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Molluscum Contagiosum Virus Topoisomerase: Purification, Activities, and Response to Inhibitors

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Molluscum contagiosum virus (MCV), the only member of the *Molluscipoxvirus* genus, causes benign papules in healthy people but disfiguring lesions in immunocompromised patients. The sequence of MCV has been completed, revealing that MCV encodes a probable type I topoisomerase enzyme. All poxviruses sequenced to date also encode type I topoisomerases, and in the case of vaccinia virus the topoisomerase has been shown to be essential for replication. Thus, inhibitors of the MCV topoisomerase might be useful as antiviral agents. We have cloned the gene for MCV topoisomerase, overexpressed and purified the protein, and begun to characterize its activities in vitro. Like other eukaryotic type I topoisomerases, MCV topoisomerase can relax both positive and negative supercoils. An analysis of the cleavage of plasmid and oligonucleotide substrates indicates that cleavage by MCV topoisomerase is favored just 3′ of the sequence 5′ (T/C)CCTT 3′, resulting in formation of a covalent bond to the 3′ T residue, as with other poxvirus topoisomerases. We identified solution conditions favorable for activity and measured the rate of formation and decay of the covalent intermediate. MCV topoisomerase is sensitive to inhibition by coumermycin A1 (50% inhibitory concentration, 32 μM) but insensitive to five other previously reported topoisomerase inhibitors. This work provides the point of departure for studies of the mechanism of function of MCV topoisomerase and the development of medically useful inhibitors.

Molluscum contagiosum virus (MCV) is one of nine poxviruses that causes diseases in humans (3). The virus infects human keratinocytes, producing a distinctive pathology. Cells near the surfaces of lesions become many times larger than normal and become filled with a granular mass called molluscum bodies. Untreated lesions in healthy people usually disappear spontaneously within several months (4, 15). MCV infection is more common in AIDS patients than in the general population (as high as 5 to 18% [4]), and the consequences are much more severe (14, 17). As many as 33% of AIDS patients with $CD4^+$ counts of less that 100 cells/mm^3 may be infected (4). In immunocompromised patients, papules can form dense crops that are disfiguring and untreatable.

Among the MCV genes identified in the recently completed DNA sequence was one encoding a putative type I topoisomerase (23). Type I topoisomerases catalyze the formation of transient nicks in DNA which result in DNA relaxation (5). Studies in many systems reveal that such a DNA relaxation activity is important for efficient DNA replication, transcription, and probably other functions (7, 31).

All the poxviruses studied to date encode a topoisomerase (6, 10, 12, 23). In the case of vaccinia virus topoisomerase, the most thoroughly studied model, it has been shown that replication requires topoisomerase function (29). Vaccinia virus topoisomerase is a 34-kDa protein that binds to the sequence 5′ (T/C)CCTT 3′ and cleaves just 3′ of the 3′-most T, forming a covalent phosphotyrosine intermediate (20, 27, 30). Amino acid residues important for topoisomerase function have been identified and characterized (2, 11, 13, 21), and an X-ray structure has been reported for an amino-terminal fragment potentially involved in DNA binding (25). Topoisomerase enzymes derived from orf virus and entomopoxvirus have also been studied in vitro (6, 12). Here we describe the purification and initial analysis of the topoisomerase of MCV.

Cloning and site-directed mutagenesis of the MCV topoisomerase gene. To clone the MCV topoisomerase gene (encoded between base pairs 104017 and 104985 [23, 24]), the coding sequence was amplified by PCR, using primers complementary to sequences at each end. MCV DNA from infected human lesions, kindly supplied by Bernard Moss (National Institutes of Health), was used as the PCR template. A band of the expected size was generated in amplification reactions and cloned into the pTA cloning vector (Invitrogen). DNA sequencing revealed that the coding region matched the reported sequence except for two positions, a leucine at amino acid 273 changed to glutamine and a glycine at position 313 changed to glutamic acid (data not shown). It is unclear whether these changes represented errors introduced in the PCR step or natural polymorphisms among different MCV isolates. In order to simplify interpretation of subsequent studies, positions 273 and 313 were changed by site-directed mutagenesis to leucine at 273 and glycine at 313 to match the published sequence.

