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Polynucleotide Ligase Activity in Cells Infected with Simian Virus 40, Polyoma Virus, or Vaccinia Virus

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The conversion of simian virus 40 (SV40) component II deoxyribonucleic acid to component I has been used to assay polynucleotide ligase in extracts of tissue culture cells. All cell types examined, including chicken, hamster, mouse, monkey, and human cells, contained adenosine triphosphate-dependent ligase. After infection of mouse embryo, monkey kidney, and HeLa cells with polyoma virus, SV40, and vaccinia virus, respectively, the enzyme activity increased, but its cofactor requirement was unchanged. In vaccinia virus-infected cells, the increased activity was localized in the cytoplasm. Ligase induction occurred in the presence of cytosine arabinoside but was prevented by puromycin. Rifampicin blocked the production of infectious vaccinia particles but had little effect on the induction of ligase.

Polynucleotide ligases have been purified from both uninfected (12, 13, 31, 40) and virus-infected *Escherichia coli* (1, 7, 37) and from mammalian tissues (26). These enzymes, which seal single-stranded breaks in duplex deoxyribonucleic acid (DNA), are thought to be involved in DNA repair and replication (10, 14, 21, 24, 29, 30). In the present study, we have found high levels of ligase activity in several types of mammalian tissue culture cells. We have also examined cells infected with vaccinia virus, which has a large DNA genome and multiplies in the cytoplasm, and with simian virus 40 (SV40) and polyoma virus, mammalian tumor viruses which contain circular DNA and replicate in the nuclei of infected cells.

MATERIALS AND METHODS

Viruses. Large-plaque polyoma virus and SV40 were grown and purified as described previously (38, 39). A stock of purified (19) vaccinia virus strain WR was kindly provided by J. R. Kates, University of Colorado, Boulder.

Cells. Suspension cultures of HeLa S3 cells were grown in Eagle spinner medium containing 10% horse serum. All of the other established cell lines were grown as monolayer cultures in Eagle medium containing 10% calf serum. Mouse embryo fibroblasts (MEF) and chick embryo fibroblasts were prepared and grown in the usual way. African green monkey kidney (AGMK) and human embryonic kidney (HEK) cells were purchased from Flow Laboratories, Rockville, Md., and were used as secondary cultures grown in Eagle medium supplemented with 10% calf serum. The mouse lymphoma, thymoma, and myeloma lines were provided by S. Sarkar, the Salk Institute for Biological Studies, La Jolla, Calif.

Preparation of ^{14}C-labeled SV40 DNA. BSC-1 cells were grown to confluence in 100-mm petri dishes, washed once with phosphate-buffered saline (PBS), and infected with 2×10^8 plaque-forming units (PFU) of SV40, i.e., a multiplicity of about 50 PFU/cell. The virus was adsorbed for 1 hr at 37 C. A 10-ml amount of Eagle medium containing 10% dialyzed fetal bovine serum was then added to each dish, and the cells were incubated at 37 C. At 24 hr after infection, 3 μc of thymidine-2-^{14}C (Schwarz BioResearch Inc., Orangeberg, N.Y.; specific activity, 45 mc/mmole) was added to each dish. At 72 hr after infection, the monolayers were washed twice with tris(hydroxymethyl)aminomethane (Tris)-buffered saline (per liter: 0.1 g of $MgCl_2 \cdot 6H_2O$, 0.1 g of $CaCl_2$, 8 g of NaCl, 0.38 g of KCl, 0.1 g of Na_2HPO_4, 3 g of Tris), and the SV40 DNA was extracted as described by Hirt (18). The viral DNA was further purified by two extractions with phenol saturated with 1 M Tris (*p*H 8.0) and by one extraction with chloroform-isoamylalcohol (24:1). After dialysis against 1 × SSC (0.15 M NaCl, 0.015 M sodium citrate) containing 0.001 M ethylenediaminetetraacetate (EDTA), 0.01 M Tris (*p*H 7.4), the DNA was centrifuged to equilibrium in CsCl-ethidium bromide as described previously (34). Fractions were collected through the bottom of the tube, and those containing the component I SV40 were pooled. The ethidium bromide was removed by three extractions with isopropanol saturated with

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CsCl (F. Cuzin et al., *in preparation*). Finally, the component I DNA was dialyzed against 0.01 × SSC, 0.0001 M EDTA and stored at −70 C. The yield was about 7 to 10 μg per petri dish, with a specific activity of 10^4 counts per min per μg. The DNA was concentrated approximately 10-fold before use.

