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Dissociation of αβ DNA Polymerase of Avian Myeloblastosis Virus by Dimethyl Sulfoxide

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The αβ DNA polymerase of avian myeloblastosis virus was treated with dimethyl sulfoxide to dissociate the enzyme subunits. The dimethyl sulfoxide-treated enzymes were passed over phosphocellulose to purify and characterize the dissociated subunits as well as to remove the dimethyl sulfoxide. RNA-directed DNA polymerase, RNase H, and nucleic acid-binding activity were monitored, as well as the subunit structure (on sodium dodecyl sulfate-polyacrylamide gels) of the various enzyme species obtained. With 30% dimethyl sulfoxide, the majority of DNA polymerase and RNase H activities as well as the α subunit were displaced from the αβ DNA polymerase position on phosphocellulose (0.23 M potassium phosphate) to the α DNA polymerase position (0.1 M). The association of DNA polymerase and RNase H activities with the α subunit suggests that α is the enzymatically active subunit in αβ. In addition to α DNA polymerase, a minor polymerase species eluted from phosphocellulose at 0.4 M potassium phosphate. The dissociated β subunit eluted from phosphocellulose at a wide range of salt concentrations (0.28 to 0.5 M potassium phosphate). The dissociated β subunit bound ^{3}H-labeled murine leukemia virus RNA and [^{3}H]poly(dT)·poly(dA) approximately 20-fold more avidly than α DNA polymerase alone. In contrast to the results with the α subunit, there was no correlation between DNA polymerase and RNase H activity profiles and the elution profile of the β subunit from phosphocellulose. These observations suggest the β subunit is either enzymatically inactive or possesses limited DNA polymerase and RNase H activity when compared with the α subunit.

Avian myeloblastosis virus (AMV) contains two species of RNA-directed DNA polymerase that can be resolved by phosphocellulose chromatography. The major enzyme species contains equimolar amounts of two polypeptide subunits, α and β, with molecular weights previously determined to be 65,000 and 105,000, respectively (3, 6). The minor species contains one subunit, α, which has the same molecular weight as the α subunit in αβ and is antigenically related to the two-subunit enzyme (3). In addition, there are similarities in amino acid sequence between the α and β subunits (2, 11). Subunit α can be generated from the β subunit by proteolytic cleavage (7). It is not known whether the β subunit, like α, possesses both DNA polymerase and RNase H activity (3, 14). To investigate what role β may have in proviral DNA synthesis, efforts were initiated to dissociate the αβ DNA polymerase into subunits while maintaining DNA polymerase and RNase H activity. I report here the results of experiments utilizing dimethyl sulfoxide treatment followed by phosphocellulose chromatography to achieve subunit separation. The results suggest that dissociated β is either enzymatically inactive or possesses limited DNA polymerase and RNase H activity, as compared with α.

MATERIALS AND METHODS

Materials. AMV, BAI strain A, was purified from frozen plasma virus kindly provided by Joseph Beard of Life Science, Inc., Fla., through the Virus Cancer Program of the National Cancer Institute. Sources for the radioactive and unlabeled materials were previously described (3, 4, 11). Dimethyl sulfoxide (certified spectranalyzed) and 1,4-dioxane (certified American Chemical Society) were obtained from Fisher Scientific Co., N.J. Subtiopeptidase A was from the Sigma Chemical Co. Rabbit globin mRNA was a kind gift from J. Lingrel.

Viral DNA polymerases. The α and αβ DNA polymerase of AMV were purified through DEAE-cellulose and phosphocellulose as described (3), except that the detergent-lysed virus was precipitated with solid ammonium sulfate (60% saturation) prior to the

DEAE-cellulose step. The phosphocellulose-purified αβ DNA polymerase was further purified as described below on a Sephadex G-100 column (1.6 by 80 cm) equilibrated with 20 mM Tris-hydrochloride (pH 8.0), 0.2 M KCl, 0.02% Nonidet P-40 (NP-40), 0.1 mM EDTA, 5 mM dithiothreitol, and 10% glycerol. First, the enzyme was precipitated with solid ammonium sulfate (60% saturation), was dialyzed against the above equilibrating buffer containing 20% glycerol, and was then passed over the Sephadex column at a flow rate of 18 ml/h. The peak fractions of DNA polymerase activity were pooled and concentrated by cyclic dialysis against glycerol (3). The purified enzymes were stored in a 50% glycerol buffer at −20 C.

Preparation of viral RNA. AMV 60–70*S* RNA was isolated from purified AMV by sodium dodecyl sulfate-phenol-chloroform extraction and zonal centrifugation (1). [^{3}H]uridine-labeled Moloney murine leukemia virus (MLV) 60–70*S* RNA was prepared as previously described (12).

DNA polymerase assays. Four different templates were used to assay DNA polymerase activity at 37 C. The incubation time was 15 min unless stated otherwise. The amount of polymer product formed was determined by assay on Whatman DEAE-cellulose paper disks (3). Enzymatic activity was linear with each template for at least 20 min. All reaction mixtures (0.1 ml) contained 20 mM Tris-hydrochloride (pH 8.0), 5 mM dithiothreitol, 10 mM $MgCl_2$, and 40 mM KCl.

Assay 1. Reaction mixtures contained 8 μg of poly(A), 1.6 μg of $(dT)_{12-18}$, and 90 μM [^{3}H]TTP (300 counts/min per pmol).

Assay 2. Poly(dA-dT) (3 μg) was the template in a reaction mixture containing 90 μM [^{3}H]TTP (300 counts/min per pmol) and dATP at 100 μM.

Assay 3. AMV 60–70*S* RNA (5.0 μg) was the template in a reaction mixture containing 10 μM [^{3}H]TTP (32,000 counts/min per pmol), dATP, dGTP, and dCTP (each 100 μM).

Assay 4. Rabbit globin 9*S* mRNA (0.62 μg) with $(dT)_{12-18}$ (0.5 μg) was the template-primer in a reaction mixture containing 95 μM [^{3}H]dGTP (4,700 counts/min per pmol), dATP, dCTP, and TTP (each 100 μM).

