On the Processive Mechanism of Escherichia coli DNA Polymerase I

QUANTITATIVE ASSESSMENT OF PROCESSIVITY*

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Comparison of the rates of polymerization catalyzed by DNA polymerases in the presence of a limited and a complete complement of the four deoxynucleoside triphosphates permits accurate measurement of the processivity of polymerization. The extent of processivity, i.e. the number of nucleotides polymerized each time the DNA polymerase associates with the DNA template, may be measured in a range from 1.0 to several hundred nucleotides. As judged by this method, Escherichia coli DNA polymerase I is processive for values as great as 188, depending on conditions and primer-template structure. Higher ionic strength and lower temperatures result in lower processivity. With nicked DNA templates, at 37° and ionic strength = 0.085 M, processivity is 15 to 20. With gapped DNA templates under the same conditions, processivity is 40 to 50. Poly[d(A-T)] templates yield processivity values as high as 188, but changes in conditions can diminish values to 3.3. In contrast, DNA polymerase β from KB cells is nonprocessive (enzyme-DNA dissociation after addition of each nucleotide) under all conditions examined.

A procedure is described to compare the “static affinity”* of a stationary DNA polymerase molecule bound to DNA, with the “kinetic affinity” of a polymerase in the course of synthesis. For E. coli DNA polymerase I, the static affinity is usually severalfold greater than the kinetic affinity.

A method has also been developed to measure the average template length available to a DNA polymerase for synthesis at individual 3’ termini of any DNA substrate, and the effect of temperature and ionic strength on this available length. Results of such measurements show that the processivity values reported here are an intrinsic property of the DNA polymerase and are not determined by the available template length.

We have previously described a method to assess whether polymerization of nucleotides by DNA polymerases is processive; that is, whether a succession of nucleotides is polymerized before the enzyme is released from the template (1). This method involves measurement of the ratio of dGMP to dCMP incorporated in the course of replicating a segment of the right-hand cohesive end of phage λ DNA with the sequence

5'-G-G-G-C-G-G-C-G-3'

When applied to Escherichia coli DNA polymerase I, this analysis clearly showed the enzyme to be processive. However, the short length of the template limited measurement of extent of processivity, i.e. the average number of nucleotides polymerized after each association of the DNA polymerase with the DNA template, to values of 7 or 8.

It would be of considerable advantage if the range of processivity measurements could be expanded to larger values. It would then be possible to monitor the effects of primer-template structure and reaction conditions on the movement of the polymerase molecule during DNA synthesis.

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In an earlier publication, Gass and Cozzarelli (2) suggested that measurement of the rate of DNA synthesis in the presence of one, two, or three dNTPs compared to that measured with all four dNTPs could provide a quantitative determination of processivity over a large range of values (one to several hundred). The basic point of their analysis was that reaction rate is determined in part by processivity, and that the rate with a full as compared to a partial complement of dNTPs could be used to distinguish processivity from other factors affecting polymerization rate. However, processivity can be derived quantitatively from such an analysis only if the rate of reaction is proportional to the number of nucleotides added to individual DNA molecules. Since this proportionality was not demonstrated in the original analysis, Gass and Cozzarelli (2) pointed out that the values which they calculated for Bacillus subtilis DNA polymerases might not represent the true processivity of polymerization.

In this report, we have extended the original proposal of Gass and Cozzarelli (2) into a theory and an experimental method which have allowed us to measure unambiguously values of the processivity of nucleotide polymerization by DNA polymerases. When applied to E. coli DNA polymerase I, this analysis has confirmed that polymerization of nucleotides by this enzyme is processive. It has further indicated that under certain conditions the processivity exceeds 180 nucleotides. The results have also shown that reaction conditions and template structure can affect the processivity of this polymerase. In contrast, DNA polymerase β from KB cells (3) is nonprocessive; that is, the enzyme dissociates following addition of each nucleotide, under all of the conditions tested.

The analysis permits a comparison of the "static affinity" of a bound DNA polymerase molecule for a primer-terminus, to the "kinetic affinity" of an actively polymerizing enzyme. It also allows determination of the "average available template length"; i.e. the total number of nucleotides which may be added to each primer-terminus of a DNA template by an excess of enzyme molecules over primer-termini under specific conditions. With this new information we are able to obtain a much clearer picture of the microscopic movement of DNA polymerases as they synthesize DNA than was heretofore available.

**Theory**

With a large excess of primer-termini over enzyme molecules, nucleotide polymerization may be considered to be a two-phase, cyclic process. In the first phase, free enzyme diffuses to and binds the primer-template (4). In the second phase, the enzyme catalyzes an ordered succession of dNTP binding, nucleotide condensation, and translocation until the enzyme dissociates from the primer-template (5). The entire cycle is then repeated. We define the average increment of time elapsed during one complete cycle, from the binding and reaction of the DNA polymerase with a 3'-primer terminus capable of supporting DNA synthesis, through the dissociation and diffusion steps, to the rebinding of the enzyme to another reactive 3' terminus, as the "cycling time."

The polymerization rate when x dNTPs are present may then be expressed as:

$$P_x = \frac{E N_x}{T_x}$$  

where $P_x$ is the rate of polymerization, $E$ the number of active enzyme molecules, $N_x$ the average number of nucleotides incorporated per polymerization cycle, and $T_x$ the cycling time. This is a general expression that applies to both synthesis with a limited complement of dNTPs ($x = 1, 2, 3$ for native DNA) and synthesis with a complete complement of dNTPs ($x = 4$ for native DNA).

For native DNA, $N_x$ is the average number of nucleotides polymerized with a complete complement of dNTPs, and is therefore the processivity of polymerization. The goal of these calculations is to express $N_x$ in terms of parameters which can be measured experimentally.