Overexpression and purification of MCV topoisomerase. For overexpression of the MCV topoisomerase, the coding regions of the initial isolate and the published wild-type were transferred to the T7 expression vector pET15B (Novagen). This resulted in the fusion of a 20-amino-acid linker containing a hexahistidine tag to the amino terminus to facilitate purification [the MCV topoisomerase protein containing the His tag is henceforth referred to as (HT)MCV-TOP]. (HT)MCV-TOP was purified from the soluble fraction of lysed *Escherichia coli* cells by chromatography on Ni-chelating Sepharose. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS/PAGE) of the fractions used for subsequent studies is shown in Fig. 1, lane 2. (HT)MCV-TOP(L273Q, G131E), the product of the originally isolated DNA, was also purified by this means

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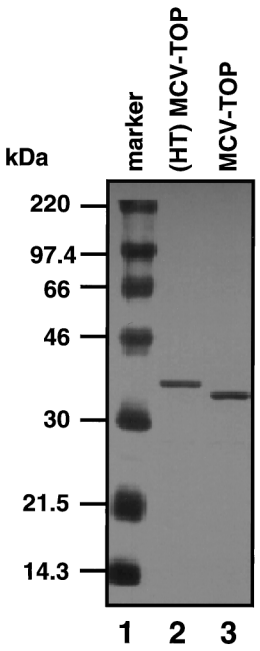

FIG. 1. Analysis of MCV topoisomerase proteins by SDS-PAGE. Lanes: 1, size markers; 2, purified (HT)MCV-TOP; 3, MCV-TOP (the same fractions as in lane 2 but lacking the amino-terminal hexahistidine tag which was removed by treatment with thrombin). MCV DNA from infected human skin, generously supplied by Bernard Moss, provided the starting material for cloning the MCV topoisomerase gene. The gene was amplified by primers FB264 (5′ CCTCCAT ATGAAACGCTTTTTTTTTTACAAG 3′) and FB265 (5′ CGCGGATCCTCA CCCCGTTTCGGGGCACCGCGC 3′) that added restriction sites for *Nde*I to the amino-terminal coding region and *Bam*HI to the carboxyl-terminal coding region. This DNA fragment was cloned into the vector pCRII (Invitrogen) to yield plasmid pBW37. The *Bam*HI-*Nde*I insert was released by cleavage with these enzymes. The purified DNA fragment was then ligated with the T7 expression vector pET15B that had been cleaved with *Bam*HI and *Nde*I, yielding pBW38. This manipulation results in the fusion of a sequence supplied by the vector encoding a hexahistidine tag to the amino-terminal coding region. Site-directed mutagenesis was carried out with the Clontech Transform site-directed mutagenesis kit according to the manufacturer's protocol. Changes (L273Q and G313E) were introduced into the MCV coding region in plasmid pBW37 by primers BW124 (5′ CCCGAAACGGGGTGAGGGCCCGCGAATCTG 3′) and BW125 (5′ GCGCTGACAGTGCTCGAGCTGGCGCGCCCGAAACGGAGT GAGGATCCGCGAATCTG 3′). To enrich for the desired products, a third primer was used (BW121, 5′ CTCGGTACCACGCGTGGCGTAATC 3′) to eliminate a restriction site in the starting vector (*Hin*dIII) and to introduce a new site (*Mlu*I). The MCV coding insert was then transferred to pET15B for overexpression in *E. coli*, yielding plasmid pBW41. Plasmids pBW38 or pBW41 were introduced into the bacterial strain BL21/DE3, which supplies T7 RNA polymerase under control of the *lacZ* promoter. Transformed cells were grown to mid-log phase at 30°C in super broth (16) and then induced to express the MCV topoisomerase by the addition of isopropyl-β-D-thiogalactopyranoside to the culture medium. Induced cells from 1.5 liters of culture were concentrated and resuspended in 25 ml of 0.5 M NaCl–5 mM imidazole–20 mM Tris-HCl, pH 7.9 (binding buffer). Protease inhibitor AEBSF (Calbiochem) was added to 25 μg/ml, and cells were lysed by treatment with 1 mg of lysozyme per ml and sonication. The lysate was centrifuged at 15,000 rpm for 30 min in a JA20 rotor (Beckman). The supernatant was filtered and applied to three 0.8-ml chelating Sepharose columns charged with nickel. The columns were washed with binding buffer and then washed with binding buffer containing 60 mM imidazole. (HT) MCV-TOP proteins were eluted with binding buffer containing 1 M imidazole. Fractions were dialyzed against 0.5 M NaCl–20 mM Tris (pH 8)–1 mM EDTA–0.1% Triton.

(data not shown). The amino-terminal linker containing the hexahistidine purification tag also contains a thrombin cleavage site for proteolytic removal of the His tag after purification. Treatment of the purified (HT)MCV-TOP with thrombin yielded a protein shortened by the expected amount (Fig. 1, lane 3; the thrombin-treated protein is referred to as MCV-TOP).