RNase H assay. The assay for RNase H activity was carried out at 37 C by using [^{3}H]poly(A)·poly(dT) and monitoring the degradation of [^{3}H]poly(A) to acid-soluble material (3). Incubation was for 30 min unless otherwise stated. The reaction mixture (0.1 ml) contained 10 mM Tris-hydrochloride (pH 8.0), 5 mM dithiothreitol, 10 mM KCl, 5 μM [^{3}H]poly(A) (50 counts/min per pmol), and 20 μM poly(dT).

Binding assay. The binding assay was performed as described below. Various parameters of the nitrocellulose filter assay technique were previously described (5). Both α and αβ DNA polymerase were retained on the nitrocellulose filters (Millipore Corp.) in the absence of any nucleic acid. No polymerase activity was detectable in the filtrates. To demonstrate that the bound polymerase was enzymatically active, the filters with bound polymerase were immersed in a DNA polymerase substrate mixture containing poly(A)·$(dT)_{12-18}$. After incubation, the filters were washed with 10% cold trichloroacetic acid containing TTP (0.001 M) and 5% sodium pyrophosphate. Because the enzymes were not in solution, the recovery of DNA polymerase activity was difficult to estimate but it appeared to be approximately 60% for both enzymes.

The reaction mixture (0.1 ml) for the binding assay contained 10 mM Tris-hydrochloride (pH 8.0), 5 mM dithiothreitol, 10 mM KCl, 10 mM $MgCl_2$, and one of the following substrates: 15 μM [^{3}H]poly(dA-dT) (1,400 counts/min per μmol); 2.5 μM [^{3}H]poly(dT) (2,500 counts/min per μmol) coupled with cold poly(dA) (1.35 μM); or ^{3}H-labeled MLV 60–70*S* RNA (2,900 counts/min per assay). The viral RNA was heat denatured for 3 min at 85 C. This was necessary to reduce the background for the nitrocellulose filter assay. After the enzyme was allowed to bind to the labeled polymer at 37 C for 3 min, the reaction mixture was diluted with 2 ml of diluting buffer and immediately filtered through nitrocellulose filters (Millipore, type HA, 0.45 μm) at a flow rate of 4 ml/min. The amount of polymer retained on the filter was directly proportional to protein concentration. The diluting buffer was the same as the reaction mixture, except that the KCl concentration was 40 mM. The filters were dried, and the amount of bound radioactive polymer was measured by counting in a toluene-based scintillation fluid.

Dissociation of αβ DNA polymerase with dimethyl sulfoxide. The αβ DNA polymerase was purified by phosphocellulose chromatography or Sephadex G-100 as described previously. The dissociation mixture consisted of 20 mM Tris-hydrochloride (pH 8.0), 5 mM dithiothreitol, 0.5 M KCl, 0.1% NP-40, 10% glycerol, and varying amounts of dimethyl sulfoxide as indicated. Low-salt-dissociation buffer was the same as above except the KCl concentration was 50 mM. All of the steps were carried out at 0 C. The final enzyme concentration in the dissociation mixture was approximately 50 μg/ml. The enzyme was always added last because exposure to dimethyl sulfoxide at higher concentrations (>50%) apparently results in a rapid, irreversible denaturation of the DNA polymerase molecule. The enzyme was usually dissociated for 10 to 20 min followed by dilution (1/13) in small aliquots in a buffer containing 10% glycerol, 5 mM dithiothreitol, 0.1% NP-40, and 10% dimethyl sulfoxide. This dilution step was necessary to decrease the salt concentration prior to chromatography on phosphocellulose. The low-salt-dissociation buffer was diluted only 1 to 1. The diluted enzyme was applied at a flow rate of 30 ml/h to a phosphocellulose column (0.9 by 6 cm) equilibrated with 10 mM potassium phosphate (pH 8.0), 2 mM dithiothreitol, 0.02% NP-40, 0.5 mM EDTA, and 10% glycerol (buffer A). Buffer B was the same except that the potassium phosphate concentration was 0.5 M. After a wash with 6 to 8 ml of buffer A, the column was developed with 60- or 80-ml linear gradient of 0.01 to 0.5 M potassium phosphate and was then washed with 5 ml of buffer B. Fraction volumes were between 1.6 and 2 ml. The salt concentration was determined conductimetrically.

Sodium dodecyl sulfate-polyacrylamide disc gel

electrophoresis. After enzymatic analysis, various fractions of the phosphocellulose-purified enzymes were pooled as indicated and dialyzed in water at 0 C for 48 h with repeated changes to remove the salt and NP-40. The samples were then dialyzed in 0.1% sodium dodecyl sulfate in water at room temperature. Samples of 5-ml volume were dialyzed for 20 min, whereas 10-ml samples were dialyzed for 10 min to obtain approximately equal amounts of sodium dodecyl sulfate inside the dialysis tubing. The samples were then lyophilized, dissolved in 70 μl of 10 mM sodium phosphate (pH 7.0) containing 1% 2-mercaptoethanol and 10% glycerol, and followed by denaturation for 10 min at 100 C. This material was subjected to sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis as described (3). The molecular weights of α and β were 62,000 and 92,000, respectively. Proteins used as standard markers were phosphorylase A, bovine serum albumin, ovalbumin, human gamma globulin, chymotrypsinogen, and cytochrome C.

