EXCISION OF THYMINE DIMERS BY PROTEOLYTIC
AND AMBER FRAGMENTS OF E. COLI
DNA POLYMERASE I

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## Summary

Excision of thymine dimers from specifically incised ultraviolet irradiated DNA by E. coli DNA polymerase I is stimulated by concurrent DNA synthesis. The 36,000 molecular-weight "small fragment" obtained by limited proteolysis of DNA polymerase I, which retains only the 5' → 3' exonuclease activity, also excises thymine dimers, but at one-tenth the rate of the intact enzyme. However, the rate of excision is increased by addition of the "large" 76,000-molecular weight fragment. With the further addition of the 4 deoxynucleoside triphosphates, permitting DNA synthesis to occur, excision approaches rates observed with the intact enzyme. The same result was obtained with a fragment of DNA polymerase I with 5' → 3' exonuclease activity that is present uniquely in polymerase I amber mutants.

Amber mutants in the structural gene for E. coli DNA polymerase I

(pol Al), though grossly defective in their polymerase I activity, retain

near normal levels of the 5' → 3' exonuclease activity that is part of the

DNA polymerase I molecule (1). Since the 5' → 3' exonuclease activity in

pol Al mutants is associated with a polypeptide whose sedimentation co-

efficient (2.8S) is very similar to the "small fragment" with 5' → 3' exo-

nuclease activity generated by proteolysis of polymerase I (2,3,4), it has

been suggested that this activity represents the amber fragment itself, or

more likely, a proteolytic cleavage product of it (1).

In exploring further the relationship of the pol Al amber peptide to

the small proteolytic fragment, we have inquired whether it can interact

with the polymerase I "large fragment" that contains the polymerase and

3' → 5' exonuclease activities of the intact enzyme (2,3,4). For these

studies, we have used as an assay for 5' → 3' exonuclease activity the

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known capacity of the enzyme to promote thymine dimer excision from appro-
priately incised ultraviolet-irradiated DNA (5). We have found that the 2.8S
peptide with 5' → 3' exonuclease activity present in pol Al mutants of E.
coli can interact with the polymerase I large fragment in a manner indis-
tinguishable from the proteolytically generated small fragment. We have
further observed that maximal rates of thymine dimer excision by polymerase
I in vitro, and very likely in vivo, require the concerted action of both
polymerase and 5' → 3' exonuclease.

## Materials and Methods

Enzymes: The T4-induced UV endonuclease was purified, as described by
Friedberg and King (6). It was stored at $4^{\circ}$ in 10mM Tris-HCl (pH 8.0),
containing 3% polyethylene glycol and 0.1mM EDTA. E. coli DNA polymerase I
(fraction VII) was kindly provided by Dr. A. Kornberg. The small (36,000)
and large (76,000) molecular-weight products of subtilisin cleavage of E.
coli DNA polymerase I were a generous gift from Dr. Hans Klenow (University
of Copenhagen). Partial purification of polymerase I-associated 5' → 3'
exonuclease from extracts of E. coli pol Al (JG 112), and the assay of DNA
polymerase I and 5' → 3' exonuclease activity were performed as described
by Lehman and Chien (1).

