Deoxyribonucleoside Triphosphate Stimulation of Exonucleolytic Activity of the Micrococcus luteus Deoxyribonucleic Acid Polymerase*

(Received for publication, December 6, 1971)

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SUMMARY

The rate of 5' to 3' exonucleolytic degradation by the Micrococcus luteus DNA polymerase is stimulated by deoxyribonucleoside triphosphates. A single deoxyribonucleoside triphosphate can stimulate 6-fold the rate of degradation of an alternating sequence DNA polymer. It is neither necessary nor sufficient that the deoxyribonucleoside triphosphate be complementary to the bases of the DNA polymer in order to stimulate the rate of exonucleolytic degradation of that polymer. The deoxyribonucleoside triphosphate is not chemically altered due to its role as a stimulator.

The Micrococcus luteus DNA polymerase has a low level of exonucleolytic activity which gives a high percentage of dinucleotides as products (1). This activity is part of the polymerase protein (2, 3). The presence of certain nucleoside triphosphates markedly stimulates the rate of exonucleolytic degradation (1, 2, 4, 5). A study of this behavior was performed in order to better understand the mechanism of DNA synthesis.

Comparative properties of the M. luteus DNA polymerase and the Escherichia coli DNA polymerase are discussed.

EXPERIMENTAL PROCEDURE

General—Many of the materials and methods are described in the preceding paper (3). They include the following: nucleotides, nucleic acids, M. luteus DNA polymerase, nuclease reactions, determinations of the products of nucleolytic degradation, and polyacrylamide gel electrophoresis.

dADP was purchased from Sigma and was purified by column chromatography containing DEAE-cellulose in the carbonate form (gift of R. W. Sweet). [C-14C]Poly(dT-dG)-poly(dC-dA), (a gift of R. W. Sweet) was synthesized as described (6).

† This work was supported in part by funds from the National Science Foundation (GB-30528X), the National Institutes of Health (1A-273), and the Jane Coffin Childs Memorial Fund (272). Supported in part by the Woodrow Wilson Fellowship Foundation and by Predoctoral Training Grant GM 00236 BCH from the National Institute of General Medical Sciences. Present address, Division of Biology, California Institute of Technology, Pasadena, California 91109.

RESULTS

Stimulation of Rate of Exonucleolytic Degradation by Single Deoxyribonucleoside Triphosphate

The rate of exonucleolytic degradation of certain double-stranded DNA polymers is increased several fold by the addition of one deoxyribonucleoside triphosphate.

Poly(dA-dT)-poly(dA-dT) — Stimulation of the rate of exonucleolytic degradation of poly(dA-dT)-poly(dA-dT) by the addition of various deoxyribonucleoside triphosphates is shown in Fig. 1. In the absence of triphosphates, degradation proceeds at a slow linear rate. Addition of dATP alone results in a 6-fold increase in the initial rate of degradation. Addition of dTTP alone increases the rate of degradation 2-fold. Under these conditions, however, the deoxyribonucleoside triphosphates serve as substrates for polymerization, resulting in a decreasing concentration of triphosphate stimulators and an increasing concentration of unlabeled poly(dA-dT). Hence the 6-fold increase in the rate of degradation observed with the addition of dATP alone is obscured by the synthetic reaction when dATP and dTTP are added simultaneously.

Fig. 1 also shows that the addition of dCTP, a nucleotide that is not complementary to either of the bases of the DNA substrate, stimulates the rate of nucleolytic degradation 5-fold.
Neither rATP nor dAMP stimulate the rate of degradation (data not shown). Pure dADP alone cannot effectively stimulate the rate of exonucleolytic degradation. Commercial preparations of nuclease diphosphates contain appreciable quantities of triphosphate, thus explaining our prior result (2). There is a small enhancement of the rate at later times in the presence of pure dADP which may be due to some stabilization of the enzyme during incubation.