RESULTS

Different physical states of AMV DNA polymerase. With the aging of phosphocellulose-purified αβ DNA polymerase preparations, the amount of α DNA polymerase that can be isolated from αβ by phosphocellulose chromatography was increased severalfold (3, 11). A typical activity profile of aged αβ DNA polymerase after passage over phosphocellulose is shown in Fig. 1A. Beginning from the left, the three peaks of DNA polymerase activity eluted at 0.1, 0.23, and 0.4 M potassium phosphate, respectively. In the remainder of this report, DNA polymerase activity that elutes from phosphocellulose at 0.1 M will be called α. In spite of the fact that, under certain conditions (see later sections), the relative molar ratio of β to α is not always maintained at 1 in the enzyme activity

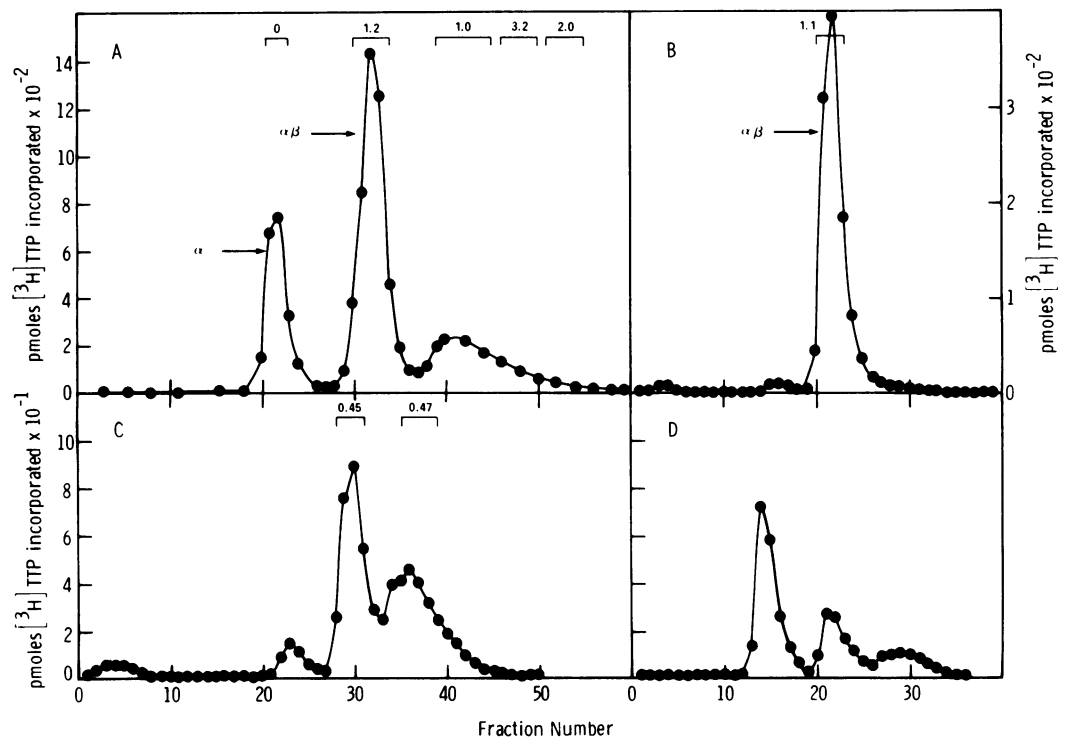

FIG. 1. *Different states of AMV DNA polymerase. (A) Several phosphocellulose-purified αβ DNA polymerase preparations (at least 5 months old) were pooled, diluted with buffer A to 30 mM potassium phosphate, and applied to a phosphocellulose column (0.9 by 15 cm) that was developed with a linear gradient. Samples (20 μl) from each fraction were assayed for 15 min as described in the text with poly(A)·(dT)$_{12-18}$. Recovery of DNA polymerase activity was 80%. (B) αβ DNA polymerase (fractions 30 to 34 of A) were pooled and concentrated by cyclic dialysis against 50 and 10% glycerol (3). This freshly prepared αβ DNA polymerase pool (130 μg) was diluted as described in A and applied to a phosphocellulose column (0.9 by 6 cm) and developed with a linear gradient. Samples were assayed as described above with 46% polymerase activity recovered. (C) Fractions (39 to 44) as shown in A were pooled and concentrated by cyclic dialysis. This enzyme preparation (180 μg) was diluted and analyzed as described in A. Recovery of DNA polymerase activity was 45%. (D) αβ DNA polymerase (40 μg) (fractions 30 to 34 of A) was dissociated with 50% dimethyl sulfoxide and passed over phosphocellulose as described in the text. Samples of these fractions were assayed as described in A.*

that elutes at 0.23 M, the peak eluting at this position will be called αβ. The sedimentation properties and the physical interaction of the α and β subunits in the third peak of polymerase activity have not been investigated, and this enzyme species will be referred to as peak III.

The association of the α and β subunits in αβ DNA polymerase and in peak III appears to be different. Repassage of freshly prepared αβ (Fig. 1A, fractions 30 to 34) over phosphocellulose yielded a single major enzyme species (Fig. 1B) (3, 11). But, repassage of peak III (Fig. 1A, fractions 39 to 45) over phosphocellulose generated three peaks of polymerase activity eluting at 0.1 M, 0.23 M, and 0.4 M potassium phosphate, respectively (Fig. 1C). These results suggest a weaker association between the α and β subunits in this polymerase species than in αβ. Treatment of αβ (control shown in Fig. 1B) with 50% dimethyl sulfoxide prior to chromatography on phosphocellulose resulted in the same three peaks of polymerase activity (Fig. 1D). α DNA polymerase rechromatographs on phosphocellulose as a single peak eluting at 0.1 M potassium phosphate (data not shown) (3).

Various fractions, as indicated in Fig. 1, were pooled and the proteins present were analyzed by sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis. Reexamination of the molecule weights of α and β using this technique demonstrated that the subunit sizes were 62,000 and 92,000, respectively. The amount of β relative to α in the various pools was determined by making densitometric tracings of sodium dodecyl sulfate-polyacrylamide gels stained with Coomassie blue and measuring the area of tracings of stained protein bands (3). The bars in Fig. 1 indicate the fractions pooled and the numbers represent the relative molar ratio of β to α.