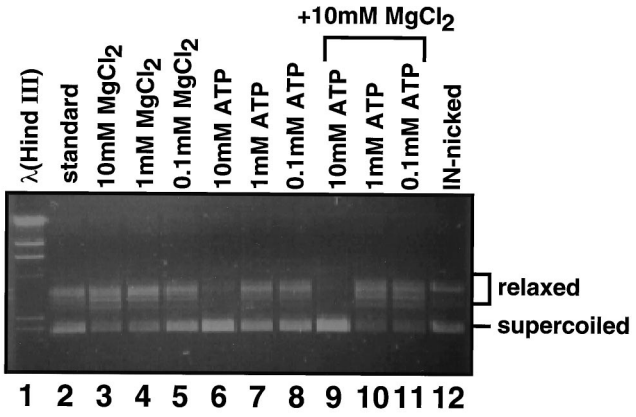

FIG. 2. Effects of ATP and $MgCl_2$ on the relaxation activity of (HT)MCV-TOP. DNA products were analyzed by electrophoresis in the absence of ethidium bromide; DNAs were subsequently visualized by ethidium bromide staining. Aliquots of reaction products were also analyzed on gels containing ethidium bromide, which confirmed that the relaxed products were covalently closed and not nicked (data not shown). Standard assay conditions for assaying relaxation of supercoiled plasmids were 200 mM potassium glutamate, 20 mM Tris-Cl (pH 8), 1 mM dithiothreitol, 0.1% Triton, 1 mM EDTA, 0.3 μg of plasmid DNA, and various amounts of MCV topoisomerase in a 20-μl volume. Reaction mixtures were incubated 5 to 30 min at 37°C, and the reaction was stopped by the addition of protein gel loading dye containing 0.5% SDS. Reaction products were separated by electrophoresis on agarose gels containing Tris-acetate-EDTA and visualized by staining with ethidium bromide.

DNA relaxation by MCV topoisomerase proteins. (HT) MCV-TOP, (HT)MCV-TOP(L273Q, G313E), and MCV-TOP were assayed for DNA relaxation activity. Column fractions were incubated with negatively supercoiled pUC19 plasmid, and the products were analyzed on native agarose gels. Figure 2, lane 2, displays the mobility of relaxation products formed after incubation with (HT)MCV-TOP. The product DNA migrates more slowly, since the relaxed form is less compact than the substrate negative supercoil. To provide a marker for the mobility of relaxed DNA, pFB312 was partially nicked by treatment with HIV-1 integrase protein and subjected to electrophoresis in an adjacent lane (Fig. 2, lane 12) (1, 26). Such plasmid relaxation assays demonstrated activity for the (HT-)MCV-TOP(L273Q, G313E) and MCV-TOP proteins as well (data not shown). Mock extracts from *E. coli* cells overexpressing irrelevant proteins showed no such activity (data not shown). Subsequent assays were carried out using the (HT) MCV-TOP protein.

Relaxation assays were used to identify optimal conditions for the function of (HT)MCV-TOP. Titration of different salts identified 200 mM potassium glutamate as optimal, potentially paralleling the stimulation of many DNA-protein interactions by soft anions (8). Nonidet P-40 at 0.1% stimulated slightly. Divalent cation was dispensable, so subsequent reactions were carried out in the presence of 10 mM EDTA. The pH optimum was about 8 (data not shown).

The specific activity of the topoisomerase enzyme was estimated under optimal conditions. Time course analyses indicated that 1 nM of active MCV topoisomerase could relax 57 nM of pUC19 in 10 min, corresponding to a relaxation rate of about 1.6 supercoils per molecule per second (data not shown). For comparison, vaccinia virus topoisomerase was found to relax 2.3 supercoils per molecule per second (28).

Topological requirements for relaxation. Topoisomerase enzymes differ in their substrate requirements for relaxation. In

particular, action on positively supercoiled substrates distinguishes among classes of topoisomerases (7). The activity of (HT)MCV-TOP was therefore tested on negatively supercoiled and positively supercoiled substrates and found to relax both, as has been reported for other poxvirus topoisomerases (6, 10, 12, 23).

