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Reversible Inactivation of the Deoxyribonucleic Acid Polymerase of Rauscher Leukemia Virus

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Rauscher leukemia virus deoxyribonucleic acid polymerase is reversibly inactivated by 6 M guanidine-hydrochloride. Gel filtration in 6 M guanidine-hydrochloride reveals that the viral deoxyribonucleic acid polymerase consists of a single polypeptide chain of approximately 70,000 molecular weight.

In earlier studies of the ribonucleic acid (RNA)-directed deoxyribonucleic acid (DNA) polymerase of Rauscher murine leukemia virus (RMuLV), a molecular weight for the enzyme was estimated, by gel filtration and sedimentation in glycerol gradients, to be 70,000 (9). Since only limited amounts of this enzyme are available, methods for determining its subunit structure, independent of enzyme activity, are difficult to use. Recently, Fleissner (3) reported that some of the proteins of avian myeloblastosis virus (AMV) could be renatured after treatment with 6 M guanidine-hydrochloride (GuHCl) followed by chromatography on an agarose column equilibrated with 6 M GuHCl (2). We took advantage of this technique to study the subunit structure of RMuLV DNA polymerase. The results presented in this communication describe the reversible inactivation of the RMuLV DNA polymerase by 6 M GuHCl and show that the active enzyme consists of a single polypeptide chain of approximately 70,000 daltons. This is in contrast to the results previously obtained with AMV DNA polymerase (5).

RMuLV and Schmidt-Ruppin Rous sarcoma virus (SR-RSV) DNA polymerases were purified as described by Ross (9). The polymerase from SR-RSV was a generous gift of J. Ross. Purified feline leukemia virus (Rickard strain) (FeLV) was obtained from Electro-Nucleonics, Bethesda, Md.

DNA polymerase activity was measured essentially as previously described (9, 10) using the synthetic template poly riboadenylic acid-oligo deoxythymidylic acid (poly rA·oligo $dT_{[12-18]}$, Collaborative Research, Waltham, Mass.) for RNA-directed activity and "activated" DNA (calf thymus DNA treated with deoxyribonuclease I) (9) for DNA-directed activity (see Table 2 for details).

Gel filtration chromatography on agarose equilibrated with 6 M GuHCl was carried out as described by Fish, Mann, and Tanford (2). Details are given in Fig. 1. The conditions used to reactivate the polymerase after treatment with GuHCl are also listed in the figure and tables.

RMuLV DNA polymerase, exposed to 6 M GuHCl, no longer had detectable activity. Enzyme activity can be restored by removing the GuHCl by dialysis. The requirements for restoration of activity by dialysis are presented in Table 1. Recovery of enzyme activity depends on the presence of Triton X-100 in the dialysis bag. Bovine serum albumin (BSA) can replace Triton X-100 to some extent. The effect of Triton or BSA may be related to enzyme sticking to the dialysis bag, since native enzyme activity is lost without either Triton or BSA by simply placing enzyme inside a dialysis bag without actually dialyzing it. Little activity is recovered unless dithiothreitol (DTT) and KCl are present in the dialysis buffer. The control enzyme is unstable relative to the GuHCl-treated polymerase. Under these conditions, BSA protects the activity of the native enzyme somewhat.

Since active enzyme could be recovered after GuHCl treatment, a molecular weight for GuHCl-treated enzyme was obtained by filtration through an agarose A-5m column (Bio-Rad Laboratories). Figure 1A is an elution profile of GuHCl-treated RMuLV polymerase from such a column. The activity of the dialyzed fractions elutes just ahead of BSA (69,000 molecular weight) and corresponds to a molecular weight of 74,000 (range from four experiments was 69,000 to 74,000). Enzyme activity as measured with "activated" DNA or poly rA·oligo dT was