**Poly(dI-dC)-poly(dI-dC)**—The rate of exonucleolytic degradation of poly(dI-dC)-poly(dI-dC) also is stimulated by the addition of one deoxyribonucleoside triphosphate as shown in Fig. 2. In the absence of triphosphates, degradation proceeds at a slow linear rate. This same rate is observed when either dGTP or dCTP is added individually. However, addition of dATP stimulates the rate 3-fold and the addition of dATP stimulates the rate 3.5-fold. The simultaneous addition of dITP and dCTP results in a slower rate of degradation than the rate observed for dATP alone. However, in this case, the synthetic reaction can occur in this case. Also, the simultaneous addition of all four complementary deoxyribonucleoside triphosphates results in an inhibition of the degradation rate.

The results obtained with poly(dA-dT)-poly(dA-dT), poly(dG-dC)-poly(dG-dC), and poly(dT-dG)-poly(dC-dA) can be summarized as follows. (a) A single deoxyribonucleoside triphosphate can stimulate the rate of exonucleolytic degradation of an alternating base sequence polymer. (b) The composition of the DNA substrate determines which deoxyribonucleoside triphosphate determines the nucleolytic reaction and determines the magnitude of the stimulation. (c) It is not necessary that a deoxyribonucleoside triphosphate be complementary to the base sequence of the DNA polymer in order to stimulate the

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**Fig. 1 (left).** Effect of deoxyribonucleoside triphosphates on the rate of exonucleolytic degradation of poly(dA-dT)-poly(dA-dT). Nuclease reaction mixtures contained 75 μM [3H]-labeled poly(dA-dT)-poly(dA-dT) (specific activity 15,000 cpm per nmole) nucleotides as specified, and 80 units per ml of M. luteus DNA polymerase, and were incubated at 37°C. At intervals, aliquots were withdrawn and assayed for acid-soluble radioactivity. No triphosphate additions (O); 200 μM dATP (A); 500 μM dCTP (○); 500 μM dITP (△); and both 200 μM dATP and 500 μM dITP (△).

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**Fig. 2 (left center).** Effect of deoxyribonucleoside triphosphates on the rate of exonucleolytic degradation of poly(dI-dC)-poly(dI-dC). Nuclease reaction mixtures contained 60 μM [3H]-labeled poly(dI-dC)-poly(dI-dC) (specific activity 3500 cpm per nmole), nucleotides as specified, and 15 units per ml of M. luteus DNA polymerase, and were incubated at 37°C. At intervals, aliquots were withdrawn and assayed for acid-soluble radioactivity. No triphosphate additions (O); 200 μM dATP (A); 500 μM dITP (△); and both dITP and dCTP (○).

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**Fig. 3 (right center).** Effect of deoxyribonucleoside triphosphates on the rate of exonucleolytic degradation of poly(dT-dG)-poly(dC-dA). Nuclease reaction mixtures contained 92 μM [3H]-labeled poly(dT-dG)-poly(dC-dA), nucleotides as specified, and 60 units per ml of M. luteus DNA polymerase, and were incubated at 37°C. At intervals, aliquots were withdrawn and assayed for acid-soluble radioactivity. No triphosphate additions (O); 500 μM dATP (A); 500 μM dCTP (△); 500 μM each of both dCTP and dATP (△); and 500 μM each of both dGTP and dCTP (○).
rate of exonucleolytic degradation of that polymer. (d) In general, purine deoxyribonucleoside triphosphates are more potent stimulators than are pyrimidine deoxyribonucleoside triphosphates. (e) A pyrimidine nucleoside triphosphate stimulates degradation most effectively when it is not contained in the DNA polymer strand which is degraded. (f) Conditions permitting DNA synthesis decrease the rate of nucleolytic degradation observed in the presence of the purine nucleoside triphosphate alone.

