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DNA-Processing Activities Associated with the Purified α, β_2, and αβ Molecular Forms of Avian Sarcoma Virus RNA-Dependent DNA Polymerase

AMNON HIZI,[1]* ARNONA GAZIT,[2] DAPHNA GUTHMANN,[2] AND ABRAHAM YANIV[2]

Department of Cell Biology and Histology[1] and Department of Human Microbiology,[2] Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel

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The RNA-dependent DNA polymerase purified from B77 avian sarcoma virus exhibited two distinct DNA-processing activities. The α and β_2 isoenzymes possessed an endodeoxyribonuclease activity capable of nicking simian virus 40 superhelical DNA, whereas the αβ isoenzyme performed as an untwisting topoisomerase. Both activities associated with the three molecular forms of the retroviral DNA polymerase were dependent on the presence of either Mn^{2+} or Mg^{2+} ions. From analysis of the denaturated DNA products, it is apparent that the α and β_2 isoenzymes introduced two nicks, one per each strand in the superhelical simian virus 40 DNA molecules, whereas the αβ polymerase converted these supercoiled molecules to the relaxed covalently closed circular form. The notion that the DNA-processing activities are located on the DNA polymerase molecules was supported by the following: (i) the three isoenzymes were of a high purity; (ii) the activities cosedimented in glycerol gradients with the DNA polymerase activities of the α, β_2, and αβ molecular forms; and (iii) immunoglobulin directed against the purified polymerase immunoprecipitated the DNA-processing activities. Chemical treatments of the DNA polymerase molecules (with pyridoxalphosphate, iodoacetamide, and sulfhydryl reagents), which inhibited the polymerase activity, also suppressed the endonucleolytic and topoisomerase activities, suggesting that cystein and amino groups play an important role in the active sites of the DNA-processing activities as well.

RNA-dependent DNA polymerase purified from a variety of avian and mammalian RNA tumor viruses has been shown to possess three enzymatic activities—RNA-dependent DNA polymerase activity, DNA-dependent DNA polymerase activity, and RNase H (21). All three activities are believed to be expressed by the same protein molecules. Other activities attributed to this enzyme, namely PP_i exchange and pyrophosphorolysis (20) are presumably associated with the catalysis of the DNA polymerization reaction.

Recently, a new group of enzymatic activities has been found to be associated with the DNA polymerase purified from avian myeloblastosis virus. The first one was an unwinding-like activity capable of removing hydrogen bonds from duplex nucleic acid molecules (2). The second was an endodeoxyribonucleolytic activity capable of nicking supercoiled DNA (4). This latter activity was associated only with the αβ isoenzyme, and evidence was presented that the active site for this activity is located within a unique sequence of the β subunit, which appears to be similar to the amino acid sequence of p32, a DNA endonuclease found in cores of avian myeloblastosis virus (AMV) (6, 19).

In the present paper, we report that the highly purified B77 avian sarcoma virus (ASV) RNA-dependent DNA polymerase exhibits two distinct DNA-processing activities, namely, an untwisting topoisomerase and an endodeoxyribonuclease, which are associated with the different molecular forms of the enzyme. The former activity is expressed by the αβ isoenzyme, whereas the latter is expressed by both the α and the β_2 isoenzymes.

MATERIALS AND METHODS

Preparation of DNA species. Supercoiled simian virus 40 (SV40) DNA (form I) was extracted from BSC-1 cells infected with a low multiplicity (0.001 PFU per cell) of plaque-purified SV40 by the method of Hirt (8). After treatment with phenol and chloroform-isoamyl alcohol, the superhelical DNA was purified by isopycnic centrifugation in cesium chloride containing ethidium bromide (18). The linear (form III) SV40 DNA was obtained after treatment with *Eco*RI restriction endonuclease in 100 mM Tris-hydrochloride, 50 mM NaCl, and 6 mM $MgCl_2$, final pH 7.9, for 30 min at 37°C.