The ratio of polymerization rates with a limited versus a complete complement of dNTPs for native DNA ($P_{x;4}$) may be expressed as:

$$P_{x;4} = \frac{E N_x T_x}{N_x T_x} \quad (x = 1, 2, 3)$$  

or

$$P_{x;4} = \frac{E N_x T_x}{N_x T_x} \quad (x = 1, 2, 3)$$  

Values of $N_x$ may ultimately be expressed in terms of $N_x$ and two statistical factors. The expressions differ for different DNA templates and complete derivations of these values are given under "Appendix." The $T_x/T_4$ ratio must be determined for individual experiments with the use of an inhibitor which binds the DNA polymerase for a short period of time during the first phase of the polymerization cycle. The presence of the inhibitor would increase the cycling time, $T_x$, by an increment, $T_{x,i}$, such that:

$$T_{x,i} = T_x + T_{x,i}$$  

where $T_{x,i}$ is the cycling time in the presence of the inhibitor. The inhibitor should be chosen such that the value of $T_x$ is not affected by the value of $x$. This requirement can be verified experimentally since processivity determined from $P_{x;4}$ and $P_{x;4}$ would not be equivalent unless $T_x$ were independent of $x$ (see "Results").

Using the definition of $T_x$, we can express $T_x/T_4$ in terms of rates of reaction. First, it follows from Equation 1 that:

$$T_x = \frac{E N_x}{P_x}$$  

With this form we can relate the cycling times for inhibited and uninhibited reactions directly to measurable rates:

$$T_{x;4} = \frac{E N_x}{P_{x;4}} \quad \frac{P_{x;4}}{P_{x;4}} = \frac{P_x}{P_{x;4}}$$  

where $P_{x;4}$ is the rate of reaction when the inhibitor is present. We may then substitute for $T_{x;4}$ from Equation 4:

$$T_{x;4} = \frac{E N_x}{P_{x;4}} \quad \frac{P_{x;4}}{P_x} = \frac{P_{x;4}}{P_x}$$  

We can then solve for $T_x$, as follows:

$$T_x = \frac{P_x}{P_{x;4}} T_{x;4}$$

1 Synthesis in the presence of a limited and complete complement of dNTPs on some types of synthetic DNA require different values of $x$. Poly(dA-T)$_4$, for example, uses one dNTP for limited synthesis and two dNTPs for complete synthesis. The following expressions are derived for native DNA containing all four nucleotides, but apply equally well to polymers of two or three nucleotides, if the terms are substituted with appropriate values of $x$. These experiments cannot be performed with homopolymers which require only one of the four dNTPs, because no comparison of rates under limited dNTP versus complete dNTP conditions can be made.
From Equation 7, when four dNTPs are used in the reaction:

\[
\frac{T_4 + T_1}{T_4} = \frac{p_4}{p_{A,i}}
\]  

We can now substitute for \( T \), in Equation 8 by using the value of \( T \), in Equation 9:

\[
\frac{T_4 + \frac{p_x T_x}{x,i} - T_x}{T_4} = \frac{p_4}{p_{A,i}} \quad (x=1,2,3)
\]

We can now solve for \( T_x/T_4 \), which is:

\[
\frac{T_x}{T_4} = \left\{ \frac{p_4}{p_{A,i}} - 1 \right\} \quad (x=1,2,3)
\]

Returning to Equation 3, it is possible to relate the measured reaction rates \( P_x, P_a, P_x,i, \) and \( P_{4,i} \) to the processivity of polymerization as follows:

\[
P_{x:4} = \left\{ \frac{N_x}{N_{4,i}} - 1 \right\} \left\{ \frac{p_x}{p_{A,i}} - 1 \right\} \left\{ \frac{p_{A,i}}{p_{4,i}} - 1 \right\}
\]

This expression is rearranged as follows:

\[
N_4 = N_x \left\{ \frac{p_x}{p_{A,i}} - 1 \right\} \left\{ \frac{p_{A,i}}{p_{4,i}} - 1 \right\}
\]

\[
N_4 = N_x \left\{ 1 - \frac{1}{p_x} \right\}
\]

\[
N_4 = N_x \left\{ 1 - \frac{1}{p_{A,i}} \right\}
\]

Note that all right-hand terms are either calculated or experimentally determined.

Equation 13 may also be rewritten as follows:

\[
N_4 = \left( \frac{N_x}{p_x} \right) \left( \frac{T_x}{T_4} \right) = \frac{N_x}{p_{x:4} \cdot T_x}
\]

where \( P_{x:4} = P_{x,4}T_x/T_4 \). Since only a single triphosphate is labeled in all experiments, a correction is made to allow use of the ratio of radioactivity incorporation rates \( R_x/4 \). This factor \( (L) \) is explained under "Theory" and relates \( R_x/4 \), to \( P_{x:4} \) as follows:

\[
R_{x:4} = L \frac{P_x}{P_{x:4}}
\]

This expression is incorporated into Equation 15 to yield:

\[
N_4 = \frac{L N_x}{R_{x:4}}
\]

The theoretical relationship between \( N_4 \) and \( R_{x:4} \) is shown for calf thymus DNA in Fig. 1 and for poly[d(A-T)] in Fig. 2. In order to use these figures to determine enzyme processivity, it is necessary to calculate the value of \( R_{x:4} \), which is:

\[
R_{x:4} = R_x \cdot \left( \frac{T_x}{T_4} \right)
\]

where \( R_{x:4} \) is the ratio of label incorporated in limited \( (x = 1, 2, \) or \( 3) \) as compared to complete \( (x = 4) \) reactions and \( T_x/T_4 \) is calculated from Equation 11.

There are two practical limitations to such an analysis. (a) Cross-contamination of one dNTP with another will produce an erroneously high polymerization rate during limited synthesis, and as a consequence, the apparent processivity will be correspondingly lowered. Commercial preparations of dNTPs are available in sufficient purity such that this is not usually a problem; however, unlabeled dNTPs used in our experiments have been further purified as described under "Materials and Methods." We have found that various combinations of dNTPs can be used in similar experiments giving...
essentially the same results (data not shown). This serves as additional evidence for purity beyond normal chromatographic checks.

