Proc. Natl. Acad. Sci. USA
Vol. 78, No. 5, pp. 2678–2682, May 1981
Biochemistry

Formation of a covalent complex between the 80,000-dalton adenovirus terminal protein and 5′-dCMP *in vitro*

(adenoviral DNA replication/5′-terminal protein/protein–nucleic acid complex)

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ABSTRACT **An *in vitro* adenovirus DNA replication system catalyzed the formation of a covalent complex between an 80,000-dalton protein and 5′-dCMP in the presence of [α-^{32}P]dCTP, $MgCl_2$, ATP, and adenovirus (Ad) DNA with a protein covalently bound to the 5′ end of each strand (Ad DNA-prot). The requirement for Ad DNA-prot in this reaction was similar to that for *in vitro* DNA replication. When dATP, dTTP, and the 2′,3′-dideoxynucleoside triphosphate (ddNTP) ddGTP were included in the reaction mixture, an elongated complex was detected, which consisted of an 80,000-dalton protein bound to a 26-base oligonucleotide. Formation of the elongated product, but not of the protein-dCMP complex, was inhibited by ddATP, ddCTP, or ddTTP. The requirements for formation of the protein-dCMP complex, the nature of the linkage between protein and dCMP, the size of the protein, and the existence of elongated forms indicated that the protein associated with the complex was identical to the 80,000-dalton Ad terminal protein found on replicating DNA molecules as described by Challberg *et al.* [Challberg, M. D., Desiderio, S. V. & Kelly, T. J., Jr. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5105–5109].**

Adenovirus (Ad) DNA is a linear, double-stranded DNA molecule with a protein (terminal protein) covalently bound to the 5′ terminus of each strand (Ad DNA-prot) (1–3). The terminal protein is a viral gene product (4) synthesized as an 80,000- to 87,000-dalton precursor (5), which is processed to yield the 55,000-dalton protein found on the termini of DNA isolated from Ad virions. Both *in vivo* (6, 7) and *in vitro* studies (8–10) indicate that protein is bound to progeny DNA molecules early in the replication process. However, the question of exactly when protein becomes attached has not been resolved because of the lack of a method for detecting progeny molecules less than several hundred nucleotides in length.

We have used a modification (10–12) of the *in vitro* Ad DNA replication system described by Challberg and Kelly (13, 14) to study the binding of protein to nascent strands of DNA. In this communication we show that the *in vitro* system, in the presence of dCTP, catalyzes the formation of a covalent complex between an 80,000-dalton protein and 5′-dCMP, the 5′-terminal nucleotide of both strands of Ad DNA. Upon addition of dCTP, dATP, dTTP, and the 2′,3′-dideoxynucleoside triphosphate (ddNTP) ddGTP, a complex is formed between an 80,000-dalton protein and an oligonucleotide corresponding to the 5′-termini of Ad DNA. Our results support the model first proposed by Rekosh *et al.* (15) in which terminal protein serves as a primer for Ad DNA replication.

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MATERIALS AND METHODS

The growth of HeLa S3 cells, infection with Ad2, and preparation of [^{14}C]thymidine-labeled Ad DNA-prot and Ad2-deproteinized DNA were as described (12).

Preparation of Extracts. HeLa cell nuclei and crude cytoplasmic extracts were prepared as described (13). For the preparation of crude nuclear extract, nuclei were washed twice in 10 mM Tris·HCl, pH 7.5/10% (wt/vol) sucrose and extracted with 0.3 M NaCl in the same buffer. For some experiments, the crude extracts were passed through DEAE-cellulose as described (8) to remove nucleic acids.