RNA-dependent DNA polymerase. The αβ, α, and β_2

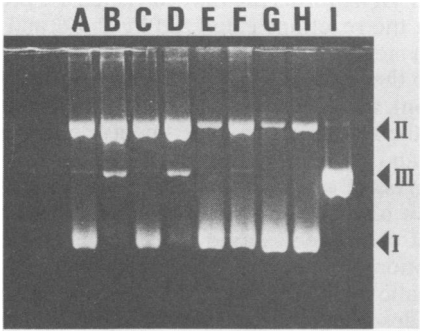

FIG. 1. Untwisting topoisomerase activity associated with the αβ form of ASV DNA polymerase. The purified polymerase (950 polymerase units) was incubated with 1 μg of supercoiled SV40 DNA at 37°C for either 0.5 h (lanes A, C, and E) or 5 h (lanes B, D, and F), in the presence of no divalent cation (lanes E and F), 5 mM $MgCl_2$ (lanes A and B), or 0.5 mM $MnCl_2$ (lanes C and D). Control incubations without enzyme were performed in the presence of $MgCl_2$ for either 0.5 h (lane G) or 5 h (lane H). Lane I: SV40 form III DNA marker (after treatment with *Eco*RI restriction endonuclease).

molecular forms of the B77 ASV RNA-dependent DNA polymerase were purified as described by Hizi and Joklik (9), by use of sequential chromatography on DEAE-cellulose, phosphocellulose, and polyuridylic acid-cellulose. The enzyme forms were highly purified, as judged from their pattern after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (9). The enzyme was assayed for the polymerase activity by monitoring the poly(rA)·oligo(dT)-directed incorporation of [^{3}H]dTMP into the acid-insoluble fraction (9). One polymerase unit was defined as the amount of enzyme which catalyzed the polymerization of 1 pmol of dTMP into acid-precipitable material during 30 min at 37°C.

Assay of the endodeoxyribonuclease activity. The DNase reactions were performed, unless otherwise stated, in a final volume of 20 μl of 10 mM Tris-hydrochloride, 100 mM NaCl, 10% glycerol, 2 mM dithioerythritol (DTE), and either 0.5 mM $MnCl_2$ or 5 mM $MgCl_2$, final pH 7.4. The incubation was carried out at 37°C as indicated in the text. The reactions were terminated by the addition of sodium dodecyl sulfate and EDTA to final concentrations of 1% (vol/wt) and 12 mM, respectively. The samples were prepared for electrophoresis by the addition of 10% sucrose and bromophenol blue as marker.

The DNA fragments were separated by electrophoresis under nondenaturing conditions on 1.4% agarose (SeaKem) horizontal slab gels. The electrophoresis was conducted, unless otherwise stated, at room temperature for about 16 h at 40 V in a solution of 40 mM Tris-hydrochloride, 20 mM sodium acetate, and 2 mM EDTA, final pH 7.8. After electrophoresis, the gels were stained for 30 min at room temperature with an ethidium bromide solution of 1 μg/ml. Gels were visualized and photographed under shortwave UV light.

RESULTS

DNA relaxation activity associated with the αβ ASV RNA-dependent DNA polymerase. Highly purified ASV DNA polymerase was examined for its activity on superhelical SV40 DNA (form I) in the absence or in the presence of either 0.5 mM $MnCl_2$ or 5 mM $MgCl_2$ (Fig. 1). This activity can be visualized from the conversion of the supercoiled DNA to the relaxed circular DNA in the reactions containing either Mg^{2+} or Mn^{2+}, to about the same extent (lanes A through D). On the other hand, very little enzymatic activity could be detected when these divalent cations were absent from the reaction mixture (lanes E and F). Some spontaneous relaxation of form I DNA, far below the enzyme-catalyzed reaction, occurred when no enzyme was present in the incubation mixture (Fig. 1, lanes G and H). This process, which is time dependent, was probably due to the reduction of the superhelical DNA by the dithioerythritol, since the conversion of form I to circular DNA in these controls was totally blocked when the reducing agent was absent in the incubation mixture and all the detectable DNA was migrating as superhelical DNA (not shown). Such a phenomenon of single-strand

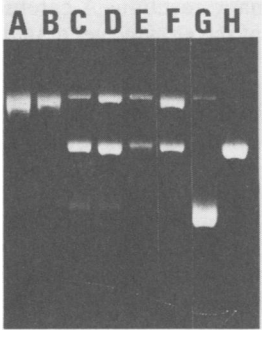

FIG. 2. DNA-processing activities associated with the αβ, α, and β_2 isoenzymes of ASV DNA polymerase. The three purified molecular forms of the ASV polymerase were incubated with 0.5 μg of SV40 supercoiled DNA in the presence of either 5 mM $MgCl_2$ (lanes A, C, and E) or 0.5 mM $MnCl_2$ (lanes B, D, and F) for 4.5 h. Lanes A and B, with 400 polymerase units of αβ polymerase; lanes C and D, with 350 polymerase units of the α polymerase; lanes E and F, with 210 polymerase units of the β_2 isoenzyme. Lane G, control of the superhelical DNA incubated with no enzyme for 4 h, in the presence of 5 mM $MgCl_2$; lane H, SV40 form III DNA marker.

