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Terminal Cross-Linking of DNA Strands by an Enzyme System from *Escherichia coli* Infected with Bacteriophage T4*

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Abstract: An enzyme system, purified 560-fold from *Escherichia coli* infected with bacteriophage T4, catalyzes the formation of a phosphodiester bond between the original 5′-phosphoryl end-group of a DNA strand and a 3′-hydroxyl group of the complementary strand. The product, a terminally cross-linked, spontaneously renaturable DNA duplex, has been characterized by chromatographic analysis, by sedimentation analysis, and by enzymatic digestion. Essential components of the enzyme system, which requires both ATP and Mg^{++}, include the T4-induced DNA ligase and a component found in extracts of uninfected *E. coli*, which is probably an exonuclease.

Covalent joining of the complementary strands of a DNA duplex has been accomplished with a variety of agents,[1] such as ultraviolet irradiation, bifunctional alkylating agents, and nitrous acid. This cross-linking confers upon the DNA an unusual physical property; it cannot be denatured irreversibly. Although the denaturing treatment may break all the interstrand hydrogen bonds, the two complementary strands of such a duplex remain joined through their covalent cross-link, and removal of the denaturing agent results in rapid spontaneous renaturation. Small fractions of the DNA's isolated from many natural sources also have this property.[1]

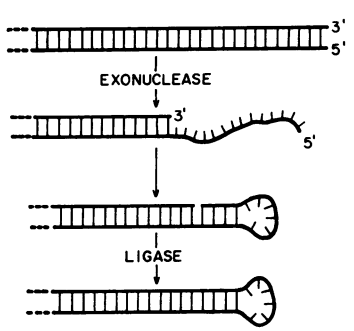


FIG. 1.—Postulated mechanism for the terminal cross-linking of DNA.

This report describes an enzyme system, isolated from *Escherichia coli* infected with bacteriophage T4, which converts duplex DNA to a spontaneously renaturable form. Evidence is presented (*1*) demonstrating that this enzyme system catalyzes the formation of terminally located phosphodiester cross-links between the complementary strands of a DNA duplex, and (*2*) suggesting a possible reaction mechanism based entirely on the actions of known enzymes. In this proposed scheme (Fig. 1), an exonuclease degrades the 3′-end of one strand until it exposes a short sequence of unpaired nucleotides which happens to be complementary to the 5′-

terminal sequence of the same strand. Then base pairing between these two regions brings the 3′- and 5′-ends together so that they can be esterified by DNA ligase. Of special interest is the finding that the phage-induced DNA ligase,[2] which alone catalyzes only *intra*-strand joining, plays an essential role in the joining of complementary strands.

Experimental Procedure. Materials: ^{3}H- and ^{32}P-T7 phage DNA's were isolated as previously described[3] from phages prepared by the method of Thomas and Abelson.[4] T4 DNA polymerase (Fraction VI)[5] and *E. coli* DNA polymerase (Fraction 7)[6] were gifts of Dr. P. T. Englund; T4 DNA ligase (Fraction VII)[7] was a gift of Dr. C. C. Richardson. Hydroxylapatite (Bio-Gel HT) was obtained from Bio-Rad Laboratories. Other unlabeled and terminally-labeled DNA preparations, enzymes, and reagents were those used previously.[3, 7] Concentrations of DNA are expressed as equivalents of DNA phosphorus.