Incision of UV-Irradiated DNA: [$^{3}$H] T7 DNA (5 to 10 x $10^{4}$ cpm/μg) was pre-
pared as described previously (6). Reaction mixtures for incision (1.0-1.5ml)
contained 150-375 nmoles (nucleotide) of [$^{3}$H]T7 DNA (irradiated at 3,000
ergs/mm$^{2}$), 10mM EDTA and 300 to 1500 units of T4 UV endonuclease. Incuba-
tion was at $37^{\circ}$ for 90 min. Based on a thymine dimer content of 1%, this
amount of enzyme was in excess of that required to nick all dimer sites.
Reactions were terminated by the addition of an equal volume of cold re-
distilled phenol (pH 7.0); the DNA was extracted twice with phenol and then
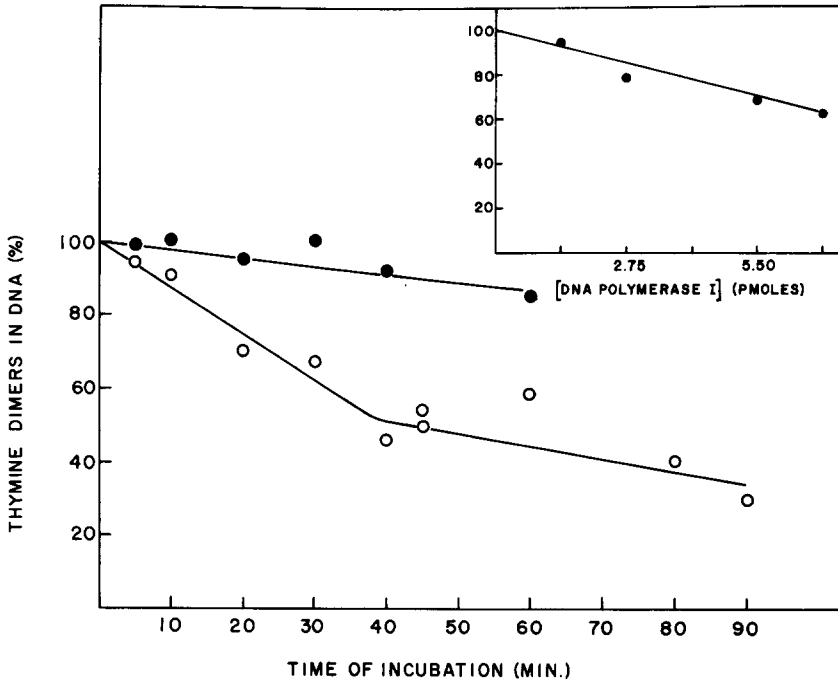dialyzed against 50 mM Tris-HCl (pH 8.0).

Figure 1.  Kinetics of thymine dimer excision by DNA polymerase I.
Details of the incubation and measurement of thymine dimers
in DNA are described in the text.  1.4 pmoles  enzyme ●;
6.9 pmoles enzyme O.  Inset figure shows the excision of
dimers as a function of DNA polymerase concentration.

Excision of Thymine Dimers:  Incubation mixtures (0.2ml) contained

10 nmoles (nucleotide) incised T7 DNA, 20mM Tris-HCl (pH 7.5) 20mM po-

tassium phosphate (pH 7.5), 50 µg/ml bovine serum albumin, 10mM N-ethyl-

maleiamide, 1.5 µg E. coli tRNA, 7mM MgCl, and the amounts of enzyme in-

dicated in the figure legends.  Incubation was at $37^{\circ}$.  When deoxynucleo-

side triphosphates (0.05M) were added, N-ethylmaleiamide was omitted.  Re-

actions were terminated by the addition of 0.2ml of cold 10% trichloro-

acetic acid.  After 15 min. at $0^{\circ}$ the tubes were centrifuged at 5,000 xg for

15 min. and the content of thymine and thymine dimer in the acid-precipitable

fraction was determined by formic acid hydrolysis, followed by two-dimensional

thin-layer chromatography as described previously (7).  Each experiment in-

cluded a control sample of incised DNA incubated for 60 min. at $37^{\circ}$ with an
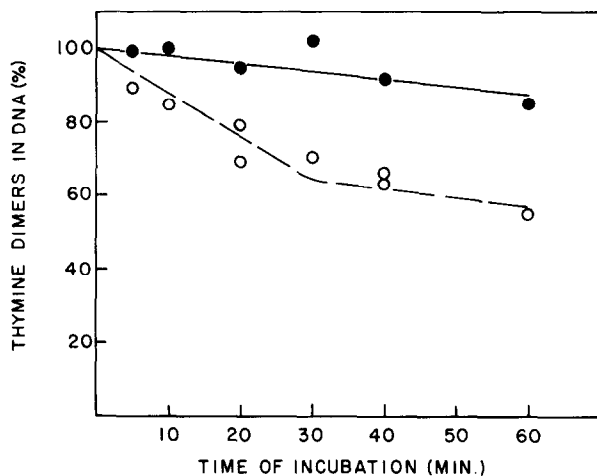
Figure 2.  Effect of addition of deoxynucleoside triphosphates on rate
of thymine dimer excision by DNA polymerase I.  1.4 pmoles of
enzyme were used.  No deoxynucleoside triphosphates ●; tri-
phosphates added O.

excess (130 µg) of T2-induced thymine dimer excision exonuclease (8), to

estimate the maximum extent of excision (80 to 90%).  The control values

were normalized to 100% and the data were corrected accordingly.