Effects of ATP and Mg^{2+}. Poxvirus topoisomerases are reported to differ in their response to Mg^{2+} and ATP. Vaccinia virus topoisomerase is stimulated, whereas orf virus topoisomerase is unaffected (6, 19, 28). The response of (HT)MCV-TOP was therefore tested (Fig. 2). Relaxation under standard conditions (200 mM potassium glutamate) is shown in lane 2. The addition of $MgCl_2$ at 10 mM, 1 mM, or 0.1 mM had little effect (lanes 3 to 5). The addition of 10 mM ATP was inhibitory (lane 6), while addition of 1 mM or 0.1 mM had no effect (lanes 7 and 8). The addition of 10 mM $MgCl_2$ did not influence the response to ATP (lanes 9 to 11). Repetition of this experiment in the presence of 100 mM NaCl or no added salt instead of the 200 mM potassium glutamate yielded similar results (data not shown). Evidently (HT)MCV-TOP differs from vaccinia virus but resembles orf virus topoisomerase in not responding to $MgCl_2$ and ATP.

Specificity of DNA cleavage. The formation of the covalent complex between topoisomerases and substrate DNA can be monitored by assaying DNA cleavage. For most cellular topoisomerases, the covalent intermediate is short-lived and specific inhibitors must be added for the intermediate to accumulate. Poxvirus topoisomerases differ, however, in that the intermediate accumulates in vitro, and cleaved DNA products can be studied after stopping reactions by the addition of SDS (27).

To investigate the sequence specificity of cleavage by (HT)MCV-TOP, preferred sites were mapped in pUC19 DNA. Linear pUC19 DNA was labeled separately on each DNA strand at the 3′ end, and the labeled DNA was incubated with (HT)MCV-TOP. Reaction products were separated on alkaline electrophoresis gels and visualized by autoradiography (Fig. 3A). Seven sites of cleavage were observed (a to g in Fig. 3A) and mapped on the primary DNA sequence (Fig. 3B). Each of these sites contains a 5′ (T/C)CCTT 3′ sequence matching the favored cleavage site reported for other poxvirus topoisomerases (Fig. 4A and data not shown) (6, 30).

Polarity and strand specificity of covalent complex formation. The polarity of the DNA cleavage involved in covalent complex formation was investigated by using an oligonucleotide substrate (MCV sub a) that matched the most prominent cleavage site (Fig. 4A). To investigate cleavage specificity, oligonucleotide MCV sub a was separately labeled on the 5′ and 3′ ends of the top and bottom DNA strands. Each substrate was then incubated with (HT)MCV-TOP, and reactions were stopped with SDS to trap the covalent intermediate. Reaction products were separated by SDS-PAGE and visualized by autoradiography.

In this gel system, covalent complexes of (HT)MCV-TOP bound to cleavage products migrated with the mass expected for the sum of the molecular weights. Unreacted substrates and free cleaved strands migrated at the buffer front with the bromophenol blue marker dye. It can be seen that only MCV sub a labeled at the 5′ end of the bottom strand became bound to protein (Fig. 4B, lane 4). The observed slowly migrating band displayed the mobility expected for (HT)MCV-TOP (37 kDa) plus the 5′ DNA fragment (4.6 kDa). These data are consistent with the idea that MCV topoisomerase, like other poxvirus topoisomerases, forms a covalent bond between the enzyme and a 3′ end in the substrate DNA.

The other half of the cleaved bottom strand is expected to be free of bound protein. Reactions were carried out with the label on the 3′ end of the bottom strand, and the length of DNA products was analyzed by electrophoresis on DNA sequencing-type gels (Fig. 4C, lanes 2 and 3). As a control, the expected cleavage product was synthesized, end labeled, and analyzed in an adjacent lane (Fig. 4C, lane 1). This indicated that the product is as expected for cleavage just 3′ of the conserved 5′ CCCTT 3′ sequence (Fig. 4C). No cleavage was detected at the second 5′ CCCTT 3′ sequence in MCV sub a, indicating that further unidentified sequence features are also important (only the preferred cleavage site is underlined in Fig. 4A).

Kinetic analysis of cleavage by (HT)MCV-TOP. To begin to elucidate the kinetics of (HT)MCV-TOP action, the rates of formation and decay of the covalent complex were measured. (HT)MCV-TOP was titrated into reactions containing 50 nM MCV sub a labeled at the 5′ end of the bottom strand. Reactions were incubated for 1 min at 37°C, and production of the covalent complex was monitored by SDS-PAGE (Fig. 5A). A plateau was reached at 1,500 nM topoisomerase, at which point about 30% of the substrate was in the covalent form.