(b) If the average length of template is shorter than the extent of processivity, then the observed rate of polymerization with a full complement of dNTPs will be lower and the ratio of polymerization rates will be increased, again leading to an artificially low processivity.

It is possible to measure the average template length available to a DNA polymerase at each 3' end of the template under conditions of an excess of enzyme molecules over primer-termini and measurement of polymerization extents rather than rates. A similar equation then applies to calculate available template length, as applied to calculate processivity. In this case:

$$A_{X:4} = \frac{A_{X}}{A_{4}} \quad (x=1,2,3) \quad (19)$$

where $A_{X:4}$ is the ratio of extents of label incorporation with a limited and with a complete complement of dNTPs, $A_x$ and $A_4$ are the average number of nucleotides which can be maximally added to a 3' terminus of the DNA substrate with a limited and complete complement of dNTPs present, respectively. The relationship between $A_{X:4}$, $A_x$, and $A_4$ is exactly the same as the relationship between $R_{X:4}$, $N_x$, and $N_4$ (see above). If $A_x > N_4$ for a given experiment it may be assumed that template length is limiting the measured processivity of DNA polymerization. If $A_x > N_4$, the processivity which is measured may be assumed to be a result of the natural propensity of the DNA polymerase to dissociate under the conditions of measurement.

**MATERIALS AND METHODS**

Nucleotides and Polynucleotides—Unlabeled dNTPs were purified as described by Hall and Lehman (6). Purity was checked on polyethyleneimine plates (Brinkman Co.) developed in 1.2 M and 1.5 M LiCl solvents. [3H]dTMP (50 Ci/mmol) was purchased from New England Nuclear Corp. or Amersham-Searle Corp. Calf thymus DNA was purchased from P-L Biochemicals or Sigma Chemical Co., and ColEl DNA was isolated according to the method of Blair et al. (7). Poly(dA-T) was prepared as described by Modrich and Lehman (8). Activation of calf thymus DNA and preparation of ColEl DNA with nicks and gaps were performed as described by Uyemura and Lehman (9). Poly(dA-T) was activated by a procedure similar to that used for calf thymus DNA. Concentrations of polynucleotides are expressed as molarity of nucleotides.

Enzymes—DNA polymerase I from *Escherichia coli* was Fraction VII (10). DNA polymerase β from KB cells (3) was a gift of Dr. T. Wang and Dr. D. Korn (Stanford). Deoxyribonuclease (bovine testis) was obtained from Sigma Chemical Co. Micrococcal nuclease was obtained from Boehringer Mannheim Biochemicals. All enzyme dilutions were made in a buffer containing 40 mM Tris/HCl (pH 8.5), 0.2 mg/ml of bovine serum albumin, 20% glycerol, and 20 mM NaCl.

DNA Polymerase Assays—Polymerase assays were conducted essentially as described by Wu et al. (11). Changes in reaction conditions are described in the legends to figures and tables when appropriate. The deoxyribonucleoside triphosphate concentration (10 μM) used in experiments with *Escherichia coli* DNA polymerase I was sufficient to insure that the reaction rate was not limited by nucleotide binding; it was, however, low enough to minimize cross-contamination. The dNTP concentration used for experiments with β polymerase was 6 μM. The reaction rate was dependent on dNTP concentration in this range, but because of the nonprocessive mechanism, it was still possible to observe dependence of the reaction rate on DNA concentration (see "Results").

In general, where rates were compared in reactions with limited and complete complements of dNTPs, and in the presence and absence of inhibiting DNA, the overall ionic strength, the relative concentrations of various salts and the DNA concentrations in each reaction were identical to within 6%. Also, when comparing reactions containing inhibitor DNA with those with no inhibitor, DNA concentrations varied by a maximum of 6%. The relative concentrations of 3'-hydroxyl versus 3'-phosphoryl DNA termini will be considered under "Results."

Preparation of "Inhibitor DNA"—In order to measure processivity unambiguously, it is necessary to use an inhibitor of DNA synthesis, which can increase cycling time without affecting the mechanism of polymerization during the time between binding of the polymerase to an active DNA substrate and its dissociation from that substrate (see "Theory"). Richardson et al. (12) have shown that if DNA is partially digested with micrococcal nuclease, the resulting 3'-phosphoryl-terminated DNA is an effective inhibitor of DNA polymerase I. Use of this DNA as the inhibitor avoids potential interference with DNA synthesis at 3'-hydroxyl termini that might occur if a synthetic product were used as the inhibitor. Since the inhibiting termini are not removed by the 3'→5' exonuclease activity of DNA polymerase I (12), the inhibitory capacity is unchanged during the reaction.

Inhibitor DNAs were prepared from supercoiled ColEl DNA, native calf thymus DNA, and poly(dA-T). A typical reaction mixture (0.5 ml) contained 0.6 mM DNA, 2 mM CaCl₂, 50 mM glycine (pH 9.0), 50 mM potassium phosphatase, and 3 μg of nuclease. The amount of DNA and nuclease were varied for different experiments as explained under "Results." The potassium phosphate was required to inhibit the small amount of phosphatase in the nuclease preparation. Reactions were incubated at 37°C for 20 min and stopped by addition of EDTA to 4 mM followed by incubation at 72°C for 10 min. The digested DNA was stored at -20°C.

The extent of micrococcal nuclease digestion required to produce effective inhibition was determined by assessment of the inhibition of polymerization on calf thymus DNA. With increasing micrococcal nuclease digestion, the number of inhibitory (3'-phosphoryl) ends will exceed the number of active (3'-hydroxyl) ends, proportionately inactivating the template for DNA synthesis. As shown in Fig. 3, the effectiveness of the calf thymus DNA as a primer-template decreased to 1% of its initial capacity after treatment with 6 μg/ml of micrococcal nuclease. Use of larger amounts of micrococcal nuclease caused increased polymerization rates. This effect is presum-
ably the result of contamination of the micrococcal nuclease with phosphatase. In fact, omission of inorganic phosphate from the micrococcal nuclease reaction resulted in no greater than 60% inhibition of polymerization. For processivity assays, sufficient micrococcal nuclease was added to decrease rate of synthesis by approximately 99%.