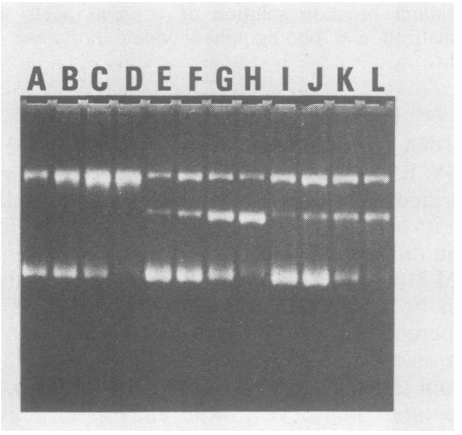

FIG. 3. Time dependence of the DNA-processing reactions. SV40 form I DNA (0.5 μg) was incubated, in the presence of 5 mM $MgCl_2$, with 150 polymerase units of αβ polymerase (lanes A through D), 130 polymerase units of α polymerase (lanes E through H), or 50 polymerase units of the β_2 isoenzymes (lanes I through L). The incubation times were 45 min (slots A, E, and I), 90 min (B, F, and J), 3 h (C, G, and K), and 6 h (D, H, and L).

scissions, introduced by reducing agents, was reported for other circular or superhelical DNA (1, 13).

It is important to note that the relaxed circular DNA is undoubtedly the predominant, but not the only, DNA product of the DNA polymerase-mediated enzymatic reaction. A careful inspection revealed that the enzyme generated a whole series of DNA products which migrated on the agarose gel between form I and the circular DNA (representing supertwisted DNA molecules with decreasing degrees of superhelicity), thus suggesting that we were dealing with an untwisting topoisomerase activity (14). In addition, there was some formation of linear DNA (form III) after long incubation periods when large amounts of enzyme were assayed.

DNA endonuclease activities associated with the α and β_2 forms of ASV DNA polymerase. The α and β_2 isoenzymes of the ASV DNA polymerase, although different from the αβ polymerase in their molecular structure as well as in some fine enzymatic features, still possess all the enzymatic activities hitherto assayed (9–11). Therefore, it was of interest to assay these forms for the DNA-processing activity as well. As can be seen from Fig. 2, both the α and β_2 isoenzymes displayed an enzymatic activity capable of nicking the supercoiled SV40 DNA in the presence of either Mn^{2+} or Mg^{2+}, thus leading to accumulation of linear double-stranded DNA (form III). As in the case of the αβ polymerase, no activity was observed when neither of the two divalent cations was present in the reaction (not shown). It is very likely that the nicked circular DNA forms are only intermediate products in the reactions catalyzed by the α and β_2 isoenzymes, which do not accumulate (in contrast to the products of the αβ enzyme-mediated reaction), a conclusion which is further supported by the results of the experiments depicted in Fig. 3 and 4.

Progress of the DNA-processing reactions as a function of both the time of incubation and the amount of enzyme assayed. To establish further the notion that we were dealing with a true enzymatic reaction, we conducted the experiments described in Fig. 3 and 4. Figure 3 shows the decrease in the amount of the substrate superhelical DNA with a concomitant accumulation of the product DNA (i.e., relaxed circular DNA in the case of the αβ polymerase and form III in the case of the α and β_2 isoenzymes) as a function of the incubation time. As expected, this reaction also depended strictly on the amount of enzyme present in the reaction mixture (Fig. 4). All the activities were heat sensitive, since a pretreatment for 2 min at 90°C abolished all the detectable activity (lanes D, H, and L for the αβ, α, and β_2 isoenzymes, respectively).