Methods: (*a*) **Assays of cross-linking activity:** Assay A, the standard assay, measured the conversion of ^{32}P-T7 DNA to a spontaneously renaturable form which, after alkaline denaturation, can be adsorbed to hydroxylapatite like native DNA.[8] The reaction mixture (0.3 ml) contained 6.7 μM ^{32}P-T7 DNA (0.3–2.5 $\times$ 10^4 cpm/mμ mole), 0.33 mM ATP, 33 mM Tris buffer, pH 7.6, 3.3 mM $MgCl_2$, 17 mM 2-mercaptoethanol, 33 μg/ml bovine plasma albumin, and 0.05 to 1.0 $\times$ 10^{-2} unit of enzyme. After 30 min at 37°, the tube was chilled and 0.3 ml of 0.1 *M* Na_3PO_4–0.01 *M* Na_3EDTA (adjusted to pH 12.6 with 1 *N* NaOH) was added. All subsequent steps were performed at 60°. After incubation for 10 min, 2 ml of 0.1 *M* potassium phosphate buffer (pH 6.8)–1.0 *M* formaldehyde were added. After 10 min, 0.4 ml of a hydroxylapatite slurry (80 mg/ml) was added, and the mixture was resuspended three times. After 10 min of settling, the supernatant solution was aspirated and replaced by another 2 ml of the buffer-formaldehyde solution. The resuspended hydroxylapatite was collected on a paper filter (Whatman no. 1, 2.4-cm diameter) and washed with nine 2-ml portions of the hot buffer-formaldehyde solution. The filter was dried, and its radioactivity was determined by liquid scintillation counting.[3] Assay values were corrected[8] for the adsorption of denatured DNA (about 1%) and for the incomplete adsorption of native DNA (80–85%). One unit of enzyme is defined as the amount catalyzing the formation of 1 $\mu\mu$mole of phosphodiester cross-links in 30 min under the conditions of Assay A. Because the T7 DNA duplex contains 8.0 $\times$ 10^4 nucleotides,[9] one unit is equivalent to the cross-linking of 80 mμmoles of T7 DNA in 30 min. With the purified enzyme, activity was proportional to enzyme concentration in the range given above.

Assay B measured the incorporation of terminal 5′-^{32}P-phosphomonoesters of the DNA substrate into phosphodiesters which were detected by their resistance to alkaline phosphatase. Each reaction mixture (0.3 ml) contained sonicated T7 DNA (average mol wt = 2 $\times$ 10^5 daltons), labeled exclusively at its 5′-termini with ^{32}P-phosphoryl groups,[3, 9] and bearing a total of 1.25 $\mu\mu$moles of ^{32}P-phosphomonoesters (2–22 $\times$ 10^4 cpm). Except for the DNA substrate, other components of the reaction mixture were the same as in Assay A. After 30-min incubation at 37°, phosphatase-resistant ^{32}P was determined as described previously for the assay of DNA ligase.[7] Under the conditions of Assay B, 1 unit of enzyme (as defined above) catalyzed the formation of 90 to 100 $\mu\mu$moles of phosphodiester cross-links in 30 min.

(*b*) **Preparation of cross-linked DNA from uniformly ^{32}P-labeled DNA:** Seventeen mμmoles of freshly prepared ^{32}P-T7 DNA (1.7 $\times$ 10^4 cpm/mμmole) were incubated in a reaction mixture (0.75 ml) containing 0.05 unit of Fraction V plus other additions as in Assay A. After 90 min at 37°, 10 mμmoles of Na_3EDTA were added.

(*c*) **Preparation of cross-linked DNA from terminally labeled DNA:** Twenty mμmoles of sonicated T7 DNA (average mol wt = 1.8 $\times$ 10^5 daltons), terminally labeled with 5′-^{32}P-phosphomonoesters (2000 cpm), were incubated in a reaction mixture (3.0 ml) containing 0.1 unit of Fraction V plus other additions as in Assay A. After 90 min at 37°, the ATP was removed by dialysis,[3] the unreacted phosphomonoesters were removed with phosphatase, and the protein was extracted with phenol.[7]

(*d*) **Other methods:** The following methods were those previously used:[3, 7] alkaline denaturation of DNA, sonication of DNA, treatments of DNA with alkaline phosphatase and polynucleotide kinase, measurements of protein and of DNA concentration, enzymatic digestions of DNA, and identification of 3′- and 5′-mononucleotides. 3′-mononucleotides were further identified by their ability to serve as phosphate acceptors in the polynucleotide kinase reaction, as described by Richardson.[10]

(*e*) **Purification of the cross-linking system:** Unless otherwise stated, all steps were performed at 0 to 4°, and all buffers used for dialysis or for chromatography contained 1 mM Na_3EDTA, 10 mM 2-mercaptoethanol and 10% glycerol. The results of a typical preparation are summarized in Table 1.