Poly(dG-dC)-poly(dG-dC)—The effect of deoxyribonucleoside triphosphates on the rate of poly(dG-dC)-poly(dG-dC) degradation is shown in Fig. 4. When tested individually, dATP, dGTP, and dCTP have no effect on the rate of degradation. Simultaneous addition of both dGTP and dCTP results in a decreased rate of degradation as expected for conditions permitting synthesis. Addition of dITP alone resulted in a small increase in the rate of nucleolytic degradation. However, the simultaneous addition of both dITP and dCTP resulted in a 4-fold increase in the rate of nucleolytic degradation. These conditions permit the rapid synthesis of poly(dI-dC) (7). Poly(dI-dC)-poly(dI-dC) is degraded 10 times more rapidly than poly(dG-dC)-poly(dG-dC) (compare Figs. 2 and 4). It would be expected that the hybrid polymer poly(dG-dC)-poly(dI-dC) would be degraded at a rate intermediate to these two cases. Hence the stimulation of poly(dG-dC)-poly(dG-dC) degradation by addition of dITP and dCTP may be a result of the formation of poly(dG-dC)-poly(dI-dC). With this unique exception, the stimulation observed with the poly(dG-dC)-poly(dG-dC) conforms with the other alternating base sequence polymers. In general, however, poly(dG-dC)-poly(dG-dC) is much less susceptible to nucleoside triphosphate stimulation and its degradation can be stimulated only by dITP.

Optimal Concentration of Deoxyribonucleoside Triphosphate for Stimulation of Exonucleolytic Degradation

The effect of the concentration of dATP on the amount of stimulation of nucleolytic activity is shown in Fig. 5a. The relative increase in the rate of nucleolytic degradation of both poly(dA-dT)-poly(dA-dT) and poly(dI-dC)-poly(dI-dC) was measured. The “fold” stimulation is calculated by dividing the nanomoles of DNA substrate degraded in the presence of dATP by the nanomoles of DNA substrate degraded in the absence of dATP. For both poly(dA-dT)-poly(dA-dT) and poly(dI-dC)-poly(dI-dC), the amount of stimulation observed increases with an increasing concentration of dATP. Maximal stimulation of the nucleolytic degradation rate is achieved at 200 μM dATP and concentrations higher than 200 μM have essentially no further effect on the amount of stimulation.

A slightly different pattern was observed for several other deoxyribonucleoside triphosphates. In Fig. 5b, the amount of stimulation of poly(dA-dT)-poly(dA-dT) degradation by dGTP or by dCTP, when tested individually, is plotted as a function of their concentration. For these nucleoside triphosphates, the increase in the amount of stimulation continues through the concentration range of 200 to 500 μM.
Nonadditivity of Stimulatory Effects

The amounts of stimulation observed when deoxyribonucleoside triphosphates are tested individually are not compounded when the nucleoside triphosphates are added simultaneously. In Fig. 6, the rate of nucleolytic degradation of poly(dA-dT)-poly(dA-dT) is stimulated 3.5-fold by dTTP when tested alone, and 4.0-fold by dATP when tested alone. Simultaneous addition of both dATP and dTTP results in the same rate as that observed for dATP alone. The concentration of dATP is saturating under these conditions. Hence, the effects of dATP and dTTP are not additive. The same result was obtained with poly(dT-dG)-poly(dC-dA) for these two nucleoside triphosphates (results not shown).

The effect of simultaneous addition of dGTP and dATP on the rate of poly(dA-dT)-poly(dA-dT) degradation is shown in Fig. 7. Although there is a small increment in the rate of degradation above that observed for dATP alone, the effects of dATP and dGTP are not strictly additive. The various nucleoside triphosphates act at the same site in stimulating nucleolytic degradation. Presuming that the triphosphates are bound only to the "triphosphate binding site," this result is consistent with the finding (8) that the E. coli DNA polymerase I has only a single binding site for triphosphates.

Stability of Deoxyribonucleoside Triphosphate Stimulator—To determine whether deoxyribonucleoside triphosphate was altered chemically as a result of its stimulatory function, [3H]dATP was incubated with the enzyme in the presence and absence of poly(dA-dT)-poly(dA-dT). No breakdown was observed which could be correlated with nuclease reaction. However, the dATP was converted to dADP by the enzyme preparation at a slow linear rate (5 nmol per hour) and no other products were formed.