Dissociation of αβ DNA polymerase by dimethyl sulfoxide. As shown in the previous section, the AMV DNA polymerase can exist in a wide variety of states with respect to subunit composition, ranging from a single polypeptide, α, to different complexes composed of β and α in greatly variable relative molar ratios (Fig. 1A). Pretreatment of αβ DNA polymerase with a combination of dimethyl sulfoxide and high salt (0.5 M KCl) was sufficient to disrupt ionic and hydrophobic bonds holding the subunits together while maintaining polymerase activity (Fig. 2). The dimethyl sulfoxide-treated enzymes were passed over phosphocellulose to purify the dissociated subunits and to remove the dimethyl sulfoxide. Prior treatment of αβ with increasing concentrations of dimethyl sulfoxide (>15%) resulted in a decrease in the amount of enzyme eluted from phosphocellulose at 0.23 M potassium phosphate, with 30% dimethyl sulfoxide being optimal for dissociation and recovery of total DNA polymerase activity. Similar data were obtained with 0.05 M KCl and 30% dimethyl sulfoxide. Dissociation of αβ with 50% dimethyl sulfoxide resulted in the same DNA polymerase and RNase H activity profile as that obtained with 30% dimethyl sulfoxide (see Fig. 5 for RNase H). This higher concentration of dimethyl sulfoxide decreased the recovery of total DNA polymerase activity to approximately 30%.

α DNA polymerase was also pretreated with 50% dimethyl sulfoxide and 0.5 M KCl followed by chromatography on phosphocellulose. α DNA polymerase eluted as a single peak from phosphocellulose at 0.1 M potassium phosphate, with a 36% recovery of DNA polymerase activity (data not shown).

Effect of dimethyl sulfoxide on stability of AMV DNA polymerases. Dimethyl sulfoxide does not preferentially inactivate either DNA polymerase or RNase H activity (Table 1). Inactivation of these two activities appears to be dependent on the dimethyl sulfoxide concentration and the time of exposure to dimethyl sulfoxide. αβ DNA polymerase and RNase H activities were unstable under these high-salt conditions minus dimethyl sulfoxide. But, by decreasing the KCl concentration from 0.5 to 0.05 M KCl, the half-life of αβ DNA polymerase and RNase H activity with 30% dimethyl sulfoxide was approximately 1 h. There was no detectable difference in the catalytic properties of either α or αβ DNA polymerase isolated from dimethyl sulfoxide-treated αβ and these two enzymes isolated without treatment with dimethyl sulfoxide (Table 2) (3, 11). During polymerization, there was no effect on DNA polymerase activity using poly(A)·$(dT)_{12-18}$ with up to approximately 4% dimethyl sulfoxide present.

Subunit composition of the three DNA polymerase peaks. The subunit composition of the various eluted DNA polymerase species obtained from αβ DNA polymerase dissociated with varying amounts of dimethyl sulfoxide was investigated (Fig. 2). The amounts of α and β were calculated from the area of each subunit stained with Coomassie blue and measured by densitometric tracings of sodium dodecyl sulfate-polyacrylamide gels, as shown in Fig. 3. The amount of α DNA polymerase activity as well as the amount of the α polypeptide increased with increasing amounts of dimethyl

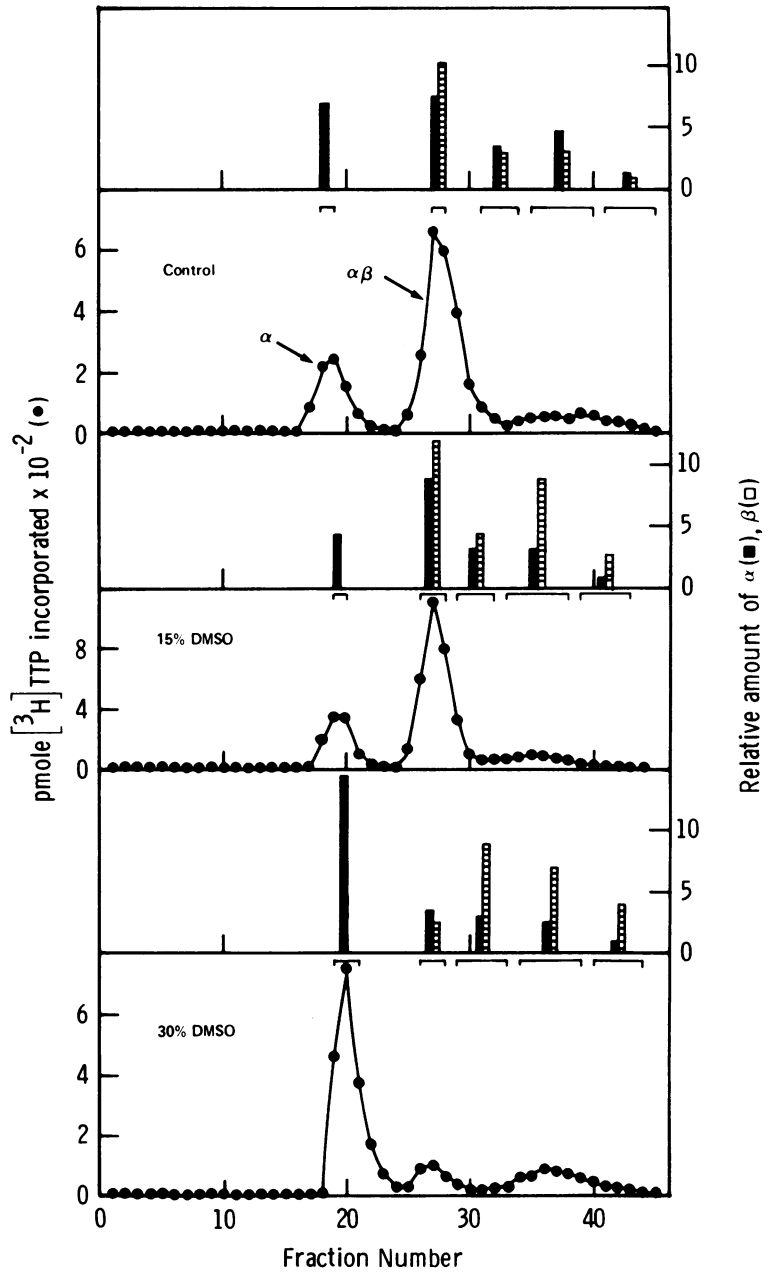

FIG. 2. *Dissociation of αβ DNA polymerase by dimethyl sulfoxide. Aged αβ DNA polymerase (4 months) (105 μg) was dissociated with 0, 15, and 30% dimethyl sulfoxide and then analyzed by phosphocellulose chromatography as described in the text. Samples (20 μl) were assayed from each fraction with poly(A)·(dT)$_{12-18}$. The first 10 fractions were assayed with 5 μl to avoid having a high concentration of dimethyl sulfoxide (DMSO) present in the reaction mixtures. Recovery of DNA polymerase activity was 67, 90, and 54%, respectively. Various fractions were pooled and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described in the text. The bars indicate the fractions pooled. The total volume of each enzyme pool was analyzed on gels. The amount of α and β after staining with Coomassie blue was determined by calculating the area from densitometric tracings (3). The area under each subunit was expressed as a histogram above the pooled fractions (■, α; □, β). The relative molar ratios of β to α are (left to right): (control), 0, 0.93, 0.66, 0.57, and 0.65; (15%), 0, 0.99, 0.92, 1.97, and 3.0; (30%), 0, 0.49, 1.86, 1.90, and 3.5.*