Preparation of single-hit component II ^{14}C-SV40 DNA. Electrophoretically pure pancreatic deoxyribonuclease (Worthington Biochemical Corp., Freehold, N.J.) was dissolved at 1 mg/ml in 0.1 M Tris (*p*H 8.0), 0.01 M EDTA, 0.06 M $MgCl_2$ (buffer A), and was stored in 0.2-ml portions at −70 C until used.

Equal volumes of buffer A and ^{14}C-SV40 component I DNA (in 0.1 × SSC, 0.001 M EDTA) were mixed; deoxyribonuclease freshly diluted 1:200,000 in buffer A from the stock solution was then added to give an enzyme concentration of 0.5 × 10^{-3} μg/ml. The mixture was incubated at 37 C. At various times (usually 5, 10, and 20 min), small samples were removed, adjusted to 0.05 M with EDTA, and assayed for components I and II on hydroxylapatite columns or alkaline sucrose gradients. (For details, see below.) While these assays were carried out, the main part of the reaction mixture was kept frozen at −20 C. From the proportion of component II present at different times of incubation, the time necessary to convert 60% of the component I to component II was determined. The remainder of the reaction mixture was then thawed, and the incubation was continued at 37 C. At the appropriate time, EDTA was added to 0.05 M and the mixture was layered on 12.0-ml, 5 to 20% neutral sucrose gradients in 0.005 M Tris (*p*H 7.7), 0.1 M NaCl, 0.01 M EDTA. Gradients were centrifuged at 31,000 rev/min for 16 hr in a Spinco SW41 rotor at 20 C. Seven-drop fractions were collected through the bottom of the tube, and 5-μliter samples of each fraction were counted directly in Bray solution. The fractions containing component II were pooled, dialyzed against 0.1 × SSC, 0.0001 M EDTA, and stored at −20 C.

Infected cell extracts: polyoma. 3T3 cells or secondary mouse fibroblasts were grown to confluence in Eagle medium plus 10% calf serum for 4 to 5 days and infected with large-plaque polyoma virus at a multiplicity of 10 PFU/cell, as described by Fried and Pitts (11). At various times after infection, the cell monolayers were washed twice with ice-cold PBS and once with ice cold TEM [0.02 M Tris (*p*H 8.0), 0.001 M EDTA, 0.005 M 2-mercaptoethanol]. The excess TEM was removed, and the monolayers were scraped with a rubber policeman into the small amount of TEM remaining on the petri dish. The extracts were stored at −70 C until used. Before enzyme assay, the extracts were thawed and centrifuged at 7,000 × *g* for 10 min at 0 C in a Sorvall SS-3 centrifuge. The supernatant fluids were assayed for enzyme activity. The above procedure is essentially that of Fried and Pitts (11).

Viral DNA synthesis was measured after 12- to 13-hr pulses of ^{3}H-thymidine (Schwarz BioResearch; specific activity, 11 c/mmole) as described by Cuzin et al. (*in preparation*).

Host DNA synthesis was measured after 12- to 13-hr pulses of ^{3}H-thymidine. The pellets obtained by the Hirt extraction (18) were dissolved in 0.1 N NaOH at 80 C for 30 min, precipitated with cold 5% trichloroacetic acid onto Whatman GF/C filter papers, and counted in a toluene-based scintillation fluid.

SV40. AGMK cells were grown and infected, and the extracts were made as described above.

Poxvirus. HeLa S3 cells were sedimented and resuspended at 10^7/ml in Eagle medium containing 5% fetal bovine serum. Vaccinia virus strain WR was added to give a multiplicity of about 250 elementary bodies per cell. The virus was allowed to adsorb for 45 min at 37 C, with intermittent shaking in the presence of various drugs where indicated. The infected cells were then washed and resuspended at 10^6/ml in prewarmed spinner Eagle medium containing 5% fetal bovine serum; this time was taken as 0 hr after infection. The infected cells were maintained in spinner culture at 37 C.

At intervals after infection, samples of the cells were pelleted, washed twice with PBS, and resuspended at 10^7/ml in TEM at 0 C. After 10 min, the swollen cells were broken by two strokes in a tight-fitting Potter-Elvejhelm homogenizer. The nuclei were removed by centrifugation at 1,000 × *g* for 5 min, and the supernatant fluids were stored at −70 C until assayed.