Conditions for Forming Protein-dCMP Complexes. Reaction mixtures (0.05 ml) contained 25 mM Hepes (pH 7.5), 5 mM $MgCl_2$, 2 mM dithiothreitol, 3 mM ATP, 0.15 μg of Ad DNA-prot, cytoplasmic extract (70 μg of protein), nuclear extract (15 μg of protein) and 0.5 μM [α-^{32}P]dCTP (200–800 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels). Except where noted, reactions contained nuclear extract from uninfected cells and cytoplasmic extract from Ad2-infected cells, both passed through DEAE-cellulose as described (8). After incubation for 60 min at 30°C, reactions were stopped by the addition of 50 μl of 0.18 M sodium pyrophosphate/0.05 M EDTA and 25 μl of 50% (wt/vol) trichloroacetic acid. The mixture was centrifuged for 10 min, and the pellet was washed with ether, dissolved in 35 μl of sample buffer [0.1 M Tris·HCl, pH 6.8/2% (wt/vol) $NaDodSO_4$/20% (vol/vol) glycerol/0.005% bromphenol blue/10% (vol/vol) 2-mercaptoethanol], and heated at 95°C for 5 min. The sample was applied to a 0.1% $NaDodSO_4$/10% (wt/vol) polyacrylamide gel with a 1-cm spacer gel. Electrophoresis was carried out at 120 V until the marker dye reached the bottom of the gel. Radioautography was for at least 17 hr at 4°C with Kodak XR-5 film.

RESULTS

Formation of a Protein–Nucleic Acid Complex. The requirements for the formation of an 80,000-dalton complex are shown in Fig. 1. Incubation of cytoplasmic extract and nuclear extract from Ad-infected HeLa cells in the presence of Ad DNA-prot, adenovirus-coded DNA binding protein (Ad DBP), ATP, ddATP, $MgCl_2$, and [α-^{32}P]dCTP resulted in formation of a ^{32}P-labeled complex. No complex was detected when Ad DNA-prot, $MgCl_2$, or either one of the protein extracts was omitted from the reaction. The omission of Ad DBP, a protein which stimulated *in vitro* DNA synthesis 2-fold (data not shown), or

Abbreviations: Ad, adenovirus; Ad DNA-prot, adenovirus DNA with a protein covalently bound to the 5′ end of each strand; Ad DBP, adenovirus-coded DNA binding protein; ddNTP, 2′,3′-dideoxynucleoside triphosphate.