TABLE 1. *Effect of dimethyl sulfoxide on stability of DNA polymerase and RNase H activity*[a]

Time (min)	Dimethyl sulfoxide (%)					
	0	15	30	0	15	30
	DNA polymerase (% of control)			RNase H (% of control)		
0	100	71	58	100	97	42
20	92	78	39	80	62	26
60	75	61	28	55	57	14
90	65	48	17	30	47	10

[a] αβ DNA polymerase (50 μg/ml) was incubated in 0.02 M Tris-hydrochloride (pH 8.0), 0.5 M KCl, 5 mM dithiothreitol, 0.1% NP-40, and varying amounts of dimethyl sulfoxide. Samples were taken at the indicated times and assayed for DNA polymerase and RNase H activity using poly(A)·$(dT)_{12-18}$ and [^{3}H]poly(A)·poly(dT), respectively. At 100%, the enzyme incorporated 684 pmol of [^{3}H]TTP and solubilized 48 pmol of [^{3}H]poly(A).

sulfoxide used for dissociation of αβ DNA polymerase (Fig. 2 and 3). This increase in α DNA polymerase was associated with a corresponding decrease in the amount of DNA polymerase activity eluting at 0.23 M potassium phosphate. This highly purified α subunit possesses all the catalytic properties of α obtained from αβ DNA polymerase preparations by different procedures (3, 11).

By dividing the total amount of DNA polymerase activity in the pooled α DNA polymerase peak by the area of α obtained from its corresponding stained gel, there was a direct correlation between activity and protein. The ratios of activity to protein for α DNA polymerase using 0, 15, and 30% dimethyl sulfoxide are 70, 91, and 102, respectively. These data suggest that the specific activity of the α subunit was not appreciably changed by exposure to dimethyl sulfoxide.

The treatment of αβ DNA polymerase with 30% dimethyl sulfoxide also resulted in a major displacement of the β subunit eluted from phosphocellulose, previously eluting at 0.23 M potassium phosphate as αβ, to higher salt concentrations. The relative molar ratio of β to α in the fractions eluting after 0.23 M increased three- to fourfold after treatment of αβ with 30% dimethyl sulfoxide, in comparison to the control enzyme (Fig. 2). There was no correlation between the DNA polymerase or RNase H activity profile and the elution profile of the β subunit from phosphocellulose, in contrast to that found with the α subunit (Fig. 2, 3, and 5). With 30% dimethyl sulfoxide-treated enzyme, 52% of the β subunit was located where only 9% of the DNA polymerase activity was present (Fig. 2, fractions 29 through 34). These observations suggest that the β subunit alone may not have polymerase or nuclease activity.

Conditions were selected to minimize proteolysis of the β subunit by a contaminating protease present in some enzyme preparations. The quantity of the 25,000-molecular-weight fragment obtained by proteolysis during dissociation of αβ with 30% dimethyl sulfoxide was decreased approximately 20% when the enzyme was dissociated in the presence of 0.5 M KCl rather than 0.05 M KCl (see Fig. 3 and data not shown). Dissociation of αβ with dimethyl sulfoxide and high salt in the presence of a protease inhibitor, phenyl-methylsulfonyl-fluoride (2 mM), had no effect on the recovery or distribution of DNA polymerase activity or the quantity of the 25,000-molecular-weight fragment eluted from phosphocellulose, in comparison to experiments in which no inhibitor was present.

Dissociation of freshly purified αβ DNA polymerase by dimethyl sulfoxide. As previously shown, 30% dimethyl sulfoxide was sufficient to dissociate aged αβ DNA polymerase (Fig. 2). However, freshly purified αβ was only partially dissociated with 50% dimethyl sulfoxide, with a 74% recovery of DNA polymerase activity (Fig. 4B). Figure 4A shows the DNA polymerase activity profile of αβ without dimethyl sulfoxide treatment. These results suggest that the association of α and β is weakened upon storage, allowing them to be more easily dissociated. As observed with 30% dimethyl sulfoxide-treated enzyme, the α and β subunits are displaced from the original eluting position at 0.23 M. The major eluting peak of β from phosphocellulose does not correspond with the three peaks of polymerase activity, again suggesting that the β subunit is enzymatically inactive (Fig. 4B). These enzyme species had the same catalytic properties as the enzymes shown in Fig. 5. There was no 25,000-molecular-weight fragment generated when this enzyme preparation was denatured with dimethyl sulfoxide, suggesting that no contaminating protease was present.

Catalytic properties of dissociated αβ DNA polymerase subunits. After treatment with 30% dimethyl sulfoxide, αβ was chromatographed on phosphocellulose and the fractions were assayed with different templates and substrates (Fig. 5). The ratio of DNA polymerase activity with poly(A)·$(dT)_{12-18}$ or poly(dA-dT) (data not shown) and RNase H activity with

TABLE 2. *Comparison of the catalytic properties of dimethyl sulfoxide-treated AMV DNA polymerases*[a]

Enzyme	Dimethyl sulfoxide %	pmol of [^{3}H]TTP incorporated		Solubilized RNase H (counts/min)	Retained binding (counts/min)
		Poly(A)·$(dT)_{12-18}$	70S RNA		
α	0	240	0.08	922	422
	30	616	0.18	1,622	1,214
	50	695	0.24	1,839	1,763
$\alpha\beta$	0	626	1.84	1,365	3,710
	30	110	0.33	287	899
	50	94	0.23	214	972

[a] $\alpha\beta$ DNA polymerase was exposed to 0, 30, and 50% dimethyl sulfoxide and then chromatographed on phosphocellulose as described in the text (see Fig. 2). In each case, α and $\alpha\beta$ DNA polymerase peaks were pooled and then assayed for polymerase activity with poly(A)·$(dT)_{12-18}$ and AMV 60–70S RNA, for RNase H with [^{3}H]poly(A)·poly(dT), and for binding with [^{3}H]poly(dA-dT). Proportionality between reaction velocity and enzyme concentration was determined for each fraction with each template or substrate by assaying at two different enzyme concentrations. The values shown were obtained using 20 μl of enzyme for polymerase activity and 10 μl for the other two assays.