The inhibitor should be competitive with respect to template DNA. This would indicate that the inhibitor is interacting with the DNA polymerase as an inactive template, lengthening the time for diffusion of the polymerase from one 3' terminus capable of supporting DNA synthesis to the next, and so increasing the cycling time. The inhibitor should be noncompetitive with respect to dNTP utilization for DNA synthesis, indicating that the sequential process of nucleotide additions, and therefore the processivity, is not being altered by the inhibition. As shown by the double reciprocal plots of reaction rate in the presence of varying amounts of inhibitor (Fig. 4), micrococcal nuclease-treated inhibitor DNA inhibits competitively with respect to activated calf thymus DNA substrate, but noncompetitively with respect to dNTPs.

RESULTS

Processivity of Escherichia coli DNA Polymerase I

General Considerations — Processivity measurements were first made under standard DNA polymerase assay conditions at 37°C (see legend for Table I). Four reactions were run simultaneously: Reaction 1, a mixture containing primer-template DNA (for DNA synthesis), inhibitor DNA, and a limited complement of dNTPs; Reaction 2, a mixture containing a complete complement of dNTPs with the same components as Reaction 1; Reaction 3, a mixture containing a limited complement of dNTPs and primer-template DNA only; and Reaction 4, a mixture containing a complete complement of dNTPs and primer-template DNA only. Rates of these reactions were used to calculate relative cycling times and processivity.

The relative concentrations of enzyme and DNA in all reactions were maintained such that the amount of DNA synthesis was less than 10% of the amount of synthesis that the DNA could support with a large excess of E. coli DNA polymerase I.

The concentration of activated DNA in inhibited reactions was lower than in the uninhibited reactions. This was done: (a) to increase inhibition by raising the inhibitor/activated DNA ratio; (b) to compensate for the higher activity of the DNA polymerase in the uninhibited reaction so the DNA would react to less than 10% of its capacity; and (c) to help compensate for any effects the concentration of 3'-phosphoryl ends might have on processivity. Both untreated and micrococcal nuclease-treated DNAs present in processivity experiments contained a low level of active 3'-hydroxyl primer-termini. Since synthesis on this DNA contributed slightly to the processivity value, all DNAs in any single determination were from the same source (e.g., synthesis on ColEI DNA was inhibited by 3'-phosphoryl termini of nicked ColEI DNA).

Nicked DNA Templates — Processivity and relative cycling times were measured with nicked ColEI DNA and activated calf thymus DNA as primer-templates (Table I). Activated calf thymus DNA may contain gaps (areas of single strands) in addition to nicks (breaks with no nucleotides removed), while nicked ColEI DNA contains essentially no gaps (9). Processivity was determined by comparing rates of synthesis with one and four dNTPs, as well as with three and four dNTPs. With nicked ColEI DNA, both conditions resulted in processivity values of approximately 20. Similarly, with calf thymus DNA, processivity values were approximately 23.

The values obtained by comparing polymerization with one versus four dNTPs and three versus four dNTPs were essentially the same. A slightly greater difference was evident between values for nicked ColEI DNA obtained with one and three dNTPs, than would be expected on the basis of statistical error. This may be due in part to a small error in the value for the base composition of ColEI DNA. Slightly higher

1 It was conceivable that the processivity could be affected by concentration of active or inhibiting DNA termini. At relatively low DNA termini concentrations, an individual enzyme molecule might have a tendency to rebind the same primer-terminus that it had just released. At higher DNA concentrations, or when inhibiting termini are present, less rebinding would occur, resulting in a lowering of processivity. Effects of concentration of calf thymus DNA termini on processivity were tested by measuring reaction rates in the presence of a limited and complete complement of dNTPs over a range of DNA concentrations. It was found that in reactions containing activated and inhibitor DNA in the same concentration ratios as in processivity measurements (4.3% activated DNA), there was essentially no rate dependence on DNA concentration (<15%) above 7.85 μM DNA. In reactions containing activated and untreated DNA (19% activated DNA), similar results were obtained above 6.6 μM DNA. Thus, comparisons of reactions having differences in concentration of 3' termini are valid for the concentration range in which we have measured processivity (approximately 0.06 μM DNA).

2 Base composition of ColEI DNA was calculated from relative equilibrium centrifugation banding position of this DNA with E. coli DNA. Possible errors in base composition used to calculate processivity could result in an error in processivity determination as much as 7 to 8%.

3 Base composition of ColEI DNA was calculated from relative equilibrium centrifugation banding position of this DNA with E. coli DNA. Possible errors in base composition used to calculate processivity could result in an error in processivity determination as much as 7 to 8%.
Processivity and relative cycling time of E. coli DNA polymerase I with nicked ColEl DNA, activated calf thymus DNA, and gapped ColEl DNA

All values are shown with standard deviations.

<table>
<thead>
<tr>
<th>Primer-template</th>
<th>dNTPs in limited reaction</th>
<th>(T/T_c)</th>
<th>No. of determinations</th>
<th>Processivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicked ColEl DNA</td>
<td>T</td>
<td>6.8 ± 1.9</td>
<td>8</td>
<td>17.8 ± 3.3</td>
</tr>
<tr>
<td>Nicked ColEl DNA</td>
<td>T + A + C</td>
<td>5.6 ± 1.7</td>
<td>15</td>
<td>20.7 ± 3.9</td>
</tr>
<tr>
<td>Nicked ColEl DNA</td>
<td>T + G + C</td>
<td>5.3 ± 2.1</td>
<td>5</td>
<td>17.8 ± 1.0</td>
</tr>
<tr>
<td>Activated calf thymus DNA</td>
<td>T</td>
<td>4.1 ± 1.3</td>
<td>3</td>
<td>24.2 ± 1.3</td>
</tr>
<tr>
<td>Activated calf thymus DNA</td>
<td>T + A + C</td>
<td>5.2 ± 0.6</td>
<td>4</td>
<td>22.4 ± 2.7</td>
</tr>
<tr>
<td>Gapped ColEl DNA</td>
<td>T + A + C</td>
<td>1.8 ± 0.4</td>
<td>6</td>
<td>47.3 ± 5.1</td>
</tr>
</tbody>
</table>