For radioactive labeling experiments, 1 μc of ^{3}H-thymidine (Schwarz BioResearch; specific activity, 11 c/mmole) was added to 5-ml samples of the cell suspensions. After incubation for 1 hr at 37 C, the cells were washed and extracts were prepared as above. The supernatant fluids were precipitated with 5% trichloroacetic acid and counted. Plaque assays were performed on chick embryo fibroblast monolayers.

Ligase assays. The reaction mixture consisted of 0.04 M Tris (*p*H 7.7), 0.01 M $MgCl_2$, 0.01 M 2-mercaptoethanol, 10^{-4} M adenosine triphosphate (ATP), 0.10 M KCl, 0.1 μg of component II ^{14}C-SV40 DNA, and the indicated amount of cell extract in a total volume of 0.12 ml. The mixture was incubated at 30 C for 25 min, and the reaction was stopped by chilling to 0 C and by the addition of 20 μliters of 0.5 M EDTA. The proportions of components I and II were determined by one of two methods.

Sedimentation through alkaline sucrose gradients. The material to be assayed was layered on the top of alkaline sucrose gradients (5 to 20% in 0.5 M NaCl, 0.001 M EDTA, 0.3 M NaOH) which were then centrifuged at 55,000 rev/min for 75 min at 20 C in a Spinco SW56 rotor. Fractions were collected through the bottom of the tube directly into scintillation vials containing 0.5 ml of 1 M Tris buffer (*p*H 8.0). A 10-ml amount of Bray solution was added to each vial, and the samples were counted. Under these conditions of centrifugation, component I assumes a supercoiled configuration with a sedimentation coefficient of 53*S*; component II is denatured by the alkali and sediments as two peaks at 18*S* and 16*S*, which are not resolved by these short periods of centrifugation (36; Fig. 1). The relative proportions of components I and II were determined by summing the counts under the peaks.

Elution from hydroxylapatite. This method is a modification of those published previously (2–4). The ligase reaction was carried out as described above. After the reaction was stopped with EDTA, 0.38 ml

of 0.5% sodium dodecyl sulfate (SDS) was added, and the samples were immersed in boiling water for 2 min and then rapidly cooled in ice water. Under these conditions, component II is completely denatured, and component I remains native. An 0.5-ml amount of 0.2 M potassium phosphate buffer (*p*H 6.8) was then added, and the samples were kept at 0 C for an additional 10 min. The precipitate of SDS and protein was removed by centrifugation at 7,000 × *g* for 10 min at 0 C.

The supernatant fluids were applied to small hydroxylapatite (H-T, Biorad Laboratories, Richmond, Calif.) columns (0.5 ml in disposable syringes) and washed in with 0.1 M potassium phosphate buffer, *p*H 6.8. The denatured component II was eluted with two washes of 0.5 ml of 0.175 M potassium phosphate buffer (*p*H 6.8) and counted. The native component I was eluted with two washes of 0.4 ml of 0.3 M potassium phosphate buffer and counted.

Poxvirus ribonucleic acid (RNA) polymerase. The particle-associated RNA polymerase was assayed by the method of Kates and McAuslan (20).

DNA polymerases in polyoma and SV-40 infected cells were assayed by the method of Hartwell, Vogt, and Dulbecco (15). For each incubation, 50 μg of crude cell protein was used.

Protein concentrations. Protein concentrations were determined by the method of Lowry et al. (27) with bovine serum albumin as standard.

DNA was determined by Burton's method (5) with deoxyadenosine monophosphate as standard.

RESULTS

Characteristics of the reaction. The ligase assay which we have used, i.e., conversion of the single-nicked relaxed circle (component II) to the covalently closed superhelical configuration (component I), is clearly a measure of the equilibrium between endonuclease and ligase activities. In an attempt to reduce selectively the endonuclease activity in cell extracts, cultures were maintained in low-serum medium for several days before preparing extracts by freeze-thawing, sonic treatment, or homogenization. Assays were also performed in the presence of inhibitors of deoxyribonuclease I (25): soluble RNA, denatured DNA, and salts. The only procedure which inhibited nuclease effectively and selectively was the addition of KCl or NaCl to the reaction mixture. In the presence of 0.1 M KCl, the deoxyribonuclease activity of an extract of MEF containing 10 μg of protein was inhibited almost completely (Fig. 1). Even in the presence of 100 μg of crude cell protein, the deoxyribonuclease level was reduced by 80% in 0.1 M KCl. Similar results were obtained with KCl or NaCl at concentrations of 0.08 to 0.3 M. The concentration of 0.1 M KCl was chosen for the ligase assays because ligase purified from bacteriophage T4-infected *E. coli* (kindly provided by E. K. Bautz, Rutgers University, New Brunswick, N.J.) remained highly active against SV40 component II at this salt concentration.