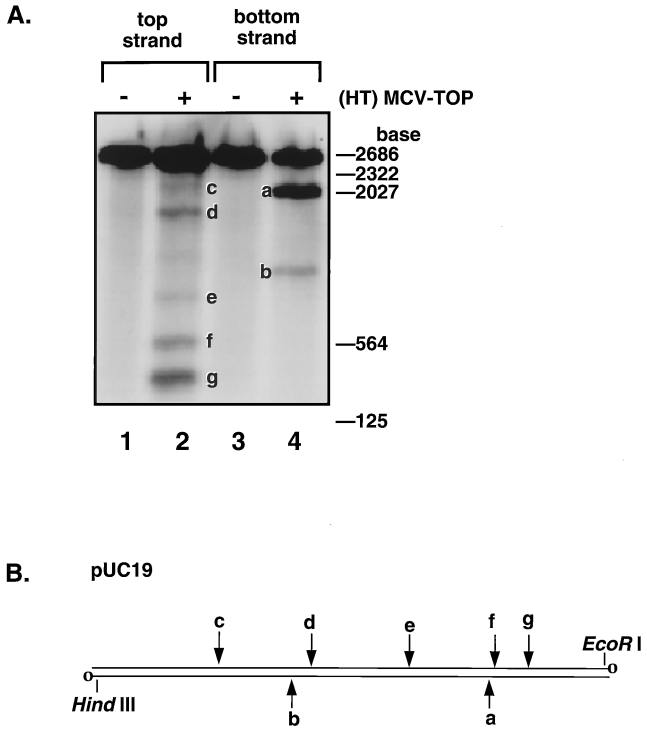

FIG. 3. Site-specific cleavage by (HT)MCV-TOP. (A) Linear pUC19 DNA uniquely labeled on each 3′ end was incubated with purified MCV topoisomerase, treated with 0.5% SDS, and separated on 1.6% alkaline agarose gels by electrophoresis. Lanes: 1, no topoisomerase; 2, 400 ng of topoisomerase; 3, no topoisomerase; 4, 400 ng of topoisomerase. To generate the cleavage substrate, pUC19 DNA was linearized with *Xba*I and 3′ end labeled with Klenow enzyme and [α-^{32}P]dCTP. Linear pUC19 labeled on one end only was then prepared by digestion with *Eco*RI (top strand) or *Hin*dIII (bottom strand) to remove the label at the undesired position. Reaction mixtures (20 μl) containing 20 mM Tris-HCl, pH 8.0, 200 mM K-glutamate, 1 mM dithiothreitol, 0.1% Triton X-100, 1 mM EDTA, and 50 ng of 3′ end ^{32}P-labeled linear pUC19 DNA plus 400 ng topoisomerase were incubated at 37°C for 5 min. Cleaved DNA products were disrupted by the addition of SDS to 0.5%, heated at 95°C for 5 min, cooled on ice, adjusted to 50 mM NaOH and 1 mM EDTA, and then analyzed by electrophoresis on a 1.6% alkaline agarose gel (16). (B) Diagram of the cleavage substrate and the approximate positions of MCV topoisomerase cleavage sites in pUC19. The site of strand labeling in each set of reactions is indicated by open circles. The cleavage sites are indicated on the top or bottom strand by arrows.