* Reaction mixtures (75 μl) contained 9.2 mM Tris/HCl (pH 8), 6 mM sodium glycine, 15 mM potassium phosphate, 4 mM MgCl₂, 1 mM NH₄Cl, 4.2 mM KCl, 10 μM dATTP (10 to 15 Ci/mmol), 18.5 μM nicked ColEl DNA, 69 μM untreated calf thymus DNA, and 6 ng of DNA polymerase I. When three dNTPs were used, mixtures were supplemented with dATP and dCTP, or dGTP and dCTP to 10 mM NH₄Cl. 4.2 mM KCl, 10 μM dATTP (10 to 15 Ci/mmol), 18.5 μM nicked ColEl DNA, 69 μM untreated calf thymus DNA, and 6 ng of DNA polymerase I. When three dNTPs were used, mixtures were supplemented with dATP and dCTP, or dGTP and dCTP to 10 mM NH₄Cl.

Processivity and relative cycling time of E. coli DNA polymerase I with activated poly[d(A-T)]

The procedures for determination of processivity and relative cycling time were the same as described in the legend for Table I. Low ionic strength reaction mixtures (75 μl) contained 4 mM MgCl₂, 6 mM Tris/HCl (pH 8.5), 1 mM NH₄Cl, 14 mM sodium glycine, 25 mM potassium phosphate, 37.0 μM of activated poly[d(A-T)], and 0.17 mM of poly[d(A-T)]<sub>les</sub>. For inhibited reactions, 18.5 μM of activated poly[d(A-T)] and 0.18 mM of poly[d(A-T)]<sub>les</sub> were used. All other components were at the same concentration as described in the legend for Table I. High salt reaction mixtures (75 μl) contained 7.5 mM MgCl₂, 91 mM potassium phosphate (pH 7.4), 14 mM sodium glycine, 2 mM Tris/HCl, and no NH₄Cl. All other component concentrations were the same as those in the low salt reactions. Values are shown with standard deviations.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Ionic strength</th>
<th>(T/T&lt;sub&gt;c&lt;/sub&gt;)</th>
<th>No. of determinations</th>
<th>Processivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>37º</td>
<td>0.110</td>
<td>0.79 ± 0.49</td>
<td>3</td>
<td>188 ± 37</td>
</tr>
<tr>
<td>5</td>
<td>0.110</td>
<td>1.86 ± 0.68</td>
<td>4</td>
<td>14.4 ± 4.9</td>
</tr>
<tr>
<td>5</td>
<td>0.312</td>
<td>1.96 ± 0.33</td>
<td>2</td>
<td>3.3 ± 0.5</td>
</tr>
</tbody>
</table>

Gapped DNA Template – The processivity of polymerization with a gapped primer-template structure was tested using nicked ColEl DNA that had been digested with exonuclease III (9). As shown in Table I, DNA polymerase I was more processive by approximately 2.5-fold on a template containing primarily gaps than on one which was completely nicked. The relative cycling time (T/T<sub>c</sub>) was substantially lower with gapped compared with nicked DNA as primer-template. This suggested that (a) the kinetic affinity for gapped DNA is greater than that for nicked DNA; (b) the static affinity for gapped structures is less than that for nicked structures; or (c) both of the above.

Poly[d(A-T)] Template – Experiments with the poly[d(A-T)] as template show that template structure, temperature and ionic strength all significantly affect processivity and relative cycling time. As shown in Table II enzyme processivity is approximately 4-fold higher with poly[d(A-T)] than with gapped ColEl DNA (Table I). The kinetic affinity of the enzyme for the alternating co-polymer was nearly equal to the static affinity.

Processivity was greatly diminished at 5º compared to 37º (Table II). Also, the relative cycling time was greater at the lower temperature, suggesting that under these conditions the static affinity of the enzyme becomes significantly higher than the kinetic affinity of the enzyme for poly[d(A-T)]. An increase in ionic strength from approximately 0.25 to 0.315 μM also reduced considerably the value of processivity, but did not affect the relative cycling time.

The striking temperature effects apparent with poly[d(A-T)] were probably the result of stabilization of the secondary structure of the co-polymer at 5º, thus preventing "slippage" or "creeping" which would have allowed further DNA synthesis by reiteration and elimination of "barriers" to polymerization (13, 15). These experiments show that the particular reaction conditions and primer-template selected for analysis can have profound effects on processivity. In this case, DNA polymerase I appeared processive for only three nucleotides at 5º and an ionic strength of 0.312 μM, yet was processive for 188 nucleotides at 37º and an ionic strength of 0.11 μM.

Experiments were attempted on gapped ColEl DNA at low temperature (5º, 0.1 μM) but the enzyme appears to have dissociated so slowly from reactive 3'-hydroxyl termini that it did not cycle and thus inhibition could not be measured accurately. Such effects have been observed using the cohesive ends of DNA as primer-template at 6º (1).

Effect of Ionic Strength on Processivity of E. coli DNA Polymerase I

Fig. 5 shows the effect of ionic strength on processivity and relative cycling time with activated calf thymus DNA as template. The values for the ratio of relative cycling times approached 1 as ionic strength was increased, but the processivity did not drop significantly until the ionic strength was increased to greater than 0.3 μM. Processivity then decreased with increasing salt to a minimum value of approximately 1.

Hence, at very high ionic strength (>0.7 μM), the enzyme...
polymerized in a nonprocessive manner. The effect of ionic strength on the activity of DNA polymerase I is also shown in Fig. 5. From these data, it is evident that while processivity must affect the overall rate of polymerization (see "Theory"), these two parameters need not show a direct correspondence.