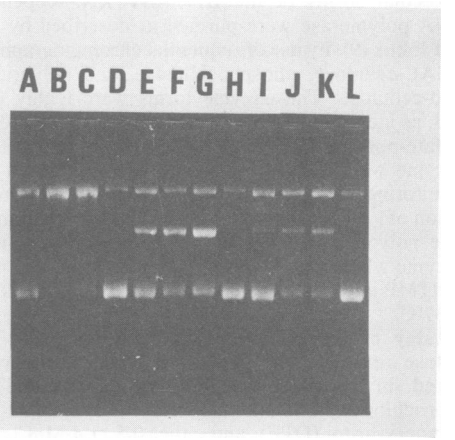

FIG. 4. Dependence of the DNA-processing reaction on the amount of DNA polymerase in the reaction mixture. SV40 supercoiled DNA (0.5 μg) was incubated for 3 h with increasing amounts of each of the purified ASV DNA polymerase isoenzymes, in the presence of 5 mM $MgCl_2$. Lanes A–D, after incubation with the indicated number of polymerase units of αβ polymerase: 100 (A), 200 (B), 450 (C), and 450 (D), after a pretreatment (2 min at 90°C) of the enzyme. Lanes E–H, incubated with the indicated number of polymerase units of α isoenzyme: 60 (E), 120 (F), 270 (G), and 270 (H), pretreated for 2 min at 90°C. Lanes I–L, after incubation with the indicated number of polymerase units of the β_2 form: 30 (I), 60 (J), 135 (K), and 135 (L), preheated for 2 min at 90°C.

Immunoprecipitation of the DNA-processing activities by the specific anti-DNA polymerase IgG. One way to support the notion that both the DNA polymerase and the DNA-processing activities are expressed by the same enzyme molecules is to demonstrate that they share similar antigenic determinants. This can be approached by two methods. The first is a direct immunoneutralization of the enzymatic activity by immunoglobulin G (IgG) prepared against the highly purified αβ ASV DNA polymerase. However, incubation of all three polymerase isoenzymes with this IgG preparation prior to the enzymatic assay, although resulting in a substantial neutralization of the RNA-directed DNA polymerase reaction, did not display any apparent effect on the endonucleolytic or topoisomerase reactions (data not shown).

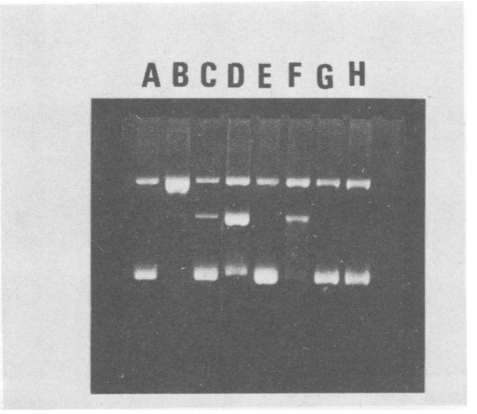

FIG. 5. Imunoprecipitation of the DNA-processing activities associated with the three DNA polymerase forms. The αβ, α, and β_2 ASV polymerase isoenzymes were treated as follows: 150 μg of either control rabbit IgG or rabbit IgG prepared against the purified ASV αβ polymerase (9) were mixed with 400 polymerase units of the enzyme, 350 polymerase units of the α form, or 210 polymerase units of the β_2 isoenzyme, in the presence of 10 mM Tris-hydrochloride, pH 7.5, 0.15 M NaCl, and 10% glycerol. The mixture was incubated for 10 min at 37°C, followed by an addition of 600 μg of purified goat IgG directed against rabbit IgG to each tube, for 10 min at 37°C and 16 h at 4°C. Then the tubes were centrifuged for 5 min at 10,000 × *g* at 4°C. One-third of the supernatant was assayed for DNA polymerase activity, and the rest was assayed for the DNA-processing activity. Under these conditions more than 90% of the αβ and α DNA polymerase activities were precipitated, whereas only 80% of β_2 polymerase was immunoprecipitated, as compared with the control rabbit IgG-treated enzyme. The DNA-processing activities were assayed by incubating 0.5 μg of the superhelical SV40 DNA with each of the samples tested, in the presence of 5 mM $MgCl_2$ for 4 h at 37°C. Lane A, αβ polymerase treated with immune rabbit IgG; B, αβ polymerase preincubated with control rabbit IgG; C, α polymerase with immune IgG; D, α polymerase with control IgG; E, β_2 isoenzyme with immune IgG; F, β_2 isoenzyme with control IgG; G, control with the preimmune rabbit IgG; H, control with the immune rabbit IgG.

The second method, a more general one, for demonstrating the presence of different enzymatically active sites on the same protein molecules is an immunoprecipitation of these activities by the specific antibodies, which is enhanced by adding a second antibody against this IgG. As demonstrated in Fig. 5, we could indeed immunoprecipitate both the DNA polymerase and DNA-processing activities of all three molecular forms of the DNA polymerase. It seems, however, that under the conditions employed the precipitation of the endonuclease activity associated with the α isoenzyme was somewhat less complete (lane C) as compared with αβ and β_2 enzyme forms (lanes A and E, respectively). The purified IgG used in this experiment was devoid of detectable DNase activity (lanes G and H) since the same spontaneous conversion of form I to form II DNA was detected when the superhelical DNA was incubated in the presence of only the reaction mixture (not shown).