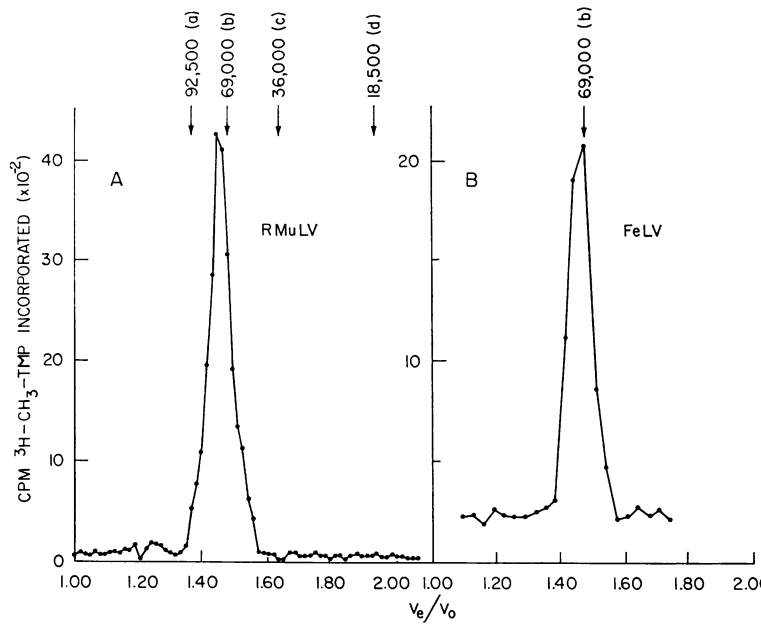

FIG. 1. *Gel filtration of MuLV and FeLV DNA polymerases in 6 M GuHCl. (A) Purified RMuLV DNA polymerase was dissolved in 0.2 ml of 6.0 M GuHCl, 10 mM DTT, and 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 8.5) and incubated at 25 C for 4 hr. Solid sucrose was added (10% w/v, final concentration), and the solution was layered onto an agarose column (Bio-Rad A-5m, 100 to 200 mesh) 83 by 1.0 cm which was previously saturated with 6 M GuHCl, 10 mM DTT, and 20 mM $NaPO_4$ buffer, pH 6.5. The enzyme was eluted with this buffer. Fractions of 1.0 ml were collected, made 0.01% in Triton X-100 (v/v), and dialyzed overnight against 1 mM Tris (pH 7.6), 0.3 M KCl, and 1 mM DTT, at 4 C. Fractions of 0.05 ml were assayed for enzyme activity, using poly rA·oligo dT as templates, as described in the text. (B) Concentrated FeLV (1.0 ml) was pelleted by centrifugation at 100,000 × g for 60 min. The pellet was dissolved in 8 M GuHCl, 10 mM DTT, 50 mM Tris (pH 8.5), 10 mM ethylenediaminetetraacetic acid, and 0.2% Triton X-100 (v/v) and heated for 20 min at 60 C. Column fractions were treated as described above. The column was calibrated with the following proteins whose subunit molecular weights are given in the figure: (a) phosphorylase a, (b) bovine serum albumin, (c) glyceraldehyde 3-phosphate dehydrogenase, and (d) β-lactoglobulin (3, 11). V_e represents the elution position of each marker (fractions were weighed), and V_o was determined using blue dextran (Pharmacia). The column was run at room temperature.*

usually recovered from the column in yields of 85%. Figure 1B is the elution profile for the DNA polymerase from disrupted FeLV. Activity elutes at a position corresponding to a molecular weight of 69,000. However, the enzyme was only recovered in 0.5 to 3.0% yield. (RMuLV DNA polymerase obtained from viruses disrupted in the same manner is recovered in approximately 70% yield.)

Some of the properties of enzyme that had been treated with GuHCl were studied and are presented in Table 2. In addition to becoming catalytically active again, the enzyme also retains immunological properties. Antibodies prepared to partially purified enzyme (8) inhibit the enzyme eluted from the GuHCl-agarose column by 89%. The control enzyme is inhibited by 67%, using the same amount of antiserum. The reason for the greater inhibition of the GuHCl-treated enzyme is not known. However, it is possible that there are catalytically inactive enzyme molecules in the native preparation that compete for the antibodies. The template preferences with respect to poly rA·oligo dT and "activated" DNA of enzyme after GuHCl-agarose gel filtration chromatography are the same as for control enzyme as also shown in Table 2. Thus, the GuHCl-treated enzyme prefers the synthetic template poly rA·oligo $dT_{[12-18]}$ over "activated" DNA to the same extent as the native polymerase. Studies of the control versus GuHCl-treated enzyme with viral RNA require larger amounts of enzyme and have yet to be completed.

The molecular weight of the RNA-directed DNA polymerase of RMuLV, estimated under nondenaturing conditions, was judged to be approximately 70,000 (9). Under denaturing conditions in 6 M GuHCl, a similar molecular weight has been obtained. The results suggest

that the DNA polymerase of RMuLV consists of a single polypeptide chain of approximately 70,000 daltons. This result was also obtained with the DNA polymerase from disrupted FeLV.

TABLE 1. *Requirements for reactivation*

Reactivation mixture[a]	Percent activity recovered after dialysis relative to untreated enzyme	
	GuHCl-treated	Control
Complete	89	11
−Triton X-100	24	8
−DTT	3	5
−KCl	31	4
+BSA−Triton X-100	75	50

[a] RMuLV DNA polymerase was incubated for 4 hr at 25 C in 50 mM tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride (*p*H 8.5); 10 mM DTT; 0.01% Triton X-100 (v/v) with or without 6 M GuHCl. The enzyme solutions were then dialyzed against 1 mM Tris-hydrochloride, 1 mM DTT, 0.3 M KCl, *p*H 7.6, (except where indicated) for 24 hr at 4 C. BSA was added to the dialysis bag (1 mg/ml). The enzyme solutions were assayed using poly rA·oligo $dT_{(12-18)}$ as template. The data represent the average of the values from four experiments. Specific assay conditions are listed in Table 2.