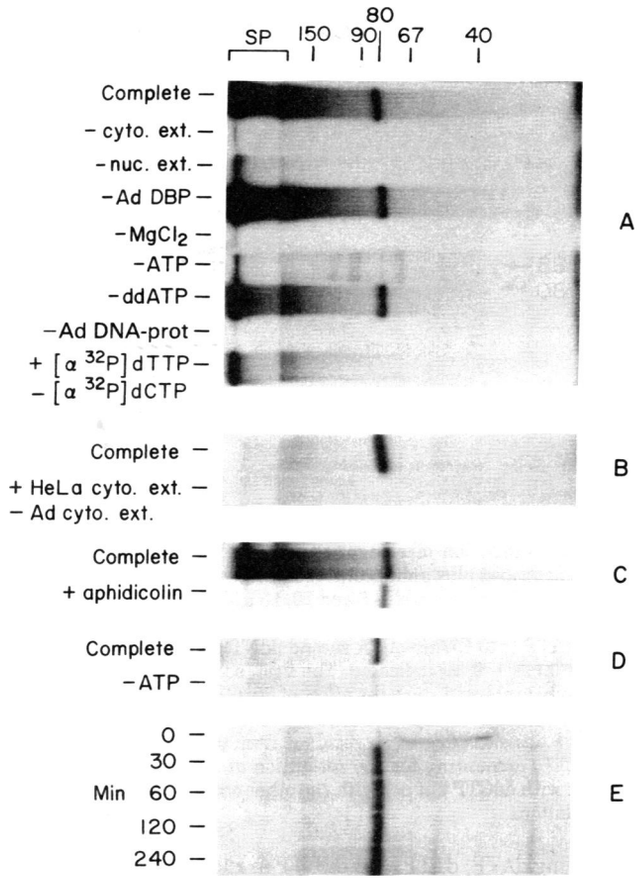

FIG. 1. Requirements for the formation of an 80,000-dalton complex. Crude extracts were used in *A*, *B*, and *C* and DEAE-cellulose fractions were used in *D* and *E*. In all cases, electrophoretic migration was from left to right. SP, spacer gel. The standards used were *Escherichia coli* RNA polymerase (150,000, 90,000, and 40,000 daltons) and bovine serum albumin (67,000 daltons), shown in kilodaltons. (*A*) The complete system contained Ad DBP (2 μg), ddATP (40 μM), and crude nuclear extract (nuc. ext.) and cytoplasmic extract (cyto. ext.) from Ad2-infected HeLa cells. Incubation was for 120 min at 30°C. (*B*) The complete reaction contained aphidicolin (100 μM), crude nuclear extract from uninfected cells, and crude cytoplasmic extract from Ad2-infected cells. HeLa cyto. ext., crude cytoplasmic extract prepared from uninfected HeLa cells. (*C*) As described in *A*. Aphidicolin was added at a concentration of 100 μM. (*D*) DEAE-cellulose fractions of cytoplasmic extract from Ad2-infected cells and nuclear extract from uninfected cells. (*E*) As described in *D*. The reaction was stopped at the time indicated by addition of 50 μl of 0.18 M sodium pyrophosphate/0.05 M EDTA. Regions of the polyacrylamide gel containing the 80,000-dalton complex were counted for Cerenkov radiation.

ddATP had no effect on the amount of complex formed (Fig. 1*A*). The omission of ATP resulted in a reduction by a factor of 2–5 in the amount of complex formed and in the appearance of one or more labeled bands not observed in the presence of ATP (Fig. 1, *A* and *D*). No complex was detected when [α-^{32}P]dTTP (Fig. 1*A*), dATP, dGTP, CTP, or UTP (data not shown) was used in place of [α-^{32}P]dCTP. Nuclear extract from uninfected cells in combination with cytoplasmic extract from Ad-infected cells supported formation of the complex, but when both nuclear and cytoplasmic extracts were from uninfected cells, no complex was observed (Fig. 1*B*).

The addition of aphidicolin (100 μM), a specific inhibitor of DNA polymerase α and of *in vitro* Ad DNA synthesis (16), did not prevent complex formation but reduced the amount of ^{32}P-labeled material migrating near the top of the gel (Fig. 1*C*). This material was also reduced by removing nucleic acids from the crude extracts by passage through a DEAE-cellulose column (Fig. 1*D*; compare to *A*). The addition of aphidicolin or the use of DEAE-cellulose fractions reduced the ^{32}P background sufficiently to permit an estimate of the amount of product formed by counting regions of gel containing the 80,000-dalton band. In a reaction containing the DEAE-cellulose fractions (Fig. 1*E*), 0, 0.67, 0.93, and 1.4 fmol of ^{32}P were incorporated into the 80,000-dalton band after 0, 30, 60, and 240 min of incubation, respectively.

Nature of the DNA Requirement for Complex Formation. Deproteinized Ad DNA did not support formation of the complex nor did Ad DNA-prot after treatment with micrococcal nuclease or with a mixture of pancreatic DNase and snake venom phosphodiesterase (Fig. 2). Therefore, both the protein and the DNA components of the Ad genome are necessary for formation of the complex. Ad DNA-prot heated at 65°C for 30 min, digested with the restriction enzyme *Xba* I, or treated with *N*-ethylmaleimide supported complex formation. Treatment with acid (0.1 M HCl; 60 min at 37°C), alkali (0.2 M NaOH; 60 min at 37°C), or heat (5 min at 100°C) resulted in loss of the ability to support complex formation. The DNA requirement for complex formation was similar to that for *in vitro* DNA replication (ref. 12; unpublished observations). The requirements for formation of the 80,000-dalton complex suggested that the complex might consist of the 80,000-dalton terminal protein bound to dCMP. This structure was confirmed as described below.

Formation of Elongated Protein–Nucleic Acid Complexes. The sequence of Ad2 DNA indicates that the first 25 nucleotides

