acid Polymerases
Lucy M. S. Chang and F. J. Bollum

J. Biol. Chem. 1973, 248:3398-3404.

Access the most updated version of this article at http://www.jbc.org/content/248/10/3398

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/248/10/3398.full.html#ref-list-1