[^{3}H]poly(A)·poly(dT) as substrate for each DNA polymerase species was relatively constant, in agreement with previous published results (Fig. 5A) (3, 11). This ratio of DNA polymerase to RNase H activity increases severalfold in peak III due to the sensitivity of RNase H activity to high salt in reaction mixtures. The ratio of these two activities with all three peaks of polymerase was very similar when assayed under the same salt concentration. $\alpha\beta$ utilizes AMV 60–70S RNA as a template approximately seven times faster than α DNA polymerase, in comparison with the ability of each enzyme to utilize poly(A)·$(dT)_{12-18}$ as template-primer (Fig. 5B) (11). The activity of peak III was similar to that of $\alpha\beta$ with AMV 60–70S RNA, although not quite as great (Fig. 5B). The inability of α DNA polymerase to effectively copy viral RNA does not carry over to other heteropolymeric RNAs such as mRNA. α DNA polymerase utilizes rabbit globin 9S mRNA coupled with $(dT)_{12-18}$ as efficiently as $\alpha\beta$ or peak III (Fig. 6A and B).

Binding properties of dissociated subunits. α and $\alpha\beta$ DNA polymerases utilize poly(A)·$(dT)_{12-18}$ and poly(dA-dT) as templates for DNA synthesis. α DNA polymerase retains [^{3}H]poly(dA-dT) on nitrocellulose filters, but $\alpha\beta$ and peak III retain this polymer on filters three- to fourfold more avidly than α DNA polymerase in comparison to the ability of each enzyme to use poly(A)·$(dT)_{12-18}$, suggesting an important binding role for the β subunit (Fig. 6A) (4, 5). Neither enzyme can utilize poly(dT)·poly(dA) effectively as a template (15, unpublished data). This property may distinguish between the active polymerase-binding site and other possible nucleic acid-binding site(s) on either the α or β subunits. As shown in Fig. 6A, α does not retain [^{3}H]poly(dT)·poly(dA) on nitrocellulose filters, whereas $\alpha\beta$ and peak III retained this labeled polymer on filters. In addition, $\alpha\beta$ or peak III bound ^{3}H-labeled MLV viral RNA approximately 30- to 40-fold more strongly than α DNA polymerase, compared with their ability to synthesize DNA using either poly(A)·$(dT)_{12-18}$ or 9S mRNA (Fig. 6A and B).

A peak of binding activity not coincidental with polymerase activity was also observed eluting at 0.32 M potassium phosphate between $\alpha\beta$ and peak III (Fig. 6A and B). This peak of binding activity was coincidental with the 25,000-molecular-weight peptide apparently generated by cleavage of the β subunit by a contaminating protease during dimethyl sulfoxide treatment (Fig. 3). It is not known whether this small peptide binds to nucleic acids or the binding is due to β present in the same region. This same molecular weight peptide can be generated by treating $\alpha\beta$ DNA polymerase with substilopeptidase A (data not shown).

DISCUSSION

$\alpha\beta$ DNA polymerase from AMV was dissociated using dimethyl sulfoxide, followed by analysis on phosphocellulose chromatography. Large quantities of highly purified α DNA polymerase possessing RNase H activity were dissociated from the $\alpha\beta$ enzyme complex while an intact β subunit was maintained. The β subunit appears to be enzymatically inactive, although it enhances the binding of α in $\alpha\beta$ to nucleic acids (4, 5, 8).

Several reagents (urea, guanidine hydrochloride, formamide, 1,4-dioxane, and dimethyl sulfoxide) were utilized for dissociation of $\alpha\beta$ DNA polymerase subunits while trying to main-