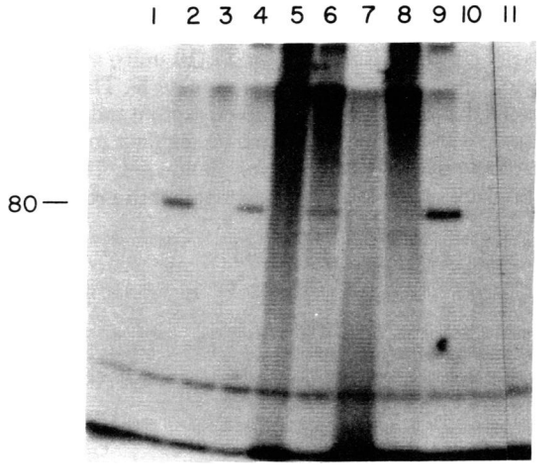

FIG. 2. The effect of various treatments of Ad DNA-prot on its ability to support formation of an 80,000-dalton complex (shown in kilodaltons). Ad DNA-prot was treated and assayed for its ability to support complex formation in reaction mixtures supplemented with aphidicolin (100 μM). Lanes: 1, products of a control reaction lacking Ad DNA-prot; 2, Ad DNA-prot, untreated; 3, Ad DNA deproteinized as described (12); 4, Ad DNA-prot heated at 65°C for 30 min; 5, Ad DNA-prot heated at 100°C for 5 min; 6, Ad DNA-prot digested with *Xba* I for 60 min at 37°C in the presence of 40 mM Hepes, pH 7.5/4 mM $MgCl_2$/8 mM NaCl; 7, Ad DNA-prot exposed to 0.2 M HCl for 60 min at 37°C, neutralized with 1 M NaOH, and dialyzed against 10 mM Tris·HCl, pH 7.5/10 mM NaCl/1 mM EDTA; 8, Ad DNA-prot exposed to 0.1 M NaOH for 60 min at 37°C, neutralized with 1 M HCl, and dialyzed as in lane 7; 9, Ad DNA-prot exposed to 5 mM *N*-ethylmaleimide for 5 min at 0°C, followed by addition of dithiothreitol to 10 mM; 10, Ad DNA-prot digested with micrococcal nuclease (20 units/ml) for 60 min at 37°C in the presence of 2 mM $CaCl_2$/40 mM Hepes, pH 7.5, followed by the addition of ethylene glycol bis(β-aminoethyl ether)-*N*-*N*-*N'*-*N'*-tetraacetic acid to 20 mM; 11, Ad DNA-prot digested with pancreatic DNase (50 μg/ml) plus snake venom phosphodiesterase (50 μg/ml) for 60 min at 37°C, followed by incubation at 65°C for 10 min.

from the 5'-terminus contain no guanine residues (17, 18). Therefore, according to the proposed model of Ad DNA replication, chain termination by ddGTP should result in the formation of a polydeoxyoligonucleotide 26 nucleotides in length bound to an 80,000-dalton protein. The formation of this product is shown in Fig. 3. When [α-^{32}P]dCTP, dATP, dTTP, and ddGTP were added to the reaction mixture, the ^{32}P-labeled 80,000-dalton band was detected together with a new band of 88,000-daltons. No 88,000-dalton band was detected when ddGTP was omitted, but a large amount of radioactivity remained near the top of the gel (Fig. 3*A*), presumably due to extensive elongation of DNA chains in the absence of the chain terminator. Formation of the 88,000-dalton band was not inhibited by 100 μM aphidicolin, a concentration that diminished the rate of *in vitro* Ad DNA synthesis by 90% (Fig. 3*B*).

The formation of the 88,000-dalton complex was inhibited by the addition of a second ddNTP to reactions containing dATP, dTTP, dCTP, and ddGTP (Fig. 4). When the second addition was ddCTP, a product intermediate in size between 80,000 and 88,000 daltons appeared, presumably due to chain termination by ddCTP. In these reactions, the second ddNTP had no effect on the formation of the 80,000-dalton product. These results suggest that ddNTPs can efficiently terminate nascent DNA chains before elongation to the 26th nucleotide and also that elongation is not necessary for the formation of an 80,000-dalton product.