TABLE 1. *Purification of the cross-linking system.*

Fraction	Total activity (units)	Specific activity (units/mg)
I. Extract	121	0.46
II. Phase partition	101	0.98
III. DEAE-cellulose	49	27
IV. Ammonium sulfate	17	35
V. Bio-Rex 70	2.1	250

Preparation of extracts: *E. coli* ER22 (endonuclease I^-)[11] was infected with bacteriophage T4 *am* N82, a gene 44 mutant,[12] and collected 1 hr after infection. Phages and cells were grown, extracts were prepared from 2 gm of a frozen cell paste, and cell debris was removed, all as previously described.[7] Na_3EDTA (2 mM) was added to the cell suspension prior to sonication.

Phase partition:[13] To the extract (Fraction I, 12 ml) were added 1.38 ml of a 20% solution of Dextran T 500 (Pharmacia) and 3.86 ml of a 30% solution of polyethylene glycol (Carbowax 6000), followed by 4.03 gm of NaCl which was added over a 10-min period with stirring. The mixture was stirred for 2 hr, and the phases were separated by centrifugation at 10,000 × *g* for 10 min. The clear upper phase (13 ml) was collected as Fraction II.

DEAE-cellulose chromatography: A column (2 cm^2 × 12.5 cm) was prepared of DEAE-cellulose (Cellex-D, Bio-Rad Laboratories, 0.70 mEq/gm) and washed with 1250 ml of a 0.05 *M* potassium phosphate buffer solution, pH 7.5, from which glycerol was omitted. Fraction II (104 mg of protein) was dialyzed for 2.5 hr against each of two 500-ml portions of 0.3 *M* potassium phosphate buffer, pH 7.5. The dialyzed protein solution (19 ml) was immediately diluted with 95 ml of 10 mM 2-mercaptoethanol–1 mM Na_3EDTA and applied to the column which was operated at 60 ml/hr. The column was washed with 50 ml of 0.05 *M* potassium phosphate buffer, pH 7.5, and the enzyme was eluted with 500 ml of the buffer containing a linear gradient of 0 to 0.5 *M* KCl. Fifteen-ml fractions were collected and immediately assayed at levels of 1 and 2 μl per standard assay. Of the activity applied to the column, 66% was recovered in the first 75 ml of eluate. Fractions having an activity greater than 1.0 unit/ml were pooled to give Fraction III (30.2 ml). This unstable fraction was treated with ammonium sulfate within 4 hr.

Ammonium sulfate fractionation: Solid ammonium sulfate (15.6 gm) was added gradually with stirring to Fraction III (30.2 ml). The resulting mixture, 75% saturated with ammonium sulfate, was stirred for 30 min, allowed to stand 12 hr, and then centrifuged (30,000 × *g* × 30 min). The supernatant solution was discarded. Buffer solutions that were 65 and 55% saturated with ammonium sulfate were prepared by the addition of solid ammonium sulfate (8.6 and 7.0 gm, respectively) to 10-ml portions of 50 mM potassium phosphate buffer (pH 7.5)–10 mM 2-mercaptoethanol-1 mM Na_3EDTA-10% glycerol. The precipitate formed in the 75% saturated solution was stirred for 20 min in 3 ml of the 65% saturated solution, centrifuged as before, and re-extracted with 3 ml of the 55% saturated solution. The final supernatant

solution was then dialyzed for 2 hr each against two 200-ml portions of 0.05 *M* potassium phosphate buffer, pH 6.5, to yield Fraction IV (4.2 ml), which was promptly applied to a Bio-Rex 70 column.