This putative modification of the stimulator is not coupled with nucleolytic degradation since: (a) the same rate is observed in the presence and absence of DNA; (b) the rate of conversion of dATP to dADP is only 20% of the rate of nucleolytic degradation under similar conditions; (c) gel electrophoresis of the enzyme preparation demonstrated that the enzyme which converts dATP to dADP and the polymerase (nuclease) are different proteins (Fig. 8). The protein which catalyzes the conversion of dATP to dADP is located at Rf 0.24. The polymerase and nuclease activities have identical Rf values of 0.69, 0.33, and 0.12 (3) in this gel electrophoresis system. Assay of the gels for nuclease activity in the presence and absence of dATP by the gel slice method showed that the nuclease activity was stimulated by dATP when resolved from the dATPase activity (results not shown).

Thus it is concluded that the dATPase activity is a trace contaminating enzyme and that the stimulation of nucleolytic degradation does not require the breakdown of the nucleoside triphosphate to the diphosphate form.

It should be noted that the nucleoside diphosphokinase activity which is associated with DNA polymerase (9) does not affect this assay for dATPase activity. The dATPase assay involves only one nucleoside triphosphate which, in the nucleoside diphosphokinase reaction, would not yield a net increase of triphosphate.

In the cases where a deoxyribonucleoside triphosphate stimulator is complementary to the base sequence of the DNA substrate, it is possible that a limited end addition reaction could occur at the 3'-hydroxyl end of the DNA substrate. During the dATPase assay in which [3H]dATP was incubated with the M. luteus DNA polymerase in the presence of poly(dA-dT)-poly(dA-dT) no detectable DNA synthesis occurred (<1 nmol per ml). A very limited DNA synthesis reaction, however, does occur under these conditions but is detected only when radioactive dATP of very high specific activity is used.1 When [α-32P]dATP (specific activity 1 × 106 cpm per nmole) was incubated with poly(dA-dT)-poly(dA-dT) and the M. luteus DNA polymerase, a small amount of [32P]dAMP was rapidly incorporated into DNA. The reaction ceased when the amount of dAMP incorporated was equal to the calculated number of 3' termini present in the poly(dA-dT)-poly(dA-dT) sample. This limited 3' end-addition reaction of a single nucleotide is expected for an alternating base sequence polymer when a single complementary nucleoside triphosphate is provided. A limited end-addition reaction has been reported for the E. coli DNA polymerase I with native DNA (10) and is being utilized for DNA sequencing with T-4 DNA polymerase (11).

The role of deoxyribonucleoside triphosphate stimulation is not strictly a result of its role in the limited end-addition reaction since the concentration of deoxyribonucleoside triphosphate required for maximal stimulation of the exonuclease rate is 200 μM (or greater in some cases). This concentration is at least 10-fold higher than the concentration required to saturate the polymerase for the end-addition reaction. Furthermore, certain purine deoxyribonucleoside triphosphates, which are not complementary to the DNA substrate, stimulate exonucleolytic degradation. However, the end-addition reaction might be a clue to determining why a complementary pyrimidine nucleoside triphosphate, which may be incorporated at the 3'-terminus, is unable to stimulate nucleolytic degradation. This would require that the nuclease at the 3'-end of a DNA polymer have a significant effect on the 5' to 3' exonuclease activity.

Product Distribution in Presence and Absence of dATP

To determine whether the stimulation of nucleolytic degradation by nucleoside triphosphates affected the proportion of mononucleotides, dinucleotides, and trinucleotides produced, [3H]poly(dA-dT)-poly(dA-dT) was degraded in the presence and absence of dATP.

1 M. J. Ryan and R. D. Wells, unpublished data.
Table I shows that the same relative distribution of label is observed in both the absence and presence of dATP. This is true although only 28% of the poly(dA-dT)-poly(dA-dT) substrate was degraded in the absence of dATP, whereas 98% degradation was observed in its presence in the same time period.