These results support the idea that the polymerase molecules carry separate active centers for the DNA polymerase, on the one hand, and for the DNase or untwisting topoisomerase reactions, on the other. It is interesting to note that similar results (i.e., a weak immunoneutralization but a substantial immunoprecipitation) were also reported for the αβ form of AMV DNA polymerase (4).

Glycerol density gradient of the α, β_2, and αβ DNA polymerase isoenzymes. To support further the notion that the DNase or relaxation of supercoiled DNA activities are physically associated with the three molecular forms of the ASV DNA polymerase, we carried out the experiment depicted in Fig. 6. It is apparent that the DNA polymerase activities of each of the isoenzymes cosedimented with either the DNase or the topoisomerase activities of the α, β_2, or αβ isoenzymes, respectively, indicating similar molecular weights and, hence, a possible physical association.

Analysis of the denaturated DNA products of the DNA polymerase-associated activities. To study further the nature of the DNA-processing activities associated with the different molecular forms of the ASV DNA polymerase, we analyzed the DNA products after denaturating them (Fig. 7). The DNA products were denatured in alkali at 90°C for 1 min, rapidly chilled on ice, and electrophoresed at 4°C to prevent the reformation of double-stranded DNA. Under these conditions the bulk of the DNA did not reanneal (lanes B and D). It is evident that the predominant products of the αβ polymerase-mediated