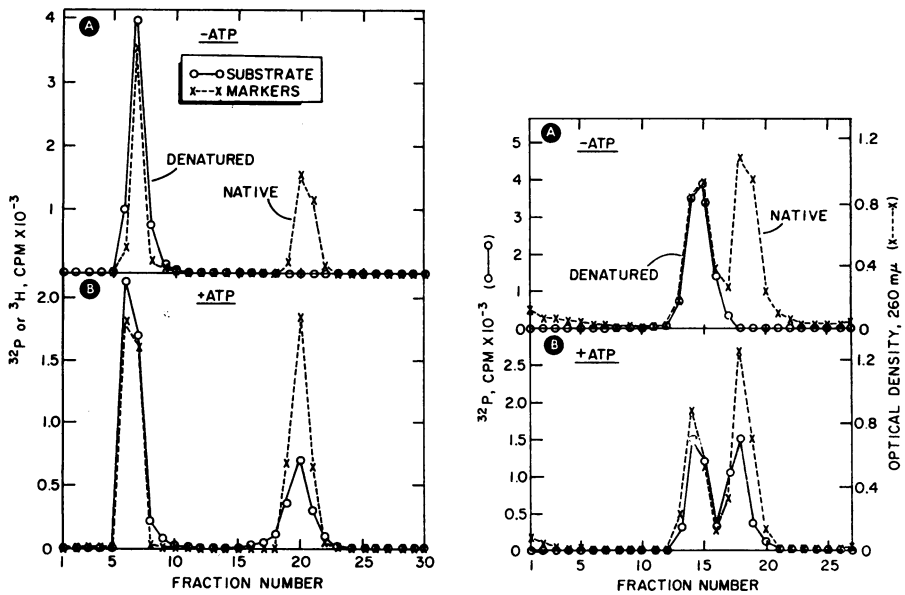
Bio-Rex 70 chromatography: A column (0.18 cm^2 × 5.5 cm) was prepared of Bio-Rex 70 (Bio-Rad Laboratories, 100–200 mesh, sodium form). It was washed first with 2 ml of 0.95 *M* KCl–0.05 *M* potassium phosphate buffer, pH 6.5, followed by 50 ml of 0.05 *M* potassium phosphate buffer, pH 6.5. Fraction IV (0.48 mg of protein) was applied to the column which was operated at 3 ml/hr. The column was then washed with 2 ml of the equilibrating buffer, followed by 18 ml more of the buffer containing a linear gradient of 0 to 0.5 *M* KCl. Fractions of 1 ml were collected. Of the activity applied to the column, 15% was eluted between four and six resin bed volumes of eluate, appearing in two fractions of equal specific activity. These fractions were pooled, and an equal volume of glycerol was added slowly with stirring to yield Fraction V (3.6 ml). Fraction V lost about 50% of its activity during 1 month of storage at −20°.

Results. Appearance of cross-linking activity after phage infection: *E. coli* B (10^9 cells/ml) was infected with T4 wild-type bacteriophage (five per cell), and cross-linking activity was measured in cell extracts by Assay A. Before infection, there was no measurable activity (less than 0.006 unit/mg of protein). At 3, 5, and 10 minutes after infection, levels of activity were 0.13, 0.23, and 0.27 unit per milligram respectively, following which there was a rapid decline to 0.07 unit per milligram at 30 minutes. This apparent decline was accompanied by an increase in nuclease activity which interfered with the assay. T7- and T4-infected cells had similar values at 20 minutes after infection (0.16 unit/mg).

Properties of the purified enzyme preparation: The optimal pH range for the reaction is 6.8–7.6 in Tris-HCl buffer. Fraction V requires both ATP and Mg^{++}; omission of either from a standard reaction mixture containing 2.5 × 10^{-3} unit of Fraction V resulted in greater than a 95 per cent decrease in activity. Omission of 2-mercaptoethanol caused a 25 per cent decrease in activity. The reactions reached an apparent limit when 39–42 per cent of the DNA became cross-linked; addition of more enzyme or ATP at this point led to no further reaction.

Spontaneous renaturability of the DNA product: T7 DNA uniformly labeled with ^{32}P was incubated with the enzyme system until 40 per cent was cross-linked (Assay A). The DNA was then denatured with alkali, neutralized, and studied by hydroxylapatite chromatography (Fig. 2) and by equilibrium centrifugation in neutral CsCl density gradients (Fig. 3). In each case (Figs. 2*B* and 3*B*), a large fraction of the DNA still behaved like native T7 DNA. However, after control incubations from which ATP was omitted (Figs. 2*A* and 3*A*), the DNA was completely denaturable. Similar studies performed without prior denaturing treatments showed the product to be indistinguishable from native DNA.