Hence, the stimulation of nucleolytic degradation increases the rate of formation of all the various types of products. In the preceding paper (3) it was shown that the majority of exonuclease activity observed on double-stranded DNA was a result of 5’ to 3’ exonucleolytic degradation which produced mononucleotides, dinucleotides and trinucleotides. Thus, it is apparent that deoxyribonucleoside triphosphates stimulate the rate of 5’ to 3’ exonucleolytic degradation.

**Effect of Deoxyribonucleoside Triphosphates on Poly(dA)-poly(dT) Degradation**

The stimulation of degradation of the poly(dT) strand of poly(dA)-poly(dT) by dATP, dTTP, and a combination of these two deoxynucleoside triphosphates is shown in Fig. 9. dATP alone stimulates the rate of nucleolytic degradation 2-fold. dTTP alone initially stimulated the degradation 3.5-fold but at later times, is inhibitory. A combination of both dATP and dTTP stimulates the initial rate of degradation 6-fold. Degradation of the DNA substrate is essentially complete within 30 min in this reaction.

A rigorous interpretation of these data is made complex by the fact that polymer synthesis occurs in all these reactions. Hence, although dATP stimulates the rate of nucleolytic degradation 2-fold, it cannot be concluded that this stimulation is similar to that observed with poly(dA-dT)-poly(dA-dT). The dATP may merely be used for the synthesis of poly(dA). Continuous synthesis of poly(dA) would ensure that the poly(dT) strand is in a double stranded form which, as noted previously, is a better substrate for the 5’ to 3’ exonuclease than single stranded poly(dT).

**Table I**

| Product      | dATP | +dATP |
|--------------|------|-------|
| Mononucleotide | 12.6 | 5.5   |
| Dinucleotide  | 75.2 | 30.5  |
| Trinucleotide | 11.3 | 4.6   |

**Fig. 9 (left).** Deoxyribonucleoside triphosphate stimulation of degradation of [T-^3H] poly(dA)-poly(dT). Nuclease reactions contained 30 μM poly(dA) annealed to 30 μM [H]-labeled poly(dT) (specific activity 1800 cpm per n mole), triphosphates as indicated, and 30 units per ml of *E. coli* DNA polymerase. The mixtures were incubated at 37° and at intervals, aliquots were removed and assayed for acid-soluble radioactivity. No triphosphate addition (A); 500 μM dATP (△); 500 μM dTTP (○); and 500 μM of both dATP and dTTP (△).

**Fig. 10 (center).** Effect of deoxyribonucleoside triphosphates on [A-^3H] poly(dA)-poly(dT) degradation. Nuclease reactions contained 60 μM [A-^3H] poly(dA)-poly(dT) (specific activity 12,500 cpm per n mole), triphosphates as indicated, and 30 units per ml of *E. coli* DNA polymerase. The mixtures were incubated at 37° and at intervals, aliquots were removed and assayed for acid-soluble radioactivity. No triphosphate addition (A); 1 mM dATP (○); 1 mM each of dTTP, dGTP, and dCTP (△); or 1 mM each of dATP, dTTP, dGTP, and dCTP (△).
The rate of degradation of the poly(dA) strand of poly(dA)·poly(dT) in the presence and absence of deoxyribonucleoside triphosphates is shown in Fig. 10. dATP, dTTP, and dCTP, and a combination of these two triphosphates do not show a marked stimulation of the initial rate of degradation and inhibit the rate at later times. Again, a rigorous interpretation of these results is difficult since DNA synthesis occurs in all cases.

Effect of Deoxyribonucleoside Triphosphates on Rate of M. luteus DNA Degradation

The rate of exonucleolytic degradation of native M. luteus DNA and the effect of addition of various deoxyribonucleoside triphosphates is shown in Fig. 11. The addition of dATP alone has no effect. Addition of a mixture of dITTP, dCTP, and dGTP also has no effect. However, simultaneous addition of all four deoxyribonucleoside triphosphates (dATP, dGTP, dCTP, and dTTP) results in a 5-fold increase in the rate of exonucleolytic degradation. Hence, all four deoxyribonucleoside triphosphates are required to stimulate the rate of native M. luteus DNA degradation. This effect is quite different from that observed with alternating base sequence polymers where a single deoxyribonucleoside triphosphate stimulates the rate of exonucleolytic degradation.