Under conditions in which processivity approached 1, it was possible to verify measurements of processivity by an independent means. If the static and kinetic affinity of DNA polymerase for DNA are very low, then the great majority of the time the DNA polymerase is not bound to DNA. If the binding step is by far the slowest, then cycling times must be essentially equivalent in the presence of a limited and complete complement of dNTPs. This condition manifests itself as a linear dependence of rate on DNA concentration, since the DNA is a second order reactant for the binding reaction. Fig. 6 shows that there is a nearly linear dependence of rate on DNA concentration at 0.725 μM ionic strength and 0 to 6 μM DNA concentration. Under these conditions, relative rates all show a value of approximately 1.0 for processivity.

**Effective Template Lengths of DNA**

A method is described under "Theory" for determination of the average available template length for DNA synthesis at each 3’ terminus of a substrate DNA under specific conditions. It involves calculation of extents of polymerization in the presence of both a limited and complete complement of dNTPs using an excess of the DNA polymerase. Apparent template lengths for the DNA used in the experiments described above were calculated accordingly, and are shown in Table III. In general, these lengths are several-fold higher than the values of processivity obtained for the same DNA. This result indicates that values of processivity were not limited by the available template length, but instead were properties intrinsic to DNA polymerase I under the conditions specified.

**Nonprocessivity of DNA Polymerase β from KB Cells**

The DNA polymerase β from KB cells when polymerizing on activated calf thymus DNA gave a processivity value of about one, i.e. is nonprocessive. There was an essentially

### Table III

**Effective template lengths of various DNAs**

<table>
<thead>
<tr>
<th>DNA</th>
<th>Temperature</th>
<th>Ionic strength</th>
<th>dNTPs in limited reaction</th>
<th>Effective template length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicked ColE1</td>
<td>37°</td>
<td>0.005</td>
<td>1</td>
<td>305</td>
</tr>
<tr>
<td>Nicked ColE1 + inhibitor</td>
<td>37°</td>
<td>0.085</td>
<td>1</td>
<td>325</td>
</tr>
<tr>
<td>ColE1 DNA a</td>
<td>37°</td>
<td>0.085</td>
<td>1</td>
<td>263</td>
</tr>
<tr>
<td>poly[d(A-T)]</td>
<td>37°</td>
<td>0.110</td>
<td>1</td>
<td>2000</td>
</tr>
<tr>
<td>poly[d(A-T)]</td>
<td>37°</td>
<td>0.110</td>
<td>1</td>
<td>400</td>
</tr>
<tr>
<td>poly[d(A-T)]</td>
<td>37°</td>
<td>0.110</td>
<td>1</td>
<td>336</td>
</tr>
<tr>
<td>Activated calf thymus</td>
<td>37°</td>
<td>0.085</td>
<td>3</td>
<td>345</td>
</tr>
<tr>
<td>Activated calf thymus</td>
<td>37°</td>
<td>0.235</td>
<td>3</td>
<td>284</td>
</tr>
<tr>
<td>Activated calf thymus</td>
<td>37°</td>
<td>0.385</td>
<td>3</td>
<td>58</td>
</tr>
<tr>
<td>Activated calf thymus</td>
<td>37°</td>
<td>0.475</td>
<td>3</td>
<td>18.5</td>
</tr>
</tbody>
</table>

*a* This reaction contained activated and inhibitor DNAs in the same concentration ratio as those used in uninhibited processivity measurement reactions. Aliquots (10 μl) were removed and assayed for acid-insoluble radioactivity, until the polymerization rates were essentially zero (usually 1 to 2 h). Effective template lengths were calculated from the last samples taken.

---

---

FIG. 6 (left). Effect of ionic strength on processivity and relative cycling time of *Escherichia coli* DNA polymerase I with activated calf thymus DNA. Reaction mixtures were identical with those described in the legend to Table I for calf thymus DNA, except that they were supplemented with KC1 to the ionic strengths shown. Data points are calculated from the average of 10- and 20-min measurements. The upper panel shows the values of relative cycling time; the middle panel shows the calculated processivity values; and the lower panel shows the activity of the DNA polymerase in the uninhibited reaction with a complete complement of dNTPs.

FIG. 6 (right). Effect of DNA concentration on rate of synthesis by DNA polymerase I at high ionic strength. The reaction mixture for limited synthesis (100 μl) contained 50 mM Tris/HCl (pH 7.5), 0.665 mM KC1, 10 mM MgCl2, 10 mM dithiothreitol, 0.75 μM to 0.75 mM activated calf thymus DNA, and 10 μM [3H]dTTTP (25 Ci/mmol). The reaction mixture for complete synthesis was supplemented with 10 μM each in dCTP, dGTP, and dATP. The reaction was initiated by addition of 3.3 ng of *E. coli* DNA polymerase I. After 15 min at 37°, an aliquot (25 μl) was removed for analysis. Incorporation is shown for mixtures with [3H]dTTTP alone (○) and for all four dNTPs (■).

TABLE III

**Effective template lengths of various DNAs**

The procedure for calculating the effective template length is presented under "Theory." Reaction mixtures (40 μl) to which 0.25 to 0.50 μg of DNA polymerase I were added, contained salt and buffer components identical with those used for processivity measurements under the conditions described (refer to the legend to Table I). DNA concentrations were 7.0 to 28.0 μM and reactions contained activated and untreated DNAs in the same concentration ratios as those used in uninhibited processivity measurement reactions. Aliquots (10 μl) were removed and assayed for acid-insoluble radioactivity, until the polymerization rates were essentially zero (usually 1 to 2 h). Effective template lengths were calculated from the last samples taken.
The lower processivity observed with nicked DNA substrates, compared to values obtained with gapped DNA substrates suggests that the affinity for primer-template is lower than the static affinity. The numerical value of \( T_r/T_s \) is not indicative of a quantitative relationship between kinetic and static affinity because some synthesis does occur even when a limited complement of dNTPs is used in the reaction.