The time course of the ligase reaction with BSC-1 cell extracts is shown in Fig. 2. The rate of conversion of component II to I began to decrease after 5 to 10 min of incubation, and the reaction was almost complete after 25 min. Included for comparison is an assay series measured by chromatography on hydroxylapatite. Samples of component II at zero time contained 3% of the

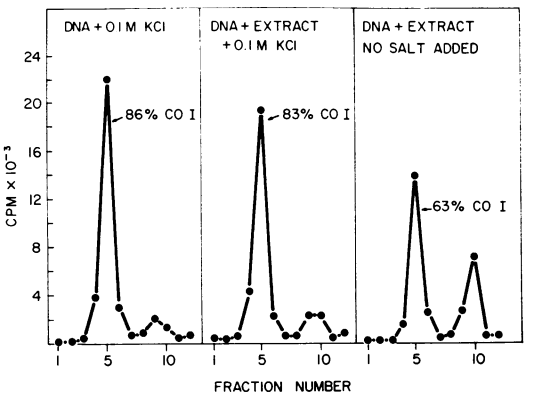

FIG. 1. *Inhibition of endonuclease activity in cell extracts by 0.1 M KCl. ^{14}C-labeled SV40 component I (CO I) DNA was incubated in the standard ligase assay with or without 0.1 M KCl and with or without MEF extract (10 μg of crude protein) and was analyzed by sedimentation through alkaline sucrose gradients.*

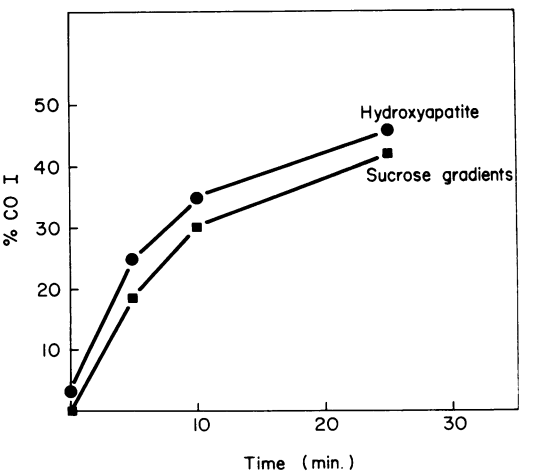

FIG. 2. *Time course of ligase reaction. The conversion of ^{14}C-SV40 component II DNA to component I (CO I) during incubation with BSC-1 cell extract (25 μg of protein) was assayed by alkaline sucrose gradient centrifugation and hydroxylapatite column chromatography.*

radioactivity with apatite adsorption properties characteristic of component I, i.e., elution in 0.3 M phosphate buffer. When this background was subtracted, the values obtained by chromatography and centrifugation were within 1 to 3% of each other.

The extent of the conversion is directly related to protein concentration (Fig. 3). The maximum amount convertible to component I varied from 45 to 76% among different preparations of component II. The cause of this variation is not understood but may be related to the proportion of component II which has a 5'-phosphorylated terminus at the nicked region. We noted that component II which was formed during storage of radioactive component I [^{3}H-labeled (10^6 counts per min per μg at -70 C) or ^{14}C-labeled (1.6×10^4 counts per min per μg at 4 C in 1 × SSC, 0.001 M EDTA)] was not repaired by ligase obtained from T4-infected *E. coli*.

Polynucleotide ligase from uninfected cells. Ligase activity was readily detected in extracts made from all types of tissue culture cells tested, including chicken, hamster, mouse, monkey, and human cells (Table 1). In every case, maximal conversion of 0.1 μg of SV40 component II to component I was observed with the addition of crude extract containing 5 to 10 μg of protein.