## Results

The excision of thymine dimers from incised irradiated T7 DNA by DNA

polymerase I was linear with respect to both time and enzyme concentration

(Fig. 1).  As expected from the known stimulation of 5' → 3' exonuclease ac-

tivity by concurrent polymerization (9), addition of the four deoxynucleo-

side triphosphates significantly increased the rate of thymine dimer ex-

cision (Fig. 2).

The rate of thymine dimer excision by the polymerase I small fragment

which retains only the 5' → 3' exonuclease activity of the intact enzyme,

was substantially less than that observed with the intact polymerase.  Thus,

approximately 10 times more small fragment than intact enzyme, estimated as

molarity of polymerase protein, or on the basis of 5' → 3' exonuclease ac-

tivity on the 5'- $[^{32}P](dT)_{300} \cdot (dA)_{5000}$ homopolymer pair, was required to

produce the same rate of excision.  However, addition of an equimolar
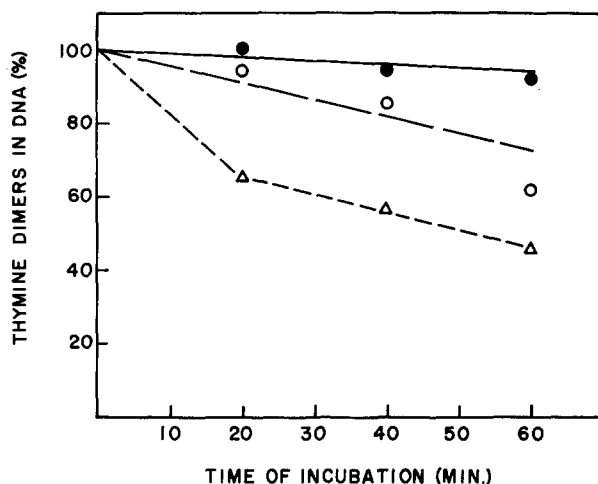
Figure 3.   Thymine dimer excision by DNA polymerase I small fragment in
            the presence and absence of large fragment and deoxynucleoside
            triphosphates.   Small fragment (7.7 pmoles) ●; small fragment
            (7.7 pmoles) plus large fragment (8.0 pmoles) 0; small fragment
            (7.7 pmoles) plus large fragment (8.0 pmoles) plus deoxynucleo-
            side triphosphates △.

amount of the polymerase I large fragment, which bears the polymerase and

3' → 5' exonuclease activities increased the rate of thymine dimer excision

by about 4-fold.   In the presence of the four deoxynucleoside triphosphates

the rate was increased even further (approximately 5-fold more) (Fig. 3).

A similar result was obtained with the 2.8S peptide containing the

polymerase I-associated 5' → 3' exonuclease partially purified from a pol A1

amber mutant.   As shown in Fig. 4, there was essentially no thymine dimer

excision by an amount of 2.8S peptide equivalent to 7 pmoles of intact

polymerase I as judged by 5' → 3' exonuclease activity on $5'-[^{32}P](dT)_{300} \cdot (dA)_{5000}$.

Addition of the large fragment resulted in a rate of excision comparable to

that obtained with approximately 3 pmoles of intact enzyme, and this rate

was further enhanced by addition of the four deoxynucleoside triphosphates.

Addition of deoxynucleoside triphosphates to either the small or large

fragment alone did not significantly affect the rate of dimer excision.

Furthermore, the differences in rates of dimer excision are not simply a re-

flection of changes in the distribution of dimers in acid-soluble and acid-

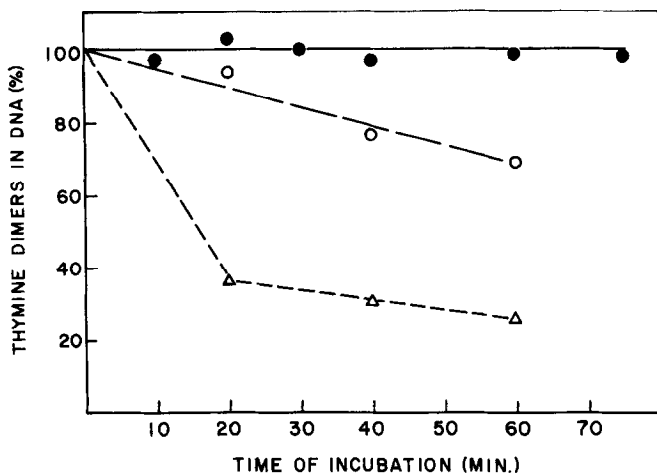insoluble oligonucleotides.   Both the intact polymerase as well as the small