Lack of Stimulation of Exonucleolytic Degradation of Single Stranded DNA

Whereas the rate of native M. luteus DNA degradation is stimulated by the presence of dATP, dTTP, dCTP, and dGTP, the rate of degradation of denatured DNA is not stimulated by the addition of these deoxyribonucleoside triphosphates (results not shown). Neither dATP alone nor dATP, dTTP, dCTP, and dGTP added simultaneously is effective in stimulating the rate of exonucleolytic degradation of denatured M. luteus DNA.

Similarly, the rate of nucleolytic degradation of poly(dT) is not stimulated by either dATP, dTTP, or a combination of these two deoxyribonucleoside triphosphates. This is in direct contrast to the stimulation observed with poly(dA) annealed to poly(dA) (Fig. 9).

It was shown (3) that single stranded DNA is primarily degraded by the 3' to 5' exonucleolytic activity. Since the degradation of single stranded DNA is not stimulated by deoxyribonucleoside triphosphates, this indicates that only the 5' to 3' exonucleolytic activity of the M. luteus DNA polymerase is stimulated by triphosphates.

DISCUSSION

A single deoxyribonucleoside triphosphate stimulates the rate of degradation of alternating base sequence polymers by the M. luteus DNA polymerase even if the nucleoside triphosphate is not complementary to the bases of the DNA substrate. The amount of stimulation observed with a given deoxyribonucleoside triphosphate is dependent on the base composition of the DNA substrate. This is particularly true for pyrimidine nucleoside triphosphates which stimulate degradation only if they are not complementary to the bases of the polymer strand degraded. For example, dCTP stimulates the rate of poly(dA-dT)·poly(dA-dT) degradation 4-fold but has no effect on the degradation of poly(dI-dC)·poly(dI-dC) or the poly(dG-dC) strand of poly(dG-dC)·poly(dG-dC). However, dTTP stimulates the degradation of the latter two polymers but has essentially no effect on the rate of poly(dA-dT)·poly(dA-dT) degradation. Since a limited end-addition reaction is possible when the deoxyribonucleoside triphosphate is complementary to a base of an alternating base sequence DNA, it would appear that when the 3'-OH terminus of a DNA polymer is a pyrimidine nucleotide and the stimulatory nucleoside triphosphate is also a pyrimidine, no stimulation is observed. However, if the 3'-OH terminal nucleotide is a purine, either pyrimidine or purine nucleoside triphosphates stimulate degradation. The nucleotide stimulator is not chemically altered as a function of its stimulatory role. Substrate concentrations, not catalytic concentrations, of nucleoside triphosphates are necessary for maximum stimulation; the presence of a stimulator does not alter the product distribution. These facts strongly suggest that the microenvironment at the 3'-OH DNA terminus binding site or the occupation of the triphosphate site (or both) influence the activity of the 5' to 3' exonuclease. The enzyme may undergo a conformational change on binding triphosphates; other studies (12) suggested that DNA polymerase is subject to conformational changes.

Although our data do not rule out the possibility that DNA synthesis can stimulate 5' to 3' exonucleolytic degradation as suggested for the E. coli DNA polymerase (13), our studies with alternating sequence polymers indicate that DNA synthesis is not required for the stimulation of the rate of 5' to 3' exonucleolytic degradation.

The M. luteus DNA polymerase is similar to the E. coli DNA polymerase I in several respects. Although the exonuclease of the M. luteus DNA polymerase is less than 1% of the polymerase activity (1), whereas the exonuclease of the E. coli DNA polymerase is 10% of the polymerase activity (14), both enzymes possess 5' to 3' exonucleolytic activities which specifically degrade double-stranded DNA. Both enzymes also possess 5' to 3' exonuclease activities which degrade single stranded DNA's to mononucleotides. The 5' to 3' exonuclease of E. coli DNA polymerase I degrades the poly(dT) strand of poly(dA)·poly(dT) to mononucleotides, dinucleotides, and small oligonucleotides (13). Kadomama and Mccarter (15) have reported that the exonucleolytic activity of the E. coli DNA polymerase also degrades poly(dA-dT)·poly(dA-dT) to yield a large proportion of dinucleotides as initial degradation products. These results are similar to the M. luteus enzyme with one exception. The 3' to 5' exonuclease activity of the E. coli DNA polymerase rapidly degrades dinucleotides to mononucleotides (15-17); dinucleotides and short oligonucleotides are resistant to further degradation by the exonuclease of the M. luteus DNA polymerase.