Structure of the Reaction Products. The susceptibility of the reaction products to various degradative enzymes was examined (Fig. 5*A*). The 80,000-dalton complex formed in the presence of [α-^{32}P]dCTP, without the addition of other nucleotides, was unaffected by treatment with pancreatic DNase, micrococcal nuclease, snake venom phosphodiesterase, or a mixture of RNase A (10 μg/ml) and RNase T1 (10 units/ml) but was not detected after treatment with proteinase K. These properties indicated that the complex contained protein and suggested that the nucleic acid moiety was small enough not to effect the mobility of the complex in a polyacrylamide gel. The 80,000- and 88,000-dalton complexes, formed together in a reaction mixture

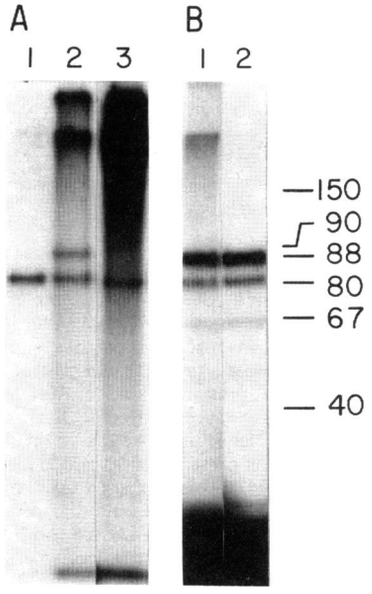

FIG. 3. Requirements for formation of elongated protein–nucleic acid complexes. Standards shown in kilodaltons. (*A*) Lanes: 1, complete reaction mixture; 2, complete reaction mixture plus dATP, dTTP, and ddGTP (20 μM each); 3, complete reaction mixture plus dATP and dTTP (20 μM each). (*B*) Lanes: 1, as in *A*, lane 2; 2, as in lane 1 plus aphidicolin (100 μM).

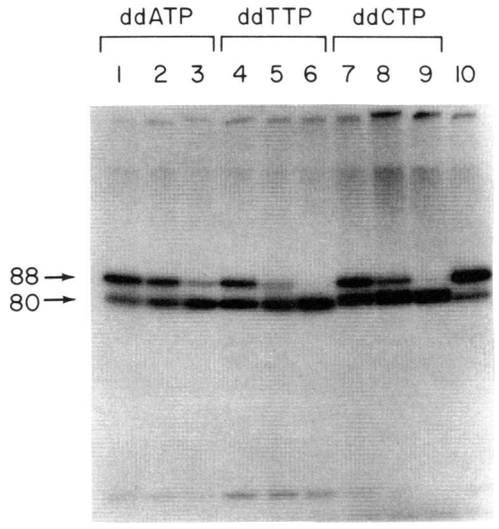

FIG. 4. Inhibition of elongation by ddNTPs. Reaction mixtures were as described plus aphidicolin (100 μM), ddGTP (40 μM), and the following additions. Lanes: 1–6 and 10, 10 μM each of dATP and dTTP and 1 μM [α-^{32}P]dCTP; 7–9, 10 μM each of dTTP and dCTP and 1 μM [α-^{32}P]dATP (410 Ci/mmol). A second ddNTP was added to the reactions in lanes 1–9, as indicated. The first, second, and third lanes in each group bracketed show the effect of 20, 60, and 200 μM of the second ddNTP, respectively. The sequence of the 5'-termini of Ad2 indicates the absence of cytosine residues from positions 7–16 and from 19–26 (17), accounting for the resolution of an intermediate length product with ddCTP but not with the other ddNTPs. Bands are shown in kilodaltons.

containing dATP, dTTP, and ddGTP in addition to [α-^{32}P]dCTP, were partially degraded by a mixture of RNase A and RNase T1 or by snake venom phosphodiesterase (presumably due to contamination of the enzyme preparations with proteases because other faster migrating bands were generated by these treatments), but neither band was detected after treatment with proteinase K. After digestion with micrococcal nuclease, the 88,000-dalton band was not detected, but the 80,000-dalton band formed in the same reaction was concomitantly intensified. Pancreatic DNase treatment resulted in the partial degradation of the 88,000-dalton band, with a region of heterogeneous material appearing between the two bands. These properties showed that the 88,000-dalton product contained protein and enough nucleic acid to alter its mobility on a polyacrylamide gel.

To determine the size of the protein moiety of the 88,000-dalton band, the complex was recovered from the gel by electroelution, digested with micrococcal nuclease, and electrophoresed on a second gel (Fig. 5*B*). Digestion with micrococcal nuclease converted the 88,000-dalton product into an 80,000-dalton band. When the 80,000- and micrococcal nuclease-treated 88,000-dalton complexes were subjected to partial proteolysis with trypsin or chymotrypsin, identical protein fragments were formed (data not shown), supporting the conclusion that the same protein is associated with the two complexes.