The cofactor requirement of the ligase from chick embryo fibroblasts, MEF, HEK cells, and continuous cultures of BHK-21, SV-3T3, BSC-1, KB, and HeLa cells was examined after extensive dialysis of extracts against TEM. For all these cell types, enzyme activity could be demonstrated only after addition of ATP to the dialyzed extracts. (See Fig. 4 in which BSC-1 extracts are shown as an example.) There was no response to nicotinamide adenine dinucleotide (NAD), the cofactor required by the *E. coli* polynucleotide ligase (32, 40). In the absence of Mg^{2+}, there was also no measureable enzyme activity. The intracellular localization of the enzyme was examined in HeLa cells. Cells were grown and broken by homogenization, and the resulting extracts were separated into nuclear and cytoplasmic fractions as described. When 2 μg of protein from each fraction was assayed, it was found that the nuclear material converted 50% of component II to component I, 10 times more than the same amount of cytoplasmic protein. The small amount of activity in the cytoplasmic fraction may result from damaged nuclei or from mitochondria. These characteristics of animal cell ligase, i.e., ATP and Mg^{2+} dependence and nuclear localization, have been reported previously for enzyme isolated from rabbit tissue (26).

TABLE 1. *Tissue culture cells examined for polynucleotide ligase activity*

Chicken	
1° Embryo	Normal
Hamster	
BHK-21	Normal
Mouse	
2° Embryo	Normal
3T3	Normal
SV-3T3	SV40-transformed
Py-tsa-3T3 (38° C)	Polyoma-transformed[a]
Py-tsa-3T3 (31° C)	Polyoma-transformed virus growth induced[a]
T1	Lymphoma
P3	Thymoma
S49	Myeloma
Monkey	
2° AGMK	Normal
BSC-1	Normal
Human	
KB	Tumor
HEK	Normal
HeLa	Tumor

[a] F. Cuzin et al. (*in preparation*).

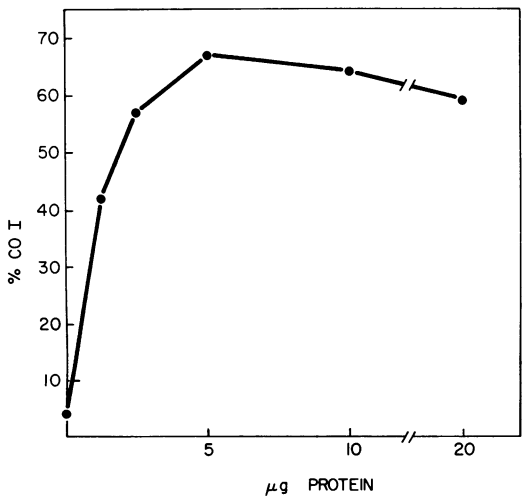

FIG. 3. *Relationship between ligase activity and protein concentration in extracts of BSC-1 cells.*

Ligase induction in virus-infected cells. Infection of resting mouse cells with polyoma virus leads to a rise in cellular as well as viral DNA synthesis (9). The activity of several enzymes involved in DNA formation also increases (15), and it was of interest to study the effect of infection on ligase activity. Confluent monolayers of mouse embryo cells were maintained in 0.5% serum for several days to reduce DNA synthesis. Under these same conditions, cellular ligase activity did

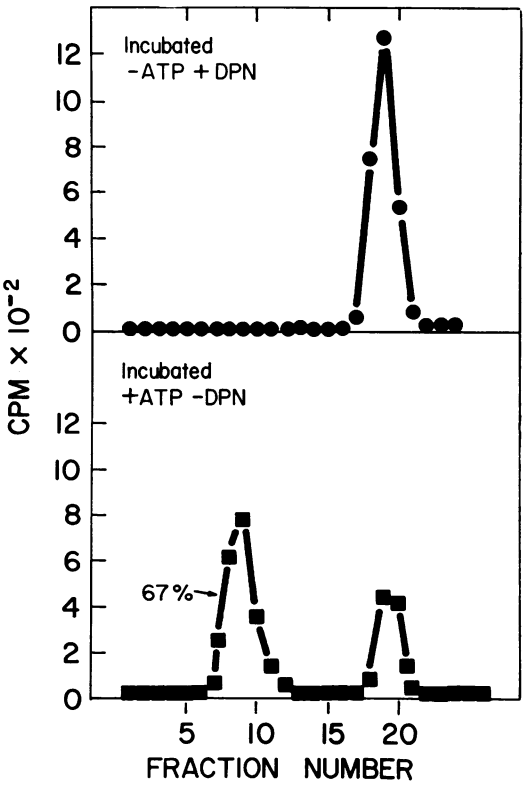

FIG. 4. *Cofactor requirement of ligase activity. Component II DNA was incubated with dialyzed BSC-1 cell extract (32 μg of protein) in a ligase assay mixture containing 10^{-4} M ATP or 10^{-5} M NAD (DPN), and the product was sedimented through alkaline sucrose gradients*

not decrease. After infection with 10 PFU of purified large-plaque polyoma virus per cell, there was a fourfold rise in cell DNA synthesis (not shown) and a three-fold increase in DNA polymerase activity (Fig. 5). The rate of incorporation of thymidine into viral DNA was maximal 24 hr after infection, and there was a twofold rise in ligase activity as compared to mock-infected cells. The cofactor dependence of the polyoma-induced ligase activity was determined after extensive dialysis of the infected cell extracts against TEM. Like the uninfected cell enzyme, the polyoma-induced enzyme showed an absolute requirement for ATP.