Sedimentation properties of the DNA product: When the above incubation mixtures were analyzed in neutral sucrose gradients,[7] without prior denaturation, no alterations of sedimentation rate were seen, indicating that there was no intermolecular joining of DNA duplexes. Under denaturing conditions, however, a covalently cross-linked DNA molecule should have a molecular weight twice that of the single strands of the untreated substrate. This property was



(*Left*) FIG. 2.—Spontaneous renaturability of the reaction product as demonstrated by hydroxylapatite chromatography. ^{32}P-labeled T7 DNA (o——o) was incubated with Fraction V in the absence of ATP (*A*), or in the presence of ATP (*B*). Following alkali denaturation and neutralization, the DNA's were adsorbed to and eluted from hydroxylapatite columns, together with native and denatured ^{3}H-T7 DNA markers (x- - -x).

Each incubation was performed as described in *Methods*. A sample containing 1.7 mμmoles of ^{32}P-labeled DNA was denatured and neutralized as for Assay A. ^{3}H-T7 DNA (24 mμmoles, 8,500 cpm) was added before denaturation and again after neutralization. The final mixture was diluted fivefold with 0.3 *M* formaldehyde and sonicated. A column (0.18 cm^2 × 5.5 cm) was prepared of hydroxylapatite and equilibrated with 10 ml of 0.02 *M* potassium phosphate buffer (pH 6.8)–0.3 *M* formaldehyde. The DNA solution was then applied to the column which was operated at 25° and at 5 ml/hr. The column was washed with 2.0 ml of the equilibrating buffer, and the DNA was eluted with 18 ml of 0.3 *M* formaldehyde containing a linear gradient of 0.02–0.50 *M* potassium phosphate buffer, pH 6.8. Fractions of 0.33 ml were collected, and recoveries of ^{3}H- and ^{32}P-labeled DNA's were 66–75%.

(*Right*) FIG. 3.—Spontaneous renaturability of the reaction product as demonstrated by equilibrium sedimentation in CsCl density gradients. ^{32}P-labeled T7 DNA (o——o) was incubated with the cross-linking system (Fraction V) in the absence of ATP (*A*), or in the presence of ATP (*B*). Following alkali denaturation, the DNA's were sedimented at pH 8.0 together with native and denatured T7 DNA markers (x- - -x).

Each incubation was performed as described in *Methods*. A sample containing 1.7 mμmoles of ^{32}P-labeled DNA was mixed with 80 mμmoles of unlabeled T7 DNA, and the mixture was denatured with alkali and neutralized (see Methods). Unlabeled native T7 DNA (160 mμmoles) was then added and the mixture added to a solution (final volume, 8.0 ml) containing 0.5 mM Na_3EDTA, 50 mM Tris-HCl buffer, pH 8.0, and CsCl (final refractive index $n_D^{25°}$ = 1.4002). The mixture was placed in a 12.5-ml cellulose nitrate tube, covered with mineral oil, and centrifuged in the no. 40 rotor of the Spinco model L ultracentrifuge at 33K rpm for 60 hr at 3 to 4°. Fractions of 0.3 ml were collected by puncturing the bottom of each tube. The recovery of denatured DNA was 65–70% and that for bihelical DNA was 70–75%, as determined both by radioactivity and optical density measurements.

demonstrated by sedimentation analysis in alkaline sucrose density gradients (Fig. 4). The spontaneously renaturable product (Fig. 4*C*, *shaded area*) sedimented 1.28 times as fast as the untreated ^{3}H-T7 DNA single strands (marker) or unreacted substrate DNA, corresponding to a molecular weight 1.9 times as

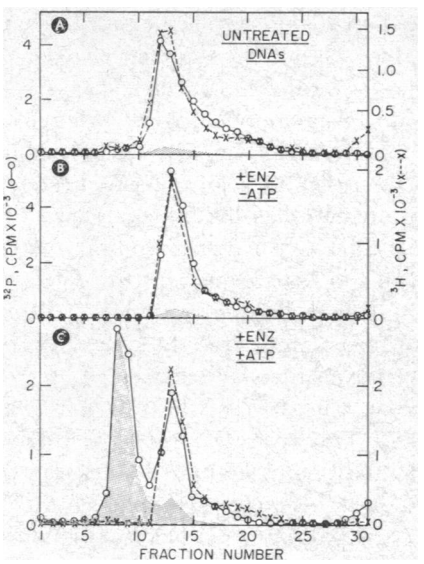


FIG. 4.—Analysis of the DNA product of the cross-linking reaction by sedimentation in alkaline sucrose density gradients. ^{3}H-T7 marker DNA (×---×) was sedimented with ^{32}P-T7 substrate DNA (○——○) which had either received no prior enzymatic treatment (*A*), or had been incubated with the cross-linking system (Fraction V) in the absence of ATP (*B*), or with the complete system including ATP (*C*). The shaded area indicates ^{32}P-DNA adsorbed to hydroxylapatite after neutralization.