The 80,000-dalton complex was isolated from the acrylamide gel by electroelution (Fig. 6*A*). Exposure of the isolated complex to alkali released material migrating as dCMP and P_i, which accounted for 72% and 28%, respectively, of the radioactivity applied to the polyethyleneimine-cellulose plate (Fig. 6*A*, lanes 2 and 3). Acid did not release ^{32}P from the complex (Fig. 6*A*, lane 4). The major product released by alkali comigrated with 5'-dCMP in chromatographic systems containing borate (not shown), distinguishing the ribo- from the deoxynucleotide. The radioactive product released by alkali was adsorbed to Norit,

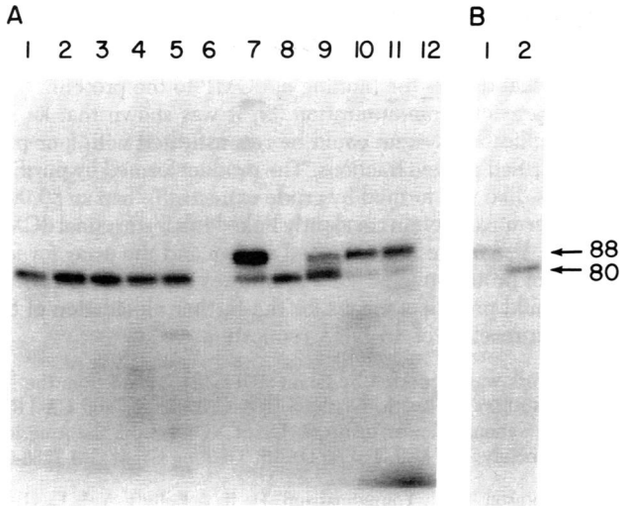

FIG. 5. Effect of various degradative enzymes on the reaction products. Bands are shown in kilodaltons. (*A*) Lanes 1–6 contained aliquots of a reaction mixture (0.2 ml) in which the 80,000-dalton complex was formed in the presence of aphidicolin (100 μM); lanes 7–12, contained aliquots of a reaction mixture (0.2 ml) in which the 80,000- and 88,000-dalton complexes were formed together with the addition of dATP, dTTP, ddGTP (40 μM each), and aphidicolin (100 μM). The reactions were stopped by heating at 65°C for 15 min. Aliquots (25 μl) were removed and treated as follows. Lanes: 1 and 7, no treatment; 2 and 8, micrococcal nuclease (20 units/ml) and 2 mM $CaCl_2$; 3 and 9, pancreatic DNase (20 μg/ml); 4 and 10, RNase A (10 μg/ml) and RNase T1 (50 units/ml); 5 and 11, snake venom phosphodiesterase (50 μg/ml); 6 and 12, proteinase K (0.4 mg/ml) and $NaDodSO_4$ (0.1%). After 60 min at 30°C, 50 μg of bovine serum albumin were added to each reaction as carrier. The samples were precipitated with trichloroacetic acid and prepared for electrophoresis. (*B*) The 80,000- and 88,000-dalton complexes were formed in a single reaction mixture as described in *A*. More than 90% of the 88,000-dalton product was recovered by electroelution for 4 hr at 200V into 25 mM Tris base/0.2 M glycine/0.1% $NaDodSO_4$/0.1 mg of bovine serum albumin per ml. The eluted material was precipitated with trichloroacetic acid and resuspended in 10 mM Tris·HCl, pH 7.5/1 mM EDTA/0.1% Triton X-100. An aliquot (2 μl) was subjected to electrophoresis with no further treatments (lane 1). A second aliquot (20 μl) was digested with micrococcal nuclease (20 units/ml) in the presence of 2 mM $CaCl_2$ for 60 min at 30°C before electrophoresis (lane 2).

but after treatment with bacterial alkaline phosphatase, crude extract of snake venom, or 5′-nucleotidase, less than 3% of the radioactivity was adsorbed to Norit (Table 1). The data show that the 80,000-dalton complex formed in the absence of ddNTPs and in the presence of 100 μM aphidicolin consists of protein covalently linked to 5′-dCMP.