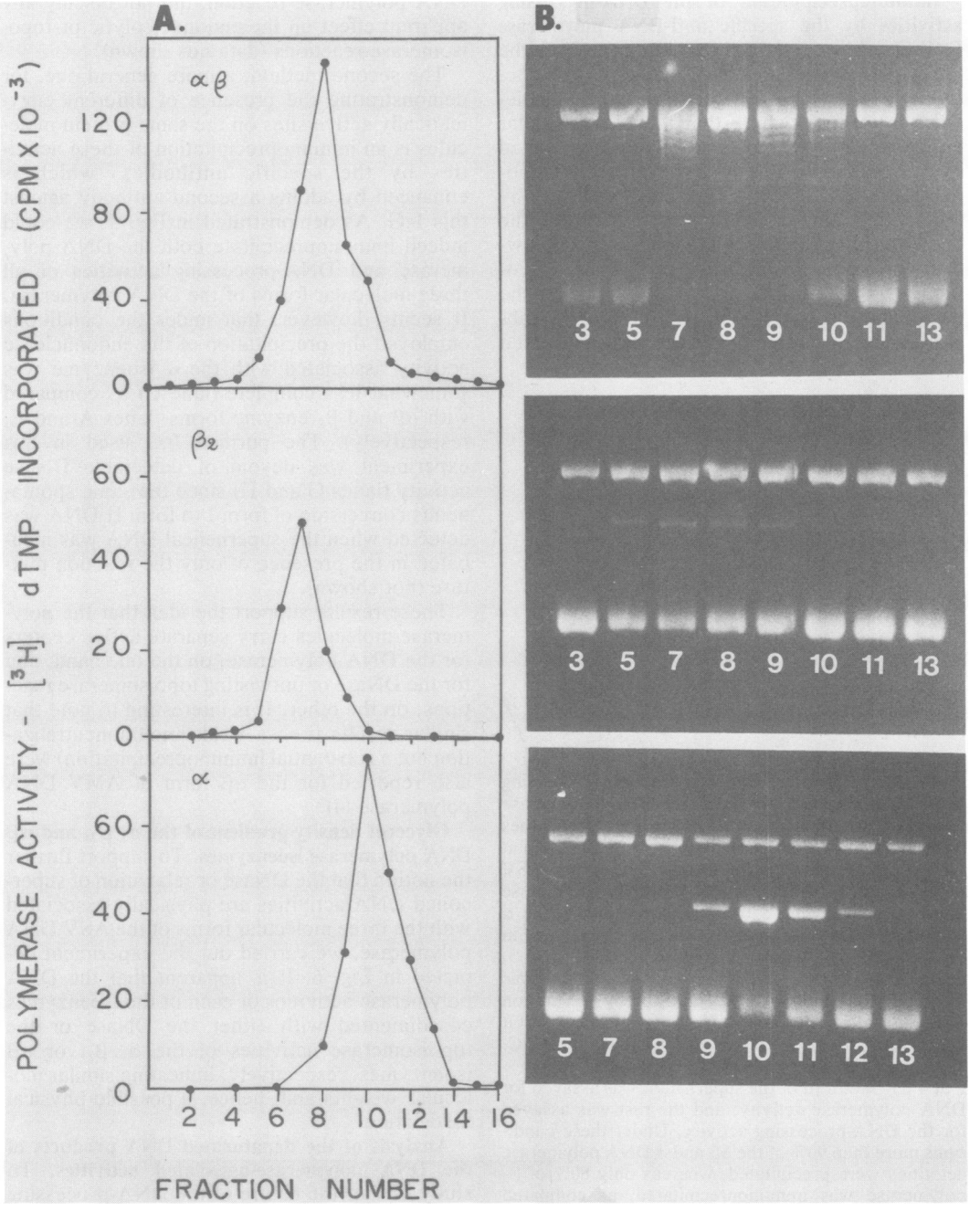

FIG. 6. Glycerol density gradients of the ASV DNA polymerase isoenzymes. Purified α, β_2, or αβ RNA-dependent DNA polymerase was layered on linear density gradients of 20 to 40% (vol/vol) glycerol in 25 mM Tris-hydrochloride, 0.05% Triton X-100, 0.4 M NaCl, and 2 mM dithioerythritol (final pH 7.4), prepared in Beckman SW50 rotor tubes. The tubes were centrifuged for 27 h at 46,000 rpm at 0°C. Fractions collected from the bottom of the tubes were assayed either for poly(rA)·oligo(dT)-directed DNA synthesis (A) or for the DNA processing activity in the presence of Mg^{2+} (B), as described in Material and Methods.

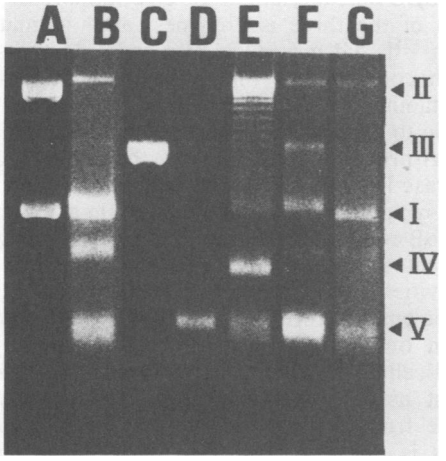

FIG. 7. Electrophoresis of the denatured DNA products derived from processing of SV40 DNA by the ASV polymerase-associated activities. DNA products were denaturated by heating the samples in 25 mM EDTA and 0.15 M NaOH for 1 min at 90°C and were rapidly chilled in an ice bath. The electrophoresis was performed at 4°C to minimize the reformation of double-strand DNA fragments. Slot A, a mixture of forms I and II SV40 DNA, undenatured; slot B, the same mixture after denaturation; slot C, *Eco*RI restriction endonuclease-treated SV40 DNA, undenatured; slot D, the same *Eco*RI-treated DNA after denaturation. Slots E–G, cleaved DNA products of the ASV polymerase-associated activities after denaturation. SV40 DNA form I was incubated in the presence of 5 mM $MgCl_2$ for 3 h, with 350 polymerase units of αβ polymerase (E), 300 polymerase units of α polymerase (F), or 150 polymerase units of the β_2 isoenzyme (G). The arrows mark the following: I through III indicate SV40 forms I, II, and III, respectively; IV, single stranded covalently closed circular DNA; V, unit-length single-stranded linear SV40 DNA.

reaction (in the presence of Mg^{2+}) are a covalently closed circular double-stranded DNA (lane E), supporting the idea that the αβ isoenzyme has an untwisting topoisomerase activity. This is further substantiated by the banding pattern of the DNA molecules between forms I and II. However, the presence of the closed circular and linear single-stranded DNA molecules suggests a minor activity of an endodeoxyribonuclease as well. Since the reducing agent present in the enzymatic mixture introduced single-stranded nicks in the DNA substrate (see above and reference 1), it seems quite likely that this minor DNase activity is due to dithioerythritol rather than to the enzyme itself. On the other hand, the products of the reaction with the α polymerase are mainly in the form of unit-length DNA (lane F), indicating the insertion of two nicks, one per strand, per superhelical SV40 DNA molecule. The pattern obtained with the β_2 isoenzyme in the presence of Mg^{2+} is similar to that of the α polymerase (lane G) with some heterogeneously degraded DNA, as visualized also when the DNA products were not denatured (Fig. 2 and 5).