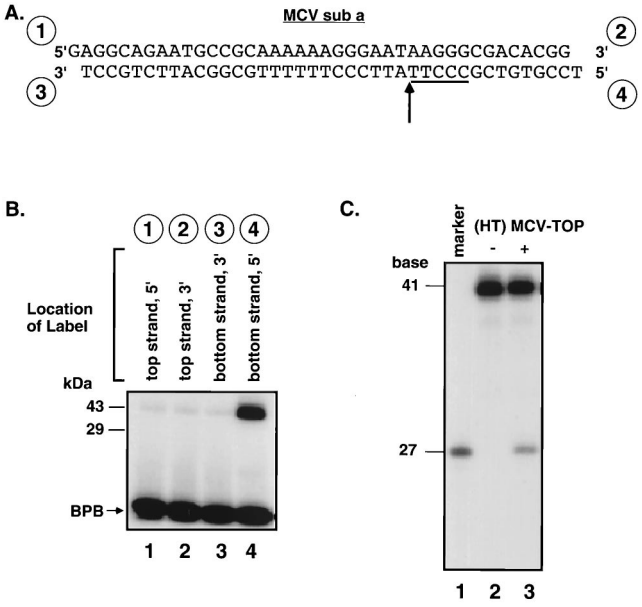

FIG. 4. Analysis of the strand specificity of covalent complex formation on the MCV sub a substrate. (A) Sequence of MCV sub a, an oligonucleotide matching the most prominent site of cleavage by (HT)MCV-TOP in pUC19 DNA (site a in Fig. 3). A consensus cleavage site, 5′ CCCTT 3′, is underlined; the favored cleavage site is marked by the arrow. (B) Strand specificity of (HT) MCV-TOP covalent complex formation. MCV sub a was separately labeled at either the 5′ end or 3′ end of each strand, covalent complexes were formed with (HT)MCV-TOP, and the products were assayed by SDS-PAGE. The numbers over each lane correspond to the DNA ends indicated in panel A. Lanes: 1, 5′ end-labeled top strand; 2, 3′ end-labeled top strand; 3, 3′ end-labeled bottom strand; 4, 5′ end-labeled bottom strand. MCV sub a was end labeled at the 5′ end by treatment of one strand with [γ-^{32}P]ATP and T4 polynucleotide kinase. After labeling, the oligonucleotide was purified in a Sephadex G-25 spin column and then hybridized to the complementary oligonucleotide by heating and slow cooling. To prepare 3′ labeled MCV sub a, the duplex oligonucleotide was treated with Klenow fragment and [α-^{32}P]dCTP. Reaction mixtures (20 μl) contained 20 mM Tris-HCl, pH 8.0, 200 mM K-glutamate, 1 mM dithiothreitol, 0.1% Triton X-100, 1 mM EDTA, 50 nM of 5′ ^{32}P-end-labeled MCV sub a, and 600 nM of MCV topoisomerase. Reaction mixtures were prewarmed at 37°C and then initiated by the addition of topoisomerase. Covalent complex products were disrupted by the addition of SDS and beta-mercaptoethanol to 0.5%, heated at 95°C for 5 min, and then analyzed on 12% polyacrylamide gels containing 0.1% SDS. Free oligonucleotide migrated with the bromophenol blue (BPB) dye marker. (C) Cleavage of MCV sub a labeled at the 3′ end of the bottom strand. Reaction mixtures (20 μl) were diluted to 1:20, adjusted to 50% formamide, heated at 95°C for 5 min, and then separated on 8% denaturing polyacrylamide gels containing 8.3 M urea in TBE buffer (16). Reaction products were denatured, separated on a DNA sequencing-type polyacrylamide gel, and visualized by autoradiography. Lane 1 contains an oligonucleotide matching the expected 27 base product of cleavage. Lane 2 displays the reaction substrate, and lane 3 contains the product of cleavage by (HT)MCV-TOP.

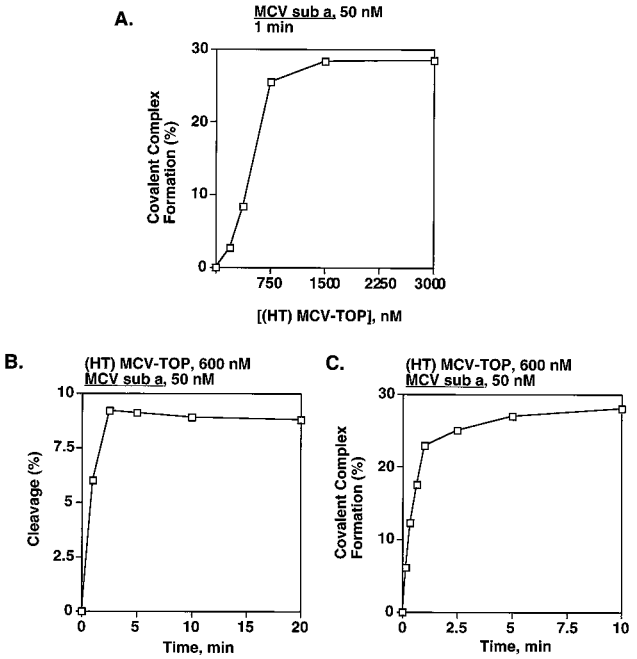

FIG. 5. Kinetics of formation of the covalent intermediate. (A) Titration of (HT)MCV-TOP with MCV sub a DNA 5′ end labeled on the bottom strand. Formation of the covalent complex was assayed by SDS-PAGE. This assay and those described below were quantitated on a Molecular Dynamics PhosphorImager. The concentrations of substrate and (HT)MCV-TOP are as indicated. (B) Time course of formation of the 3′ cleavage product accompanying covalent complex formation. (C) Time course of formation of the covalent complex. MCV sub a labeled on the 5′ end of the bottom strand was incubated with (HT)MCV-TOP, and aliquots were assayed as a function of time by SDS-PAGE.