When the products formed in the presence of dATP, dTTP, ddGTP, and [α-^{32}P]dCTP were isolated by electroelution from a polyacrylamide gel and treated with alkali, the major ^{32}P-labeled product migrated as a 26-base oligonucleotide on a second polyacrylamide gel (Fig. 6*B*). This result confirms the structure of the 88,000-dalton band. Both the length of the oligonucleotide and the observation that micrococcal nuclease rendered 83% of the ^{32}P associated with the complex acid soluble [presumably cleaving off four of the five dCMP residues in the terminal 26 nucleotides (17, 18)] suggest that its sequence is that of the 5′-termini of Ad DNA.

DISCUSSION

An *in vitro* Ad DNA replication system catalyzed the formation of a complex between an 80,000-dalton protein and 5′-dCMP. Formation of the complex required cytoplasmic extract from Ad-infected cells, nuclear extract from either infected or uninfected cells, Ad DNA-prot, $MgCl_2$, and dCTP. ATP stimulated the reaction 2–5 fold. No complex was formed when dCTP was replaced with either dATP, dTTP, dGTP, CTP, or UTP. The linkage between the protein and dCMP was alkali labile and acid stable, as is the phosphodiester bond between the terminal protein of Ad2 and the 5′-terminal dCMP residue of the DNA (3, 5, 19). The size of the complex, the requirements for its formation, and the nature of the linkage to dCMP indicate that the 80,000-dalton protein is identical to the terminal protein found on Ad DNA synthesized *in vitro* (5, 11) and on Ad H2ts1 DNA from virus grown at the nonpermissive temperature (4).

When dATP, dTTP, and ddGTP were added to the reaction, a product was detected that migrated with an apparent molecular weight of 88,000 on a polyacrylamide gel. This product was characterized as an 80,000-dalton protein covalently bound to a 26-base oligonucleotide. The 80,000-dalton proteins associated with the 80,000- and 88,000-dalton complexes were struc-