Infection of AGMK cells with SV40 virus also stimulated the formation of both cell and viral DNA (16). AGMK cultures were maintained in 0.1% serum for 4 to 5 days to reduce DNA synthesis. There was no decrease in ligase activity during this time. The cells were then infected with purified SV40 at a multiplicity of 15 PFU/cell. The rate of cell DNA synthesis increased 14-fold (not shown), and viral DNA synthesis was maximal at 38 to 51 hr after infection (Fig. 6). DNA polymerase and ligase activites increased by factors of five and two, respectively, above mock-infected cells. When cytosine arabinoside (10 μg/ml) was present in the culture medium, synthesis of both viral and cellular DNA was prevented but enzyme activity increased. Similar results have been reported previously for the SV40-induced thymidine kinase (22), DNA polymerase (23), and T antigen (33). The cofactor requirement of the SV40-induced ligase was ATP.

Vaccinia virus multiplies in the cytoplasm of infected cells (6) and is accompanied by an increase in cytoplasmic ligase activity which is easily measured against the low background levels. At 1 hr after infection of HeLa cells with 25 PFU of vaccinia virus per cell, viral DNA synthesis began in the cytoplasm (Fig. 7). An

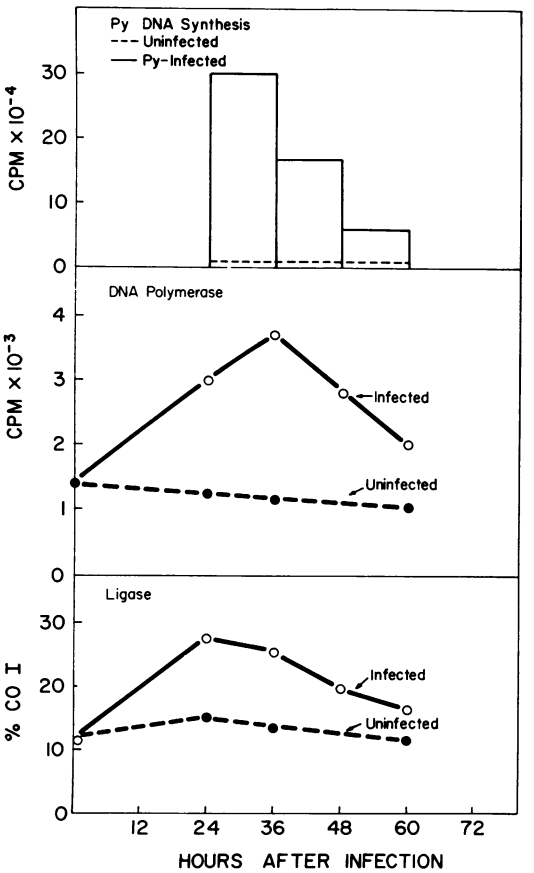

FIG. 5. *Polyoma DNA synthesis and DNA polymerase and ligase activities in mouse embryo cells. For the ligase assay, 2 μg of crude protein was used.*