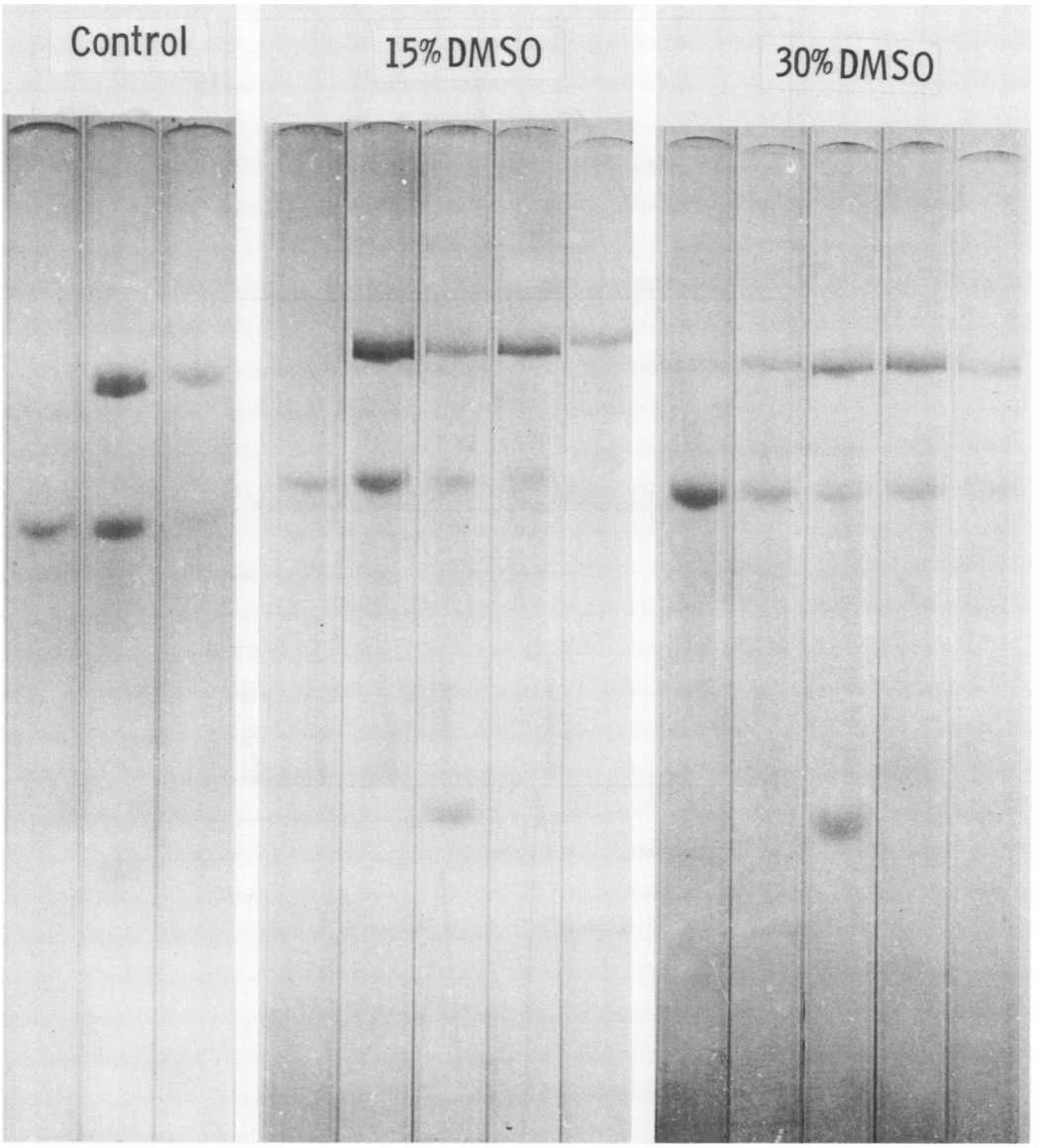

FIG. 3. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of dissociated αβ DNA polymerase. Photographs of the subunits associated with the different pooled fractions of DNA polymerase eluted from phosphocellulose obtained with treatment of αβ with 0, 15, and 30% dimethyl sulfoxide (DMSO). The bars in Fig. 2 show the fractions that were pooled for photographs except the control enzyme. The pooled fractions and the gels are read from left to right. The control enzyme (60 μg) used in this photograph was eluted from phosphocellulose with a steeper gradient than the control enzyme in Fig. 2, resulting in some overlap of αβ with peak III. In the control, from left to right the gels are α, αβ, and peak III.*

tain polymerase and nuclease activity. Only dimethyl sulfoxide in a narrow range of concentration (approximately 30 to 50%) and 15% dioxane (unpublished data) meet these criteria. The correlation of DNA polymerase and RNase H activity with the α subunit after phosphocellulose chromatography of αβ treated with 30% dimethyl sulfoxide strongly suggests that α is the enzymatically active subunit in αβ. This conclusion was further supported by the fact that there is no correlation between DNA polymerase or RNase H activity and the elution of the β subunit from phosphocellulose. From these data, it appears that the β subunit is either completely enzymatically inactive or possesses little polymerase and RNase H ac-