Less covalent complex accumulated in the presence of 100 mM NaCl or no added salt (data not shown). The plateau likely represents the state at which all substrate molecules are bound to enzyme, and the proportion of cleaved versus uncleaved substrates represents a balance between formation and decay rates of the cleaved complex.

The rate of formation of the cleaved complex was then investigated. First, MCV sub a was labeled on the 3′ end of the bottom strand to monitor the production of the free 3′ fragment after cleavage (Fig. 5B). MCV topoisomerase (600 nM) was mixed with 50 nM MCV sub a, and aliquots were withdrawn at different times and analyzed on denaturing polyacrylamide gels. The half time for formation of the cleaved product was less than 1 min.

The time for formation of the covalent complex was also measured under the same conditions and also found to be less than 1 min, as expected since DNA strand cleavage should be coupled with covalent complex formation (Fig. 5C). For unknown reasons, the final extent of the reaction measured by the two methods differed: 10% for production of the 3′ cleaved strand versus 30% for production of the cleaved complex (the yield of the 3′ cleavage reactions was variable among experiments).

The rate of decay of covalent complexes was measured by preincubating (HT)MCV-TOP with 5′ labeled MCV sub a and then challenging the reaction with a 10-fold excess of unlabeled MCV sub a (Fig. 6A). The abundance of the covalent complex declined to one-tenth of its former amount with a half time of 3 min (Fig. 6B). These data are consistent with the idea that the turnover of (HT)MCV-TOP is limited by a late step in the reaction cycle, such as product release.

Inhibition of DNA relaxation by coumermycin A1. To assay the effect of known topoisomerase inhibitors on the MCV enzyme, inhibition of the relaxation activity was tested. Conversion of the substrate supercoiled plasmid DNA to relaxed circular DNA was measured after 5 min of incubation at 37°C in the presence of different concentrations of inhibitors (Fig. 7). Coumermycin A1 inhibited MCV topoisomerase with a 50% inhibitory concentration of 32 μM (Fig. 7B). In contrast, five other topoisomerase inhibitors were inactive against MCV topoisomerase at the concentrations tested (Fig. 7A and C).

Implications. The topoisomerase of MCV resembles previously described poxvirus enzymes in many respects, though this could not have been predicted since MCV topoisomerase is only 54% identical of that of vaccinia virus. The proteins are

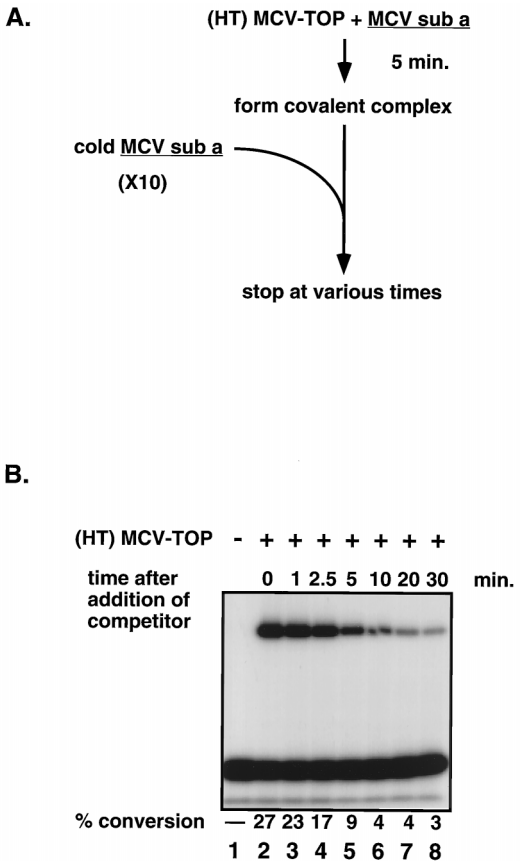

FIG. 6. Kinetics of decay of the covalent intermediate. (A) Outline of the challenge protocol. (B) Analysis of decay of the covalent complex. Reactions were stopped by treatment with SDS and analyzed by SDS-PAGE. Lanes: 1, no added (HT)MCV-TOP; 2, (HT)MCV-TOP but no added competitor; 3 to 8, (HT)MCV-TOP with a 10-fold excess of competitor. Times after competitor addition are indicated above the autoradiogram.