To 0.1 ml of each incubation mixture (1.7 mμmoles of ^{32}P-DNA; see *Methods*) were added 0.02 ml of a ^{3}H-T7 phage preparation (12,000 cpm), followed by 0.015 ml of 1.0 *N* NaOH. After 10 min at room temperature, each mixture was chilled and layered on a linear density gradient containing 4.6 ml of 5 to 20% sucrose (w/v) in 0.1 *N* NaOH–0.9 *M* NaCl–1.0 mM Na_2EDTA. Centrifugation was performed in the SW 50 rotor of the Spinco model L ultracentrifuge at 49K rpm for 2.5 hr at 4°. The bottom of each tube was then punctured, and fractions (0.15 ml) were collected into 0.35 ml of water. Radioactivity was measured in 0.2 ml samples; recoveries were 75–80%. The remainder of each fraction was assayed for spontaneously renaturable DNA, as in Assay A. Values were corrected for 80% adsorption of native DNA to hydroxylapatite (see *Methods*).

great.[14] Although the rapidly sedimenting peak might contain molecules cross-linked at both ends as well as those cross-linked at only one end, no further attempts were made in the present study to resolve these components.

When ATP was omitted from a control incubation, there was no detectable change in the sedimentation rate (Fig. 4*B*). This result also demonstrates that Fraction V is free of endonucleases that can attack double-stranded DNA.

Requirement for 5′-phosphoryl end-groups: Treatment of T7 DNA with alkaline phosphatase to remove its terminal 5′-phosphomonoesters[3] destroyed 90 per cent of its activity (Assay A with 2×10^{-3} unit of enzyme); subsequent phosphorylation with polynucleotide kinase and ATP restored greater than 95 per cent of this activity. After sonication of a 12 per cent cross-linked DNA preparation under conditions that should shear each duplex into 80 to 120 fragments, less than 1 per cent of the fragments bore cross-links; therefore, there could have been no more than a few cross-links per original intact duplex. When the DNA substrate was sonicated before cross-linking, however, 14 per cent of the fragments became cross-linked and were not measurably affected by subsequent sonication. Therefore, sonication, which is known to produce new 5′-phosphoryl and 3′-hydroxyl end groups,[9] also produces new sites for cross-linking.

Formation of 3′,5′-phosphodiester bonds during cross-linking: Sonicated T7 DNA labeled specifically with 5′-^{32}P-phosphoryl end groups was incubated with Fraction V, and the reaction was followed by Assays A and B. Cross-linking was paralleled by the conversion of ^{32}P into a phosphatase-resistant form. A sample of the 40 per cent cross-linked DNA product, containing only phosphatase-resistant ^{32}P (see *Methods*), was hydrolyzed with pancreatic DNase

and snake venom phosphodiesterase. More than 95 per cent of the radioactivity was recovered in nucleoside 5′-monophosphates. When the sample was digested by micrococcal nuclease and spleen phosphodiesterase, more than 95 per cent of the radioactivity was found in nucleoside 3′-monophosphates. Therefore, during the cross-linking reaction, phosphatase-sensitive 5′-terminal phosphomonoesters are converted into phosphatase-resistant 3′,5′-phosphodiesters.

T4 DNA ligase is an essential component of the cross-linking system: The data in Table 2 demonstrate the following: (1) The purified cross-linking system contains a large amount of DNA ligase; (2) T4 *ts* A80, a temperature-sensitive ligase mutant,[15] induces a temperature-sensitive cross-linking system; (3) T4 *am* H39X, also a ligase mutant,[15] produces no measurable cross-linking activity; and (4) although purified T4 DNA ligase and extracts of uninfected *E. coli* each have no measurable cross-linking activity, a mixture of the two does have cross-linking activity. Therefore, the cross-linking system requires T4 DNA ligase plus at least one other component that can be supplied by *E. coli* extracts.