Figure 4.  Thymine dimer excision by 2.8S peptide from E. coli pol A1.
2.8S peptide (equivalent in 5' → 3' exonuclease activity to
6.9 pmoles polymerase I) O; 2.8S peptide plus large fragment
(8.0 pmoles) ●; 2.8S peptide plus large fragment (8.0 pmoles)
plus deoxynucleoside triphosphates, △.

fragment excise dimers as oligonucleotides 4 to 8 residues long (4,5) and

we have found dimer - containing octanucleotides in the acid-soluble fraction

(data not shown).

## Discussion

Kelly et al. (5) have shown that E. coli DNA polymerase I can excise

thymine dimers from nicked UV-irradiated DNA and from the UV-irradiated

homopolymer pair $(dT)_{200} \cdot (dA)_{4000}$.  Setlow and Kornberg (4) further demon-

strated that the polymerase I small fragment is able to degrade the ir-

radiated homopolymer pair to yield products indistinguishable from that

formed by the intact enzyme.  We have confirmed these observations using

specifically nicked UV-irradiated DNA.  However, we have also found that the

rate of dimer excision catalyzed by the small fragment is only about one-

tenth that observed with the intact enzyme.  In contrast, there is no dif-

ference in 5' → 3' exonuclease activity when the two are compared with

$5'-[^{32}P](dT).(dA)$ as substrate.  Although addition of the polymerase I large

fragment to the small fragment does not affect significantly the 5' → 3'

exonuclease activity measured with the homopolymer pair (4), it markedly

enhances the rate of dimer excision from incised UV-irradiated DNA.  We

do not know the reason for this effect; however, one possibility is that

binding of the small fragment to a nick at or near a thymine dimer may be

weaker than to a nick without the dimer.  Addition of the large fragment may

then stabilize the binding of the small fragment to termini near the dimer.

The observation that addition of the four deoxynucleoside triphosphates

strongly stimulates thymine dimer excision by intact polymerase I, and by

the combined large and small fragments, is expected, since 5' → 3' exonu-

clease activity is markedly and specifically enhanced by concurrent DNA

synthesis (9).  This finding does, however, reinforce the idea that maximal

rates of 5' → 3' exonuclease activity, and hence dimer excision, require the

concerted action of both polymerase and exonuclease.  Thus the finding of

nearly normal levels of 5' → 3' DNA polymerase I-associated exonuclease ac-

tivity in extracts of pol A mutants (1) may not be sufficient to explain the

essentially normal rate of dimer excision observed in vivo (10).

The observation that the 2.8S peptide with 5' → 3' exonuclease ac-

tivity isolated from amber mutants of polymerase I is very similar, if not

identical, to the proteolytically generated small fragment in its ability

to interact with the large fragment, provides strong support for the notion

that this 5' → 3' exonuclease activity is related to the incomplete peptide

generated in E. coli pol A1 as a result of the amber mutant which it bears.

## Acknowledgments

## References

1.  Lehman, I.R., and Chien, J.R. (1973) J. Biol. Chem. 248, 7717-7723.

2.  Klenow, H., and Henningsen, I. (1970) Proc. Nat. Acad. Sci. U.S.A.
    65, 168-175.

3.  Setlow, P., Brutlag, D., and Kornberg, A. (1972) J. Biol. Chem. 247, 224-231.

4.  Setlow, P., and Kornberg, A. (1972) J. Biol. Chem. 247, 232-240.

5.  Kelly, R.B., Atkinson, M.R., Huberman, J.A., and Kornberg, A. (1969) Nature 224, 495-501.

6.  Friedberg, E.C., and King, J.J. (1971) J. Bact. 106, 500-507.

7.  Goldmann, K., and Friedberg, E.C. (1973) Anal. Biochem. 53, 124-131.

8.  Friedberg, E.C., Minton, K., Pawl, G., and Verzola, P. J. Virology (In Press).

9.  Kelly, R.B., Cozzarelli, N.R., Deutscher, M.P., Lehman, I.R., and Kornberg, A. (1970) J. Biol. Chem. 245, 39-45.

10. Boyle, J.M., Paterson, M.C., and Setlow, R.B. (1970) Nature. 226, 708-710.