Similar patterns of nicks introduced by the three ASV DNA polymerase isoenzymes were obtained when the enzymes were assayed in the presence of Mn^{2+} instead of Mg^{2+} (data not shown), the only exception being that the product of the β_2 polymerase was more homogeneous and most of it was in the form of unit-length linear SV40 DNA. This is compatible with the data presented above for the undenaturated products of the β_2 enzyme form (Fig. 2).

Effects of chemical modifications of the polymerase on the DNA-processing activities. The amino acid residues which are involved in the DNA-processing activities associated with the retroviral DNA polymerase were studied by chemical modifications of some of the residues known to take part in the active sites of the DNA polymerase reaction (Table 1). The DNA polymerase activity is very sensitive to the hydrophobic sulfhydryl reagents 5,5'-dithiobis (2-nitrobenzoic acid) and *N*-ethylmaleimide (NEM), with the latter also demonstrating some reactivity with amino groups (5, 15). Moreover, alkylation with iodoacetamide resulted in a substantial decrease in the DNA polymerase activity, probably as a result of reaction with cystein groups and possibly with amino groups, but to a lesser extent (15). Interestingly enough, we found that these treatments markedly inhibited the untwisting topoisomerase activity as well. In addition, pyridoxal phosphate, which was found to inactivate the RNA-dependent DNA polymerase activity by forming a Schiff base with reactive amino groups, without affecting the RNase H activity of the enzyme (16, 17), also inhibited the DNA topoisomerase activity. Similar results of inhibition were also obtained with the DNase activities of α and β_2 isoenzymes (not shown). Taken together, it is evident that cystein and amino groups play an important role in the active sites for the DNA-processing activities. RNAs such as tRNA, polyadenylic acid, and polyuridylic acid at concentrations as high as 200 μg/ml partially inhibited the activities of topoisomerase and DNase, probably as a result of some competition with the DNA (not shown).

DISCUSSION

The DNA polymerase isolated from B77 ASV consists of two subunits—β, with a molecular

weight of about 81,000, and α, with a molecular weight of 63,000. The amino acid sequences in the α subunit are a subset of those in β (3, 12). The αβ enzyme form, which is the predominant one in purified virions, is probably biologically the most important. However, all the enzymatic activities detected in the αβ polymerase were also found in the purified α or β_2 forms, with the latter having different fine enzymatic features, in terms of enzyme kinetics, enzyme specificity, and mechanism of enzyme-mediated catalytic reaction (9–11). In many respects the properties of the dimeric enzyme forms resemble each other much more closely than those of the α form. But in some very important respects, such as affinity to viral RNA and the size of the DNA transcripts formed from it, the αβ isoenzyme performs significantly better than either of the other two enzyme forms. It seems that, as far as the DNA-processing activities associated with the purified enzyme forms are concerned, the second category prevails, namely, the αβ enzyme form has a specificity different from that of the α and β_2 forms. The αβ molecular form behaves mainly as an untwisting topoisomerase, which leads to a substantial accumulation of the relaxed circular DNA. On the other hand, the α and β_2 isoenzymes perform as DNA endonucleases, introducing two nicks, one per each strand, thus producing linear DNA. The activities observed in presence of either Mg^{2+} or Mn^{2+} are similar, with the exception of the somewhat heterogeneous DNA produced by the β_2 isoenzyme in the presence of Mg^{2+}. One way to explain this latter phenomenon is that the β_2 polymerase has a Mg^{2+}-dependent, mixed endonucleolytic and exonucleolytic activity, which becomes strictly endonucleolytic when the Mg^{2+} is replaced by Mn^{2+}. Such a phenomenon was reported for the DNases specified by herpes simplex virus (13).