Although they have not been denatured with GuHCl, similar molecular weights have been obtained for the DNA polymerases of Mason-Pfizer monkey virus, murine mammary tumor virus, and all other mammalian C-type DNA polymerases studied so far. Thus, it appears that avian and mammalian tumor virus DNA polymerases, although quite similar in the reactions they catalyze (4), are different with respect to molecular weight and antigenicity (8, 10). AMV DNA polymerase has a molecular weight of 110,000 as determined by glycerol gradient centrifugation (4, 5). Analysis of the enzyme by polyacrylamide gel electrophoresis suggests that this enzyme may consist of two polypeptide chains of molecular weight of 69,000 and 110,000 (5). The enzyme from SR-RSV also has a molecular weight of 110,000 (1). We have not been able to reactivate SR-RSV DNA polymerase after treatment with 6 M GuHCl using the conditions described for the RMuLV enzyme.

Although the GuHCl-treated enzyme retains the same template specificity as the native polymerase with poly rA·oligo dT and "activated" DNA, it remains to be seen whether the DNA made from viral 70*S* RNA will be the same. It is possible that the renatured enzyme works less well with viral RNA or may catalyze the formation of long DNA molecules approaching the size

TABLE 2. *Comparison of template specificity and antigenicity of native and reactivated enzymes*[a]

Enzymes	Expt 1			Expt 2[b]	
	^{3}H-TMP incorporated/60 min (counts/min)[c]		Ratio (DNA/γAdT)	% Inhibition of poly rA·oligo $dT_{(12-18)}$-supported activity by antipolymerase serum	
	Poly rA.oligo $dT_{(12-18)}$ template	"Activated" DNA template		Control serum	Antiserum
Native enzyme	120,000	584	0.0049	<5	67
Renatured[d]	97,000	443	0.0046	<5	89
Renatured[e]	89,000	337	0.0037		

[a] Assay mixtures for DNA-directed and RNA-directed DNA synthesis contained in a final volume of 0.1 ml: 40 mM Tris-hydrochloride, *p*H 7.8; 2 mM DTT; and 3×10^{-5} M deoxythymidine triphosphate-*methyl-*^{3}*H* (37.5 μCi). The potassium chloride concentration was adjusted to 90 mM for all assays. For RNA-directed activity, the assay mixtures also included 1 mM manganous acetate, 3.75 μg of oligo thymidylic acid $(dT)_{(12-18)}$ per ml, and 60 μg of polyriboadenylic acid (poly rA) per ml as template. For DNA-directed activity, 5 μg of "activated" DNA; 1.5 mM magnesium acetate; and 0.25 mM deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxyadenosine triphosphate (P.L., Milwaukee, Wis.) were added. Even with purified polymerase, Triton X-100 (0.1% v/v) was included in all reaction mixtures to minimize sticking of dilute enzymes to the walls of the assay tubes. The assays were incubated for 60 min at 37 C and stopped with 10.0% trichloroacetic acid. The precipitated DNA was collected on membrane filters (Millipore Corp.) and counted in a liquid scintillation counter.

[b] Native and renatured enzymes were used at equal levels of activity (20,000 counts/min of ^{3}H-thymidine monophosphate [^{3}H-TMP] incorporated/60 min). Seventy micrograms of crude control or antiserum was used in each case.

[c] Backgrounds (290 counts/min-poly rA.oligo dT; 80 counts/min-DNA) were subtracted.

[d] Enzyme treated as described in Table 1.

[e] Enzyme eluted from agarose column, peak fraction.

of the viral RNA template, as opposed to the short pieces (6 to 7*S*) produced by the native enzyme (7).

A major caveat in the procedures is that, since only very small amounts of RMuLV DNA polymerase can be used, we have no direct evidence that the enzyme is completely denatured by 6 M GuHCl. All other enzymes that have been studied under these conditions have been found to be converted to single polypeptide chains (11). Furthermore, even after heating the RMuLV DNA polymerase at 60 C for 20 min in 6 M GuHCl, the same molecular weight was obtained. Fleissner (3), however, has found that certain proteins from AMV are aggregates in 6 M GuHCl, but dissociate in sodium dodecyl sulfate. Thus, confirmation of the molecular weight reported here will require molecular-weight determinations by methods employing sodium dodecyl sulfate. Such studies are in progress.

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