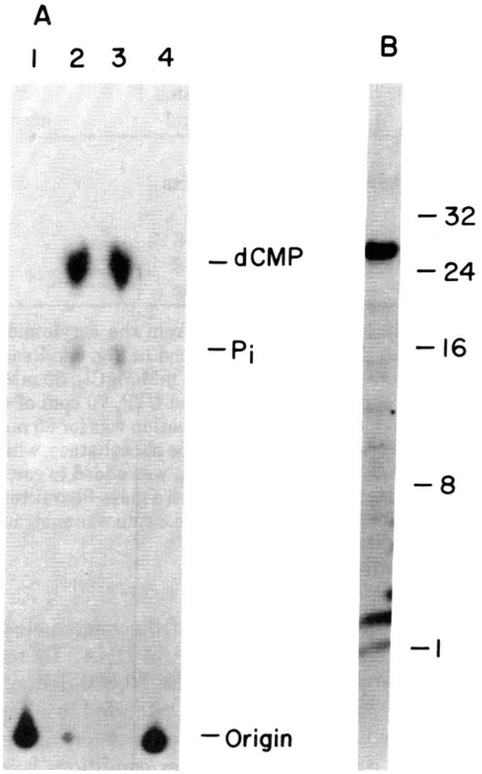

FIG. 6. Characterization of the reaction products. (*A*) The ^{32}P-labeled 80,000-dalton complex was electroeluted from a region of gel containing the 80,000-dalton band and was concentrated as in Fig. 5. Aliquots (4 μl) of the eluted material were applied to a polyethyleneimine-cellulose plate directly (lane 1), after incubation with 0.2 M KOH for 30 min (lane 2) or for 60 min (lane 3), or after incubation with 0.2 M HCl for 240 min (lane 4). Samples in lanes 2–4 were neutralized before application to the plate. The chromatogram was developed in 0.5 M LiCl. (*B*) The 80,000- and 88,000-dalton complexes were formed with the addition of dATP, dTTP (20 μM each), and ddGTP (40 μM). ^{32}P-Labeled material was electroeluted from a region of gel containing both the 80,000- and 88,000-dalton complexes and precipitated as described in Fig. 5*B*. The pellet was resuspended in 0.2 M KOH (10 μl). After incubation for 60 min at 30°C the sample was neutralized, extracted with chloroform-saturated phenol, lyophilized, and resuspended in 10 mM NaOH/0.1 mM EDTA/85% (vol/vol) formamide/0.05% xylene cyanol/0.05% bromphenol blue. The sample was applied to a 20% polyacrylamide/7 M urea gel and electrophoresed for 2 hr at 1700 V. No ^{32}P-labeled material entered the gel if the alkali treatment was omitted. Markers were [α-^{32}P]dCMP and oligonucleotides generated by the action of T4 DNA ligase on a self-complementary octanucleotide.

Table 1. Effect of enzymes on product eluted from gel with alkali

Enzyme	Addition per ml	^{32}P Norit adsorbable, %
None		91
Alkaline phosphatase	2 units	3
Snake venom phosphodiesterase	50 μg	91
Crude snake venom*	75 μg	1
5′-Nucleotidase	50 μg	3

The 80,000-dalton band was eluted from the acrylamide gel and treated with alkali for 60 min as described in Fig. 6A. Reactions (0.1 ml) were carried out in the presence of 4 mM $MgCl_2$, 50 mM Tris·HCl (pH 8.0), 2 nmol each of CMP, CDP, and CTP, 70 cpm of the eluted material, and enzyme as indicated. Incubation was for 60 min at 30°C, except for the reaction containing alkaline phosphatase, which was for 60 min at 65°C. After acidification, Norit was added to each reaction, and the adsorbed material was collected on a glass-fiber filter. Samples were counted for 10 min, and a blank of 13.3 cpm was subtracted before determining the values shown.
* *Crotalus adamenteus.*

turally related, and the sequence of the oligonucleotide was related to that of the 5′-termini of Ad2 DNA. Therefore, the 88,000-dalton product is probably the 80,000-dalton terminal protein with the nucleic acid moiety extended to a length of 26 nucleotides, at which point elongation was terminated by the incorporation of ddGMP. Even under conditions favoring formation of the 88,000-dalton complex, an 80,000-dalton product was detected on the gel. The latter product may result from either the failure of some protein-dCMP complexes to elongate, or the degradation of elongated complexes by nucleases present in the extracts. Although we cannot rule out the possibility that the protein-dCMP complex resulted from degradation of a protein-oligonucleotide complex, the observation that ddNTPs inhibited formation of the elongated form but had no effect on the formation of the 80,000-dalton product (Fig. 4) argues against this mechanism.

In a pulse–chase experiment, a fraction of the ^{32}P-labeled product formed during a brief incubation could be chased into the 88,000-dalton complex (data not shown). Our inability to chase the 80,000-dalton complex quantitatively into elongated forms may reflect its unstable association with the Ad DNA-prot template. It is possible that elongation must be coupled directly to the formation of a protein-dCMP complex.

Treatments of Ad DNA-prot that inactivated it as a template for *in vitro* DNA replication also inactivated it as a cofactor in complex formation. At least two roles can be imagined for Ad DNA-prot in the formation of a protein-dCMP complex. The DNA and the terminal proteins may provide binding sites for factors involved in the initiation reaction (13, 20). Alternatively, the DNA may serve as template, providing a 3′-terminal dGMP residue that directs the binding of dCMP to the protein.

In a previous communication (8), it was shown that an Ad DNA replication system could be reconstituted with four partially purified protein fractions. The product formed by purified fractions, like that formed by crude extracts (5), had an 80,000-dalton terminal protein covalently linked to a 5′-terminal dCMP residue. Use of the reconstituted system and the assay for formation of protein-nucleic acid complexes described in this report should provide a means for the further elucidation of the initiation reaction of Ad DNA replication.

This work was supported by Grant ROI GM13344-15 from the National Institutes of Health, Grants 5 ROI CA21622-04 and CA-11512 from the National Cancer Institute, Grant NP89L from the American Cancer Society, and U.S. Public Health Training Grant GM 7288-06.

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