Examination of the data shows that \( T_r/T_s \) is much higher on the nicked DNA substrate than on the gapped DNA substrate. This suggests that the process of continual removal of DNA ahead of the growing primer diminishes the kinetic affinity relative to static affinity.

Our experiments with the DNA polymerase \( \beta \) from \( E. coli \) cells show that regardless of temperature and ionic strength, the mechanism of this enzyme is nonprocessive \( (N_r = 1) \).

The analysis presented above allows calculation of the average template length available for polymerization beyond each primer-terminus of the substrate DNA. Results from these experiments show that processivity is not limited by the template length, but is instead an accurate indication of the propensity of DNA polymerase I to translocate along the DNA template. The ability to determine available template length may be useful in other aspects of nucleic acid research. For example, it may be possible to determine template lengths available to DNA polymerases after radiation, chemical, or mechanical damage, and during repair and recombination events.

Processivity of DNA polymerization has been actively investigated by a number of laboratories in recent years. McClure and Jovin (13) showed that with poly(dA-T) at 4°C and ionic strength approximately 0.20 \( \mu \), DNA polymerase I is essentially nonprocessive. Results at higher temperatures were consistent with increased processivity. Our findings of low processivity \( (N_r = 3.3) \) with poly(dA-T) at low temperature and high processivity \( (N_r = 188) \) at higher temperature, are in reasonable agreement with their findings.

Chang (18) and Sherman and Gefter (19) have shown that polymerase I will extend an excess of primer strands in a synchronous manner over short time intervals (several minutes). These experiments, however, cannot be interpreted quantitatively. Some ambiguity occurs because the time intervals examined were presumably much longer than the cycling time of DNA polymerase I, even if polymerization were processive for more than 100 nucleotides.

Thomas and Olivera (6) have devised a method to measure the processivity of nucleases on homopolymer templates. Although their results are not directly comparable to ours, since we cannot measure processivity on homopolymers, they have shown that at 37°C and approximately 0.09 \( \mu \) ionic strength, the 5’→3’ exonuclease of DNA polymerase I is processive for...

\[ \text{Table IV} \]

Quantitative Processivity of \( E. coli \) DNA Polymerase I

<table>
<thead>
<tr>
<th>KCl (mM)</th>
<th>DNA</th>
<th>No. of determinations</th>
<th>Ratio ( R_{r/l} )</th>
<th>Processivity ( N )</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.60</td>
<td>1.1</td>
<td>8</td>
<td>1.28 ± 0.22</td>
</tr>
<tr>
<td>20</td>
<td>0.75</td>
<td>3</td>
<td>2</td>
<td>0.92 ± 0.23</td>
</tr>
</tbody>
</table>
less than approximately 20 bases during nick translation. Such a value is consistent with our results on nicked DNA substrates.

The finding that the DNA polymerase δ from KB cells is nonprocessive is in agreement with an earlier report that an analogous enzyme purified from calf thymus extended a 3' terminus of DNA for only one nucleotide before dissociating (18).

Finally, it is clear from recent work (15, 19, 20) that proteins associated with DNA replication, for example, E. coli DNA-binding protein, can affect processivity. The simple, rapid method described here for quantitative measurement of processivity provides a useful tool to assess the effects of these proteins on the mechanism of DNA polymerases. The advantage of the method described here is that virtually no restriction is placed either on the choice of primer-template or reaction conditions.

Acknowledgment – We wish to thank Dr. I. R. Lehman for his interest and encouragement. We also thank Dr. Henry S. H. Tye for his help with the mathematics involved in the derivations, Dr. Jack Henkin for very helpful suggestions concerning our experimental approach, Mr. Philip Fay and Mr. Steven Matson for performance of a considerable number of experiments, and Miss Janice Chien for preparation of nicked and gapped ColE1 DNA. Dr. Theresa S.-F. Wang and Dr. David Korn generously provided the KB cell DNA polymerase δ used in our experiments.

APPENDIX

This section describes the calculations used to obtain the theoretical curves shown in Figs. 1 and 2. These curves show the relationship between the ratio of reaction rates with a limited versus complete complement of dNTPs and the processivity of polymerization. To simplify the analysis, the relative cycling time (T/Tδ) is assumed to be 1.0. The relationship can be corrected for other values of relative cycling time as described under “Theory” and below.

Determination of Processivity with ColE1 DNA and Activated Calf Thymus DNA – In this case, the rate of limited synthesis with one, two, or three dNTPs is compared to the rate of complete synthesis when all four dNTPs are provided. As mentioned under “Theory,” the rate of polymerization with any number of dNTPs may be generalized as follows:

\[ P_x = \frac{E}{D_x} \sum_{n=1}^{N_x} n D_n \]  \hspace{1cm} (X=1,2,3 or 4)  \hspace{1cm} (1)

where the subscript x denotes the number of dNTPs, P is the polymerization rate, E, is the number of enzyme molecules, \( N_x \) is the average number of nucleotides polymerized during each polymerization cycle, and T is the cycling time.

The term \( N_x \) may be expressed in the following manner:

\[ N_x = \sum_{n=1}^{\infty} (S_x + S^2_x + S^3_x + \ldots + S^n_x) D_n \]  \hspace{1cm} (20)

where \( S_x \) is the probability of addition of an available nucleotide at any available site, and \( D_n \) is a Poisson distribution factor,

\[ D_n = \frac{N^n e^{-N}}{n!} \]  \hspace{1cm} (21)

where \( N \) is the processivity. The value of \( S_x \) is determined by the deoxynucleoside triphosphates present and the base composition of the template. Values of \( S_x \) are shown in Table V for the cases of calf thymus DNA, ColE1 DNA, and poly[d(A-T)].