Possible role of an exonuclease: Several observations are compatible with a requirement for an exonuclease in terminal cross-linking: (1) DNA polymerase

TABLE 2. *Requirement for DNA ligase in terminal cross-linking.*

Enzyme source	Ligase activity ($\mu\mu$moles/30 min/mg protein)	Cross-linking activity ($\mu\mu$moles/30 min/mg protein)
Purified preparations:		
T4 DNA ligase	390×10^3	<5
Cross-linking system (Fraction V)	80×10^3	4600
Phage-infected *E. coli:*		
T4 *am* N82 (gene 44), control	540	42
T4 *am* H39X (gene 30)	10	<2
T4 *ts* A80 (gene 30)		
Assayed at 25°	53	7.3
Assayed at 37°	<5	<0.5
Uninfected *E. coli:*		
Minus T4 DNA ligase	<10	<2
Plus T4 DNA ligase, 8.1×10^{-3} unit	810	42

Cross-linking activity (Assay B) and ATP-dependent ligase activity are expressed as rates of phosphodiester bond formation. Ligase activity was determined as previously described,[7] but with a DNA substrate containing only internally located ^{32}P-phosphomonoesters.[3] Cells were infected with five phages per cell, and extracts were prepared by sonication, as described by Fareed and Richardson.[15] T4 *am* N82-infected cells were those used for the purification of the cross-linking system.

TABLE 3. *Inhibition of cross-linking by DNA polymerases.*

Additions	Relative cross-linking activity (%)
None	100
T4 DNA polymerase	91
Four triphosphates	74
T4 DNA polymerase + four triphosphates	<1
E. coli DNA polymerase	37
E. coli DNA polymerase + four triphosphates	<1

Cross-linking activity was determined by Assay B. Reaction mixtures (0.3 ml) contained 0.1 μmoles of KF,[5] 5×10^{-3} unit of Fraction V, plus additions: T4 DNA polymerase (0.9 unit), *E. coli* DNA polymerase (0.7 unit), or four triphosphates (20 mμmoles each of dATP, dGTP, dCTP, and dTTP). Other components were those used in Assay B. Fifteen per cent of the ^{32}P radioactivity of the substrate was released by the exonuclease activity of *E. coli* DNA polymerase.

activity, which shoul reversed any 3′-exonucleolytic degradation, also inhibits terminal cross-linking (Table 3); (2) when 0.01 unit of Fraction V is incubated in Assay A without ATP, 5 per cent of the DNA becomes acid soluble; in the presence of ATP, only 2 per cent becomes acid soluble; and (3) the glucosylated DNA of bacteriophage T4, which is resistant to many nucleases,[16] is only 10 per cent as active as T7 DNA in the terminal cross-linking reaction.

Discussion. The biological role of terminal cross-linking is uncertain, especially when we consider that T4 DNA is a poor substrate and that the reaction is blocked by polymerase activity. Nevertheless, this *intra*molecular reaction may be used as a model for *inter*molecular reactions occurring during genetic recombination. Postulated mechanisms for these latter reactions also involve nucleolytic scission, the baring of single-stranded complementary regions, and base pairing as a prelude to covalent joining by a ligase.[2] Similar mechanisms, involving terminal *intra*strand base pairing, have been proposed to explain the actions of T4 DNA polymerase[5] and of *E. coli* exonuclease III[17] on single-stranded DNA's and to explain the circularization of ϕX174 DNA.[18] Furthermore, both the T4 polymerase product[5] and naturally occurring cross-linked DNA's[1] are thought to be terminally cross-linked.

Enzymatically cross-linked DNA may also prove to be of value as an assay substrate for enzymes that break or join DNA molecules, in the same way that chemically cross-linked DNA's have been used.[2, 19] The enzymatic product has the possible advantages of possessing fewer single-strand breaks and of resembling unmodified DNA more closely.

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† Lederle Medical Faculty awardee.

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