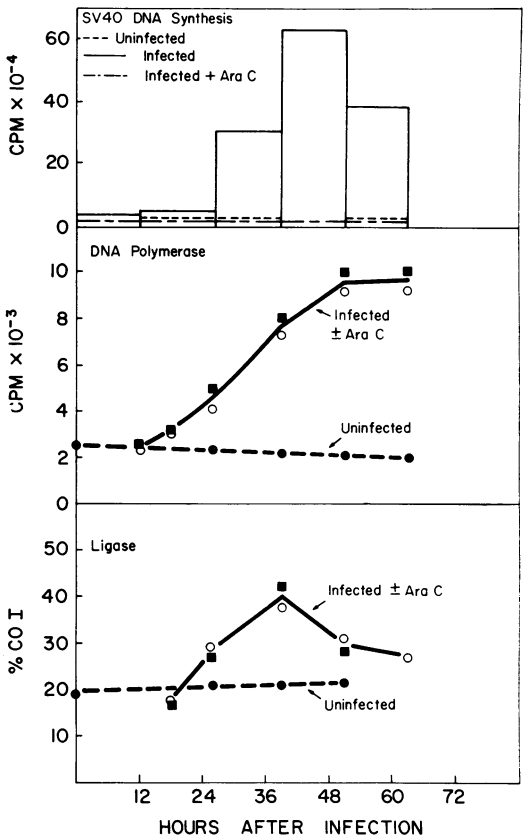

FIG. 6. *SV40 DNA synthesis and DNA polymerase and ligase activities in AGMK cells infected with SV40 virus and treated with cytosine arabinoside (10 μg/ml). Ligase was measured with 5 μg of crude protein.*

increase in ligase activity accompanied the DNA rise, and, at 3 hr after infection, there was 13 times more activity than in the cytoplasm of mock-infected cells. As in SV40-induced AGMK cells, 10 μg of cytosine arabinoside per ml prevented DNA synthesis but not ligase stimulation. Inhibition of protein synthesis by 20 μg of puromycin per ml blocked both viral DNA production and ligase induction, indicating that the increased activity in infected cells probably represents de novo protein synthesis rather than activation of a preformed enzyme. Ligase activity could not be detected in purified virus particles which had been treated with 2-mercaptoethanol and Nonidet NP-40 to activate the particle-associated RNA polymerase.

The cytoplasmic localization of the ligase in vaccinia virus-infected cells suggests that it may be virus-coded. However, the vaccinia-induced ligase, like that found in uninfected cells, is ATP-dependent. Heller et al. (17) and Subak-Sharpe et al. (35) have shown that there is a selective inhibition of vaccinia multiplication by rifampicin. Uninfected tissue culture cells grow for several days in the presence of 100 μg of the antibiotic per ml, a concentration which inhibits the yield of infectious vaccinia by more than 99%. In an effort to test whether the vaccinia-induced ligase is virus-coded, the effect of this antibiotic on virus-infected HeLa cells was studied.

In control cultures, the yield of infectious WR strain increased from 4×10^5 PFU/ml at 5 hr after infection to 1.7×10^7 PFU/ml at 24 hr. In the presence of 100 μg of rifampicin per ml, the virus yield at 24 hr after infection remained at 2×10^5 PFU/ml. However, viral DNA synthesis was reduced only 20% by 100 μg of rifampicin per ml (Fig. 7). Ligase induction was inhibited to an even lesser extent. These observations do not indicate whether the new ligase activity is virus- or cell-coded but do suggest that rifampicin inhibits a late viral function. Consistent with this hypothesis is the finding (Table 2) that the vaccinia particle-associated RNA polymerase, which is known to be responsible for early viral messenger RNA synthesis (20), is not inhibited by rifampicin in vitro.

DISCUSSION

Conversion of SV40 component II DNA to component I has proved to be a useful method for assaying polynucleotide ligase, since the substrate is easily prepared and the two forms of DNA are readily separated by simple techniques. The major disadvantage of the assay is its extreme sensitivity to contaminating endonuclease. This difficulty was overcome by the addition of 0.1 M salt to the incubation mixture, making it possible to examine ligase activity in crude cell extracts. High levels of activity were found in all types of tissue culture cells examined. In every case, the ligase activity was ATP-dependent, as reported previously for the enzyme in rabbit tissues (26).

Polynucleotide ligases from bacteriophage T4-infected *E. coli*, uninfected animal cells grown in tissue culture, or cells infected with SV40, polyoma virus, or vaccinia virus are all capable of sealing SV40 component II DNA. This broad spectrum of activity is in agreement with the postulated role of ligases in the repair of damaged DNA. Some substrate selectivity apparently does exist, since component II resulting from storage of radioactive component I was not sealed by the enzyme.