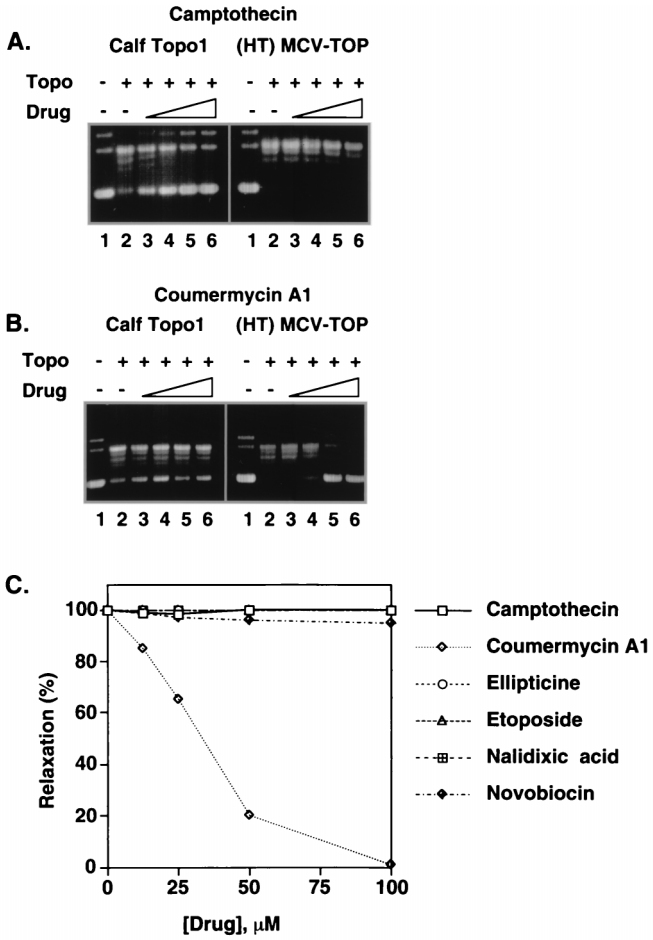

FIG. 7. Inhibition of DNA relaxation by several topoisomerase inhibitors. (A) Inhibition of calf topoisomerase I and (HT)MCV-TOP by camptothecin. Control reactions were performed without enzyme (lanes 1) or without inhibitor (lanes 2). Concentrations of inhibitor added were 12.5 μM (lane 3), 25 μM (lane 4), 50 μM (lane 5), and 100 μM (lane 6). The reaction mixtures contained a 10% (vol/vol) final concentration of dimethyl sulfoxide. (B) Inhibition of calf topoisomerase I and (HT)MCV-TOP by coumermycin A1. Lanes are the same as described for panel A. (C) Quantitation of inhibition of (HT)MCV-TOP by several topoisomerase inhibitors.

similar in size, relaxation specificity, and favored sites of action (6, 12, 28, 30). Evidently the common features previously described for the topoisomerases of the *Orthopoxvirus*, *Parapoxvirus*, and *Entomopoxvirus* B genera also hold for the divergent *Molluscipoxvirus* genus.

The sequences favored for cleavage are identical among the poxvirus topoisomerases studied. All cleave the same sites in pUC19 and favor sequences containing the pentanucleotide 5′ (C/T)CCTT 3′. However, the precise nature of the sequences required for cleavage has not been fully clarified. In pUC19 DNA, there are eight 5′ CCCTT 3′ sequences, but only four were seen as cleavage sites for MCV topoisomerase (Fig. 3). Three additional sites in pUC19 found to be favored for cleavage had the sequence 5′ TCCTT 3′ (data not shown). Two 5′ CCCTT 3′ sequences are present in oligonucleotide MCV sub a, but for unknown reasons only one is cleaved efficiently (Fig. 4C). Evidently base pairs outside the favored pentanucleotide are also important. The topoisomerase of vaccinia virus has been studied extensively, but there also the sequence determinants of cleavage have not been fully clarified (11, 20, 27). Further data in this area may be useful in identifying the favored sequences and understanding their role in the topoisomerase mechanism.

MCV topoisomerase is a potentially attractive target for the development of antiviral agents. Poxvirus type I topoisomerases are quite different from human type I topoisomerases, being smaller and only distantly related in sequence (9). The MCV topoisomerase displays an unusual response to inhibitors for a eukaryotic type I topoisomerase. It is resistant to the type I topoisomerase inhibitor camptothecin, but sensitive to coumermycin A1, which otherwise acts only on type II topoisomerases. Vaccinia virus topoisomerase displays a similar pattern of sensitivity to inhibitors (18, 22, 28). These distinctive responses raises the hope that small molecules may be found that selectively inhibit MCV topoisomerase but not human topoisomerases. Such compounds might be useful as MCV antiviral agents. Small molecules active against MCV topoisomerase might be applied topically to MCV papules, potentially circumventing many possible concerns regarding toxicity of the antiviral compound.

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