When all four dNTPs are present, \( S_{x-4} = 1 \) and the expression for \( N_x \) becomes:

\[ N_{x=4} = \sum_{n=1}^{\infty} (1 + 1^2 + 1^3 + \ldots + 1^n) D_n = \sum_{n=1}^{\infty} n D_n = N \]  \hspace{1cm} (22)

This simply shows that the average number of nucleotides incorporated per polymerization cycle with all four dNTPs is, indeed, the processivity. When one, two, or three dNTPs are present (\( x = 1, 2, \) or 3, respectively), the expression for \( N_x \) may be rewritten:

\[ N_x = \sum_{n=1}^{\infty} (S_x + S^2_x + S^3_x + \ldots + S^n_x) D_n \]

\[ = \frac{S_x}{1-S_x} + \frac{S_x^2}{1-S_x^2} + \frac{S_x^3}{1-S_x^3} + \ldots + \frac{S_x^n}{1-S_x^n} D_n \]

\[ = \frac{S_x}{1-S_x} \sum_{n=1}^{\infty} \left( \frac{n-1}{n!} \right) e^{-N} \]

\[ = \frac{S_x}{1-S_x} \sum_{n=1}^{\infty} \left( \frac{n-1}{n!} \right) e^{-N} \]

Since

\[ \sum_{n=1}^{\infty} \frac{n}{n!} e^{-N} = 1, \]

\[ N_x = 1, 2, \) or 3 = \left( \frac{S_x}{1-S_x} \right) \left[ 1 - \frac{(N_x)^N e^{-N}}{n!} \right] \]

We may now multiply the summation term by 1 in the form:

\[ \left( e^{N_x} - N_x \right) \]

\[ \left( e^{N_x} - N_x \right) \]

Thus,

\[ N_x = 1, 2, \) or 3 = \left( \frac{S_x}{1-S_x} \right) \left[ 1 - \left( e^{-N} \right)^N \right] \]

\[ \left( e^{N_x} - N_x \right) \]

\[ \left( e^{N_x} - N_x \right) \]

The generalized rate Equation 1 for \( x = 1, 2, \) or 3 can now be rewritten, incorporating Equation 23 as:

\[ P_x = \frac{E}{1-S_x} \left( 1 - e^{N_x} \right) \]  \hspace{1cm} (24)

and the rate equation for \( x = 4 \) can be rewritten, incorporating Equation 22 as:

\[ P_4 = \frac{E}{4} \]  \hspace{1cm} (25)

Thus, the ratio of polymerization rates (\( P_{x,i} \)) when comparing...
limited to complete syntheses, can be expressed using Equations 24, 25, and 2 (see "Theory") as:

\[
\begin{align*}
P_x = \frac{\left(1 - e^{-N(S_x)}\right)}{\left(1 - e^{-N}\right)}
\end{align*}
\]

When only a single deoxynucleoside triphosphate is radioactive, a correction factor \( L \) must be incorporated into the expression. The ratio of rates of radioactivity incorporated in limited versus complete synthetic reactions may then be expressed as:

\[
\begin{align*}
R = \frac{\left(1 - e^{-N(S_x)}\right)}{\left(1 - e^{-N}\right)}
\end{align*}
\]

where \( L \) is defined as the fraction of incorporated nucleotides which are labeled in the limited reaction divided by the fraction of incorporated nucleotides which are labeled in the complete reaction. Values of \( L \) for the case of label in dITP only with ColEl DNA, calf thymus DNA, or poly[d(A-T)] are shown in Table V. In order to reduce the number of variables so that the expression could be graphed, it was assumed that \( T_1/T_2 = 1 \). This means that cycling time is unaffected by omission of any of the deoxynucleoside triphosphates. Equation 10 then reduces to:

\[
\begin{align*}
R_x = \frac{L N}{S_x} \left(1 - e^{-N(S_x)}\right)
\end{align*}
\]

where \( R_x \) is defined by Equation 18. Equation 28 as well as the values shown in Table V were used to generate the curves shown in Fig. 1.

In order to use the figure to calculate processivity it is necessary to calculate \( R_x \) from:

\[
\begin{align*}
R_x = \frac{S_x}{N} \left(1 - e^{-N(S_x)}\right)
\end{align*}
\]

Since \( R_x \) and \( T_1/T_2 \) are all measurable values, it is possible to directly determine processivity from the curves in Fig. 1.

**Table V**

<table>
<thead>
<tr>
<th>Primer-template</th>
<th>dNTPs added during limited synthesis</th>
<th>( x )</th>
<th>( S_x )</th>
<th>( L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf thymus DNA or ColEl DNA</td>
<td>dTTP</td>
<td>1</td>
<td>0.278 (0.250)</td>
<td>3.80 (4.00)</td>
</tr>
<tr>
<td>dATP</td>
<td>2</td>
<td>0.536 (0.500)</td>
<td>1.80 (2.00)</td>
<td></td>
</tr>
<tr>
<td>dCTP</td>
<td>2</td>
<td>0.500 (0.500)</td>
<td>2.00 (2.00)</td>
<td></td>
</tr>
<tr>
<td>dGTP</td>
<td>2</td>
<td>0.500 (0.500)</td>
<td>2.00 (2.00)</td>
<td></td>
</tr>
<tr>
<td>dTTP, dATP, dCTP</td>
<td>3</td>
<td>0.778 (0.750)</td>
<td>1.285 (1.333)</td>
<td></td>
</tr>
<tr>
<td>dTTP, dATP, dGTP</td>
<td>3</td>
<td>0.778 (0.750)</td>
<td>1.285 (1.333)</td>
<td></td>
</tr>
<tr>
<td>dTTP, dCTP, dGTP</td>
<td>3</td>
<td>0.722 (0.760)</td>
<td>1.886 (1.333)</td>
<td></td>
</tr>
</tbody>
</table>

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Quantitative Processivity of E. coli DNA Polymerase I


On the processive mechanism of Escherichia coli DNA polymerase I.
Quantitative assessment of processivity.
R A Bambara, D Uyemura and T Choi


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