The 5' to 3' exonucleases of both the M. luteus DNA polymerase and the E. coli DNA polymerase are stimulated by deoxyribonucleoside triphosphates. The rate of degradation of naturally occurring DNA by the E. coli DNA polymerase I is stimulated several fold by the addition of four deoxyribonucleoside triphosphates (15). This is also observed with the M. luteus enzyme. Thus, for a natural template perhaps DNA synthesis is required for the triphosphate stimulation.

When the 5' to 3' exonuclease is cleaved from E. coli DNA polymerase I by proteolytic treatment (18) and separated from the polymerase fragment, no stimulation is observed unless the polymerase fragment is again added to the exonuclease fragment (19). Our observation that the stimulation of 5' to 3' exonuclease activity by deoxyribonucleoside triphosphates apparently involves a change in the 3'-OH primer terminus binding site or the triphosphate binding site, explains why the exonuclease...
The interpretation of the 5' to 3' exonuclease site on the polymerase fragment produced by proteolytic treatment of E. coli DNA polymerase I requires the polymerase fragment for stimulation by nucleoside triphosphates. The polymerase fragment presumably possesses both the triphosphate and primer terminus binding sites. Furthermore, it explains why the rate of degradation of naturally occurring DNA cannot be stimulated by a single deoxyribonucleoside triphosphate as observed for alternating base sequence polymers. In repeating base sequence polymers, the strands of DNA are able to slip with respect to each other. This slippage phenomenon permits the 3'-OH terminus of the DNA to be in close proximity with the 5' terminus even after extensive exonuclease cleavage in the absence of synthesis. With naturally occurring DNA, no slippage occurs so that after the first few exonuclease scissions, the 3'-OH terminus is no longer proximal to the 5' terminus. Since the 3'-OH-terminal nucleotide has an influence on the 5' to 3' exonuclease activity when it is bound to the polymerase, stimulation will not be observed in the case of naturally occurring DNA's unless DNA synthesis occurs.

A model was proposed for the E. coli enzyme to explain the stimulation of the rate of 5' to 3' exonuclease degradation by deoxyribonucleoside triphosphates (16, 20). The model proposes that the 5' to 3' exonuclease lies in front of the path of 5' to 3' DNA synthesis. Hence, when deoxyribonucleoside triphosphates are present which permit DNA synthesis to occur, the synthesis of DNA in the 3' to 5' direction forces exonuclease degradation in the 5' to 3' direction. The interpretation of the original experiment (16) which most strongly supports the positioning of the 5' to 3' exonuclease in front of the polymerase site is now questionable (21). Hence, it is not yet possible to exactly position the 5' to 3' exonuclease site on the polymerase protein.

The in vivo role of the 5' to 3' exonuclease remains an enigma. Although it was suggested that the 5' to 3' exonuclease of the E. coli DNA polymerase excises thymine dimers in the dark repair of ultraviolet damage (17), recent evidence suggests that other nucleases are responsible for thymine dimer excision (22, 23) although the polA mutant does have an increased ultraviolet sensitivity. We previously showed (3) that the 5' to 3' exonuclease of the M. luteus DNA polymerase has different properties than the M. luteus exomuclease which is presumably responsible for the excision of thymine dimers in vivo. Hence, the biological role of the 5' to 3' exonuclease is unclear.

Acknowledgment—We thank Mrs. Jacquelynn E. Larson for her skillful technical assistance.

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J. Biol. Chem. 1972, 247:2675-2681.

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