In cells infected with SV40, polyoma virus, or vaccinia virus, the ligase activity increases. We have no evidence that this increase in activity is

caused by the appearance of a viral-coded enzyme. In contrast to *E. coli* in which the cellular and bacteriophage-induced enzymes require NAD and ATP, respectively (1, 32, 37, 40), the ligase activity in both uninfected and virus-infected animal cells is ATP-dependent. It is likely that the ligase induced in SV40- and polyoma virus-infected cells is another of that family of host enzymes concerned with DNA formation that increase in activity after infection. In vaccinia virus-infected cells, the newly formed ligase may be virus-coded since it appears in the cytoplasm

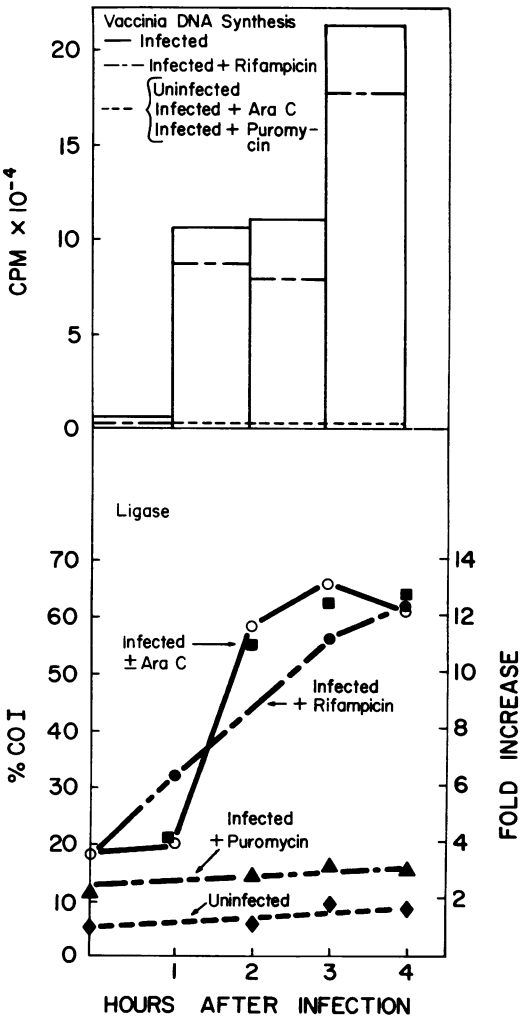

FIG. 7. *Increase in DNA synthesis and ligase activity in the cytoplasm of HeLa cells infected with vaccinia virus. Effect of cytosine arabinoside (10 μg/ml), puromycin (20 μg/ml), and rifampicin (100 μg/ml) are shown. Ligase activity was assayed in a sample of cell extract containing 0.5 μg of crude protein.*

TABLE 2. *Effect of rifampicin on vaccinia virus-associated RNA polymerase*[a]

Rifampicin concn (μg/ml)	RNA synthesis[b]
0	4.88
10	4.64
20	4.60
50	4.72
100	4.48

[a] Active particles were prepared by treating purified vaccinia virus with 2-mercaptoethanol and the detergent Nonident-P40 (20). Reaction mixtures contained 0.5 μmole each of ATP, guanosine triphosphate, cytosine triphosphate, and ^{3}H-uridine triphosphate (specific activity, 2 μc/μmole), 2.5 μmoles each of 2-mercaptoethanol and $MgCl_2$, 50 μmoles of Tris buffer (*p*H 9.0), 5 μmoles of sodium phosphoenolpyruvate, 100 μg of pyruvate kinase, and 0.25 mg of virus protein in a volume of 0.5 ml. After incubation for 1 hr at 35 C, the reaction was stopped by adding 5% trichloroacetic acid in ice. The acid-precipitable RNA product was filtered, washed with acid, and counted in toluene-Liquifluor.

[b] Expressed as nonomoles of uridine monophosphate incorporated per milligram of protein per hour.

with induction kinetics similar to those of other early enzymes which are known to be virus-coded (28).

Rifampicin inhibits the replication of poxyviruses (17, 35). In *E. coli*, the primary site of action of the antibiotic is the phosphocellulose fraction of RNA polymerase (8), and it has been suggested that rifampicin acts against poxviruses by inhibiting the particle-associated RNA polymerase (35). This seems unlikely since virus particles synthesized RNA in vitro as well in the presence of rifampicin as in its absence. Further, viral DNA synthesis and ligase induction in vaccinia virus-infected cells were only slightly affected by rifampicin. It is possible that the antibiotic acts in infected cells by binding to the newly synthesized viral RNA polymerase and either prevents expression of late functions or results in the formation of progeny virus particles which contain an inactive enzyme. In any case, it is clear that rifampicin cannot be used as a tool to determine which functions are coded for by the viral genome.

The possibility remains that there are specific ligases involved in the closure of newly replicated circular viral genomes and that these ligases are inactive in our assay. The detection of such enzymes will probably depend upon the isolation of viral mutants which fail to induce ligase activity at nonpermissive temperatures.

ACKNOWLEDGMENTS

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