As to the identity of the proteins responsible for the DNA-processing activities, on the one hand, and the DNA polymerase, on the other, the enzymes used for the experiments described are highly purified (9). Nonetheless, there can always be a possibility of the presence of traces of protein components responsible for the DNA-processing activities, although an inspection of the SDS-polyacrylamide gel electrophoresis pattern of the three isoenzymes, after iodination with ^{125}I, did not reveal significant amounts of such contaminants (not shown). Such protein contaminants, if any, should be highly immunogenic to explain the immunoprecipitation of the DNA-processing activities by the anti-αβ polymerase IgG (Fig. 5). Moreover, the fact that the anti-αβ polymerase IgG inhibited, in addition to the αβ enzyme, the β_2 and α isoenzymes as well indicates an antigenic cross-reactivity among the activities in all three enzyme preparations. Since these enzyme forms were separated extensively from one another, especially on polyuridylic acid-cellulose column chromatography (9), one must assume that such contaminants should elute from the columns in very broad peaks. This is unlikely, however. Furthermore, it is evident that the molecular weights of each of the DNA-processing activities are similar to that of the DNA polymerase isoenzymes, as determined by sedimentation on glycerol gradients (Fig. 6). Taken together, there is quite good evidence that both the DNA polymerase and the DNase activities are expressed by the same enzyme proteins. Still, rigorous proof that the DNA-processing activities, like that of RNase H, are located on the same DNA polymerase molecules must await the identification of mutants deficient in both activities.

It is of interest to compare the activities we found with those reported for other avian oncornaviral DNA polymerases. The purified AMV DNA polymerase was found to nick superhelical ColE1 plasmid DNA (4). However, there are some discrepancies between the observed features of the AMV enzyme and those found by us for the ASV enzyme. The two major differences are (i) that the AMV enzyme nicked the super-

TABLE 1. Effects of different reagents on the RNA-dependent polymerase and topoisomerase activities[a]

Reagent	Concn (mM)	Inhibition of DNA polymerase[b] (%)	Inhibition of DNA topoisomerase activity[c]
N-Ethylmaleimide	10	99.8	++
5,5'-Dithiobis(2-nitrobenzoic acid)	5	99.9	++
Iodoacetamide	10	94.8	+
Pyridoxal phosphate	2	88.1	++
Sodium pyrophosphate	2	81.5	+

[a] Purified αβ ASV DNA polymerase (180 U) was mixed with each of the listed reagents and kept on ice for 15 min. Then one half of the mixture was assayed for the poly(rA)·oligo(dT)-directed DNA polymerase activity. The other half was assayed for untwisting topoisomerase activity (SV40 form I DNA was used as a substrate) for 3 h at 37°C, conditions under which only circular DNA was detected in the control (with no inhibitory reagents).

[b] The extent of the DNA polymerase inhibition was expressed as the percentage of initial activity which was destroyed.

[c] Symbols: ++, full inhibition of topoisomerase activity (only form I DNA was detected); +, partial inhibition, i.e., both the superhelical and the relaxed circular DNA were apparent.

helical DNA only in the presence of Mn^{2+} and was ineffective when Mg^{2+} was present and (ii) that the α isoenzyme of the AMV DNA polymerase lacked any endonucleolytic activity whatsoever. On the other hand, the same AMV DNA polymerase was shown by others (2) to possess an activity capable of removing hydrogen bonds from duplex nucleic acid molecules. This activity was dependent on the presence of either Mg^{2+} or Mn^{2+} ions and was associated with both the αβ and α isoenzymes. It seems to us that in our studies we are dealing with another facet of this latter activity. However, we do not understand the discrepancies between our findings and the earlier ones (4). Moreover, it seems that the differences are not due to the different superhelical DNA molecules used as substrates, since we observed similar activities when the SV40 DNA was replaced by supercoiled DNA from either ColE1 or pBR322 plasmids (results not shown).

The most interesting question concerning the DNA-processing activities associated with the oncornaviral DNA polymerase is the biological significance of these activities. It was extensively reported that the provirus DNA synthesized in cells infected with oncornaviruses includes circular and superhelical double-stranded DNA molecules which represent the total viral genome and accumulate in the nuclei of the infected cells. This DNA is a prerequisite for the integration of the viral genome in the cellular DNA (7, 22). Therefore, it is attractive to speculate that the viral DNA polymerase plays a role in processing these DNA molecules, before they are integrated into the host cell chromosomal DNA. On the other hand, it might be that the cellular nuclear DNA, into which the proviral genomes are integrated, is nicked by the DNA polymerase prior to and during this integration process. In any case, it would be conceivable that the endodeoxyribonuclease activity associated with the α and β_2 isoenzymes is capable of recognizing specific deoxynucleotide sequences and, hence, performing as a restriction endonuclease, whereas the untwisting-topoisomerase activity of the αβ DNA polymerase may be specific for relaxing the superhelical proviral DNA.

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