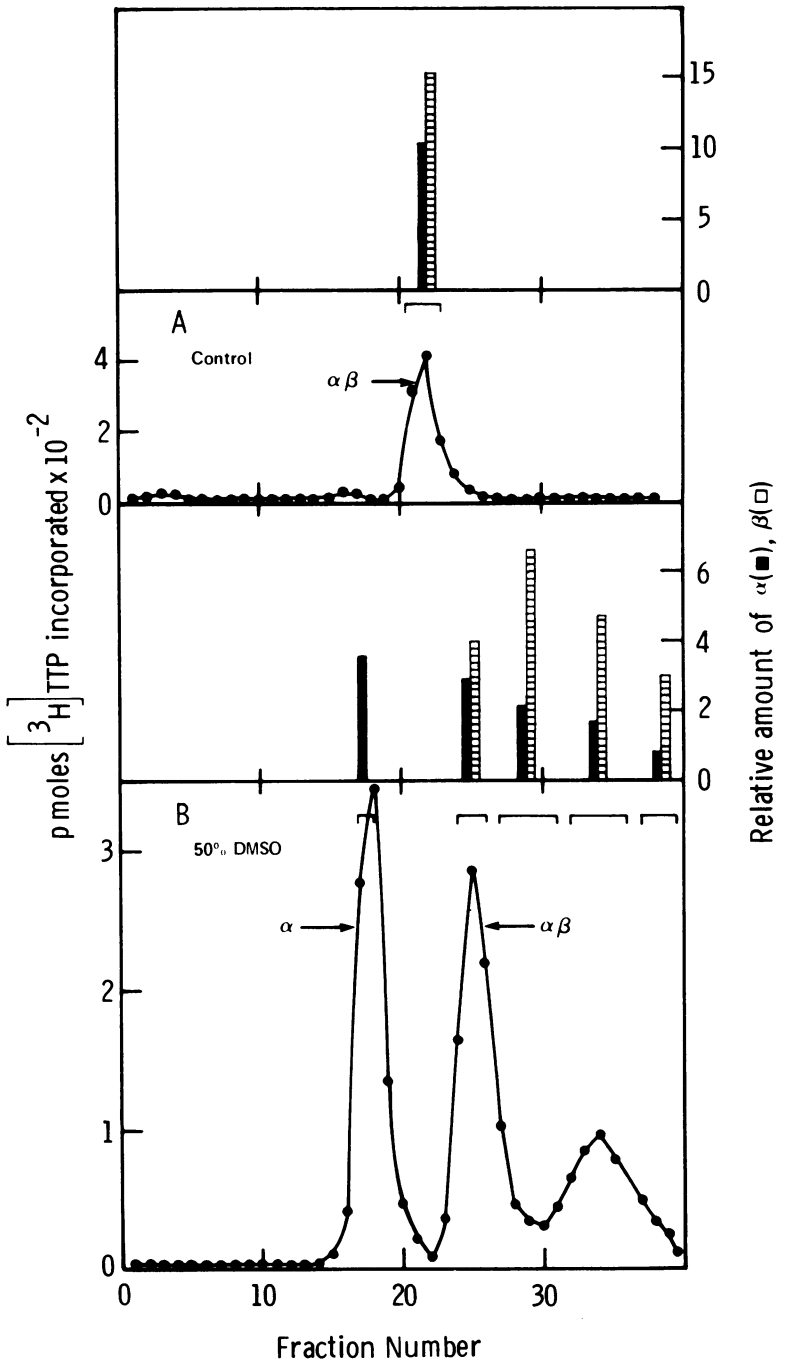

FIG. 4. *Histograms of dissociated αβ DNA polymerase subunits. (A) The αβ DNA polymerase (Fig. 1B) was pooled and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described in Fig. 2. The relative molar ratio of β to α was 1.09. (B) The same αβ DNA polymerase (300 μg) used above was subjected to dissociation using 50% dimethyl sulfoxide (DMSO) and 0.5 M KCl. The recovery of DNA polymerase activity was 74%. The subunit composition of the different polymerase peaks was analyzed as described in A with the bars indicating the fractions pooled. The relative molar ratios of β to α for the five pooled fractions (left to right) were 0, 0.96, 2.13, 2.17, and 2.33, respectively.*

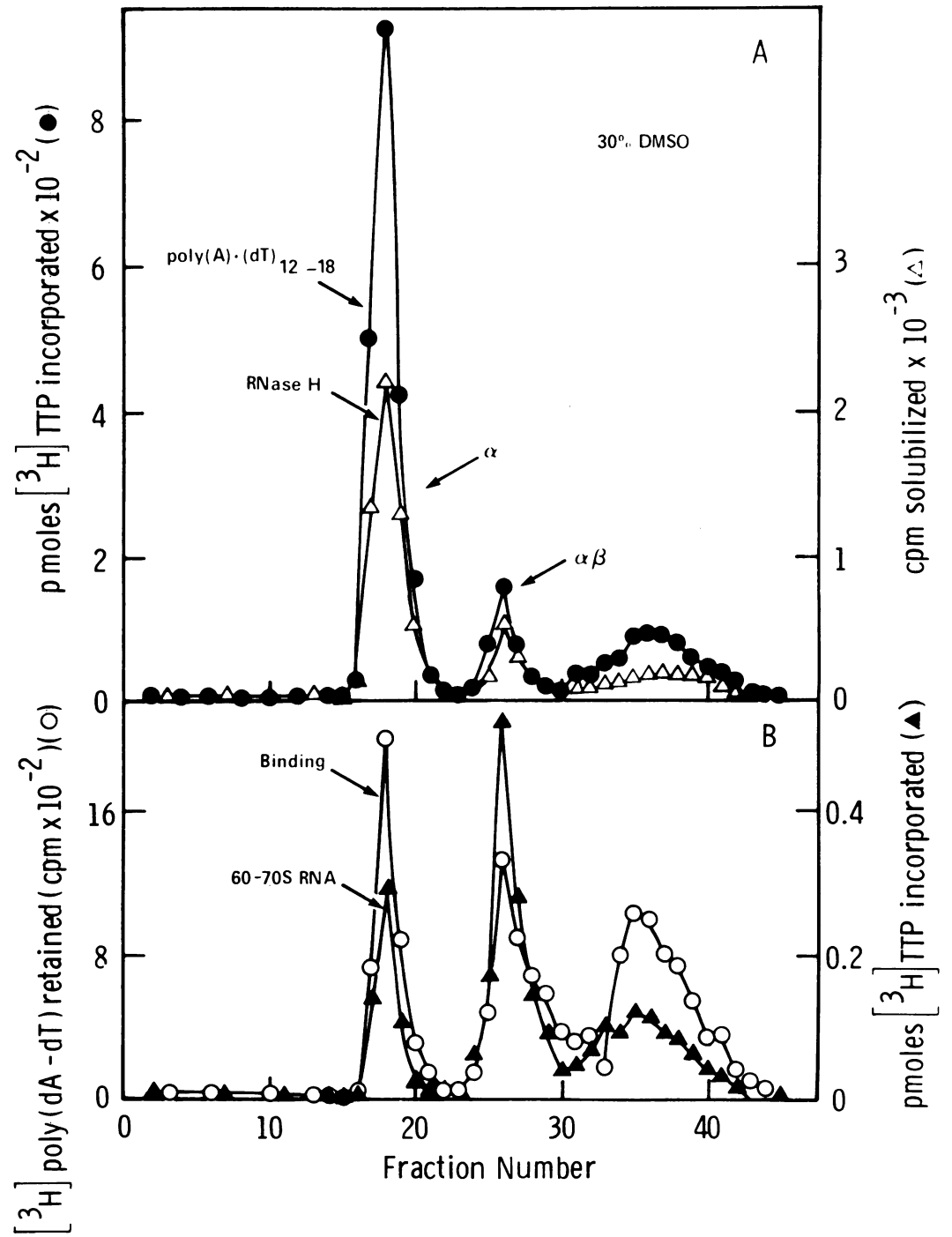

FIG. 5. *Catalytic properties of dissociated AMV DNA polymerase subunits. The same αβ enzyme preparation (105 μg) used in Fig. 2 was dissociated by 30% dimethyl sulfoxide (DMSO) with 0.5 M KCl present. After elution from the phosphocellulose column, samples were assayed as follows: (A) 20 μl for 15 min with poly(A)·(dT)$_{12-18}$ (●) and 10 μl for 30 min with [^{3}H]poly(A)·poly(dT) (△); (B) 20 μl for 30 min with AMV 60–70S RNA (▲) and 10 μl for 3 min with [^{3}H]poly(dA-dT) (○). Recovery of DNA polymerase activity with poly(A)·(dT)$_{12-18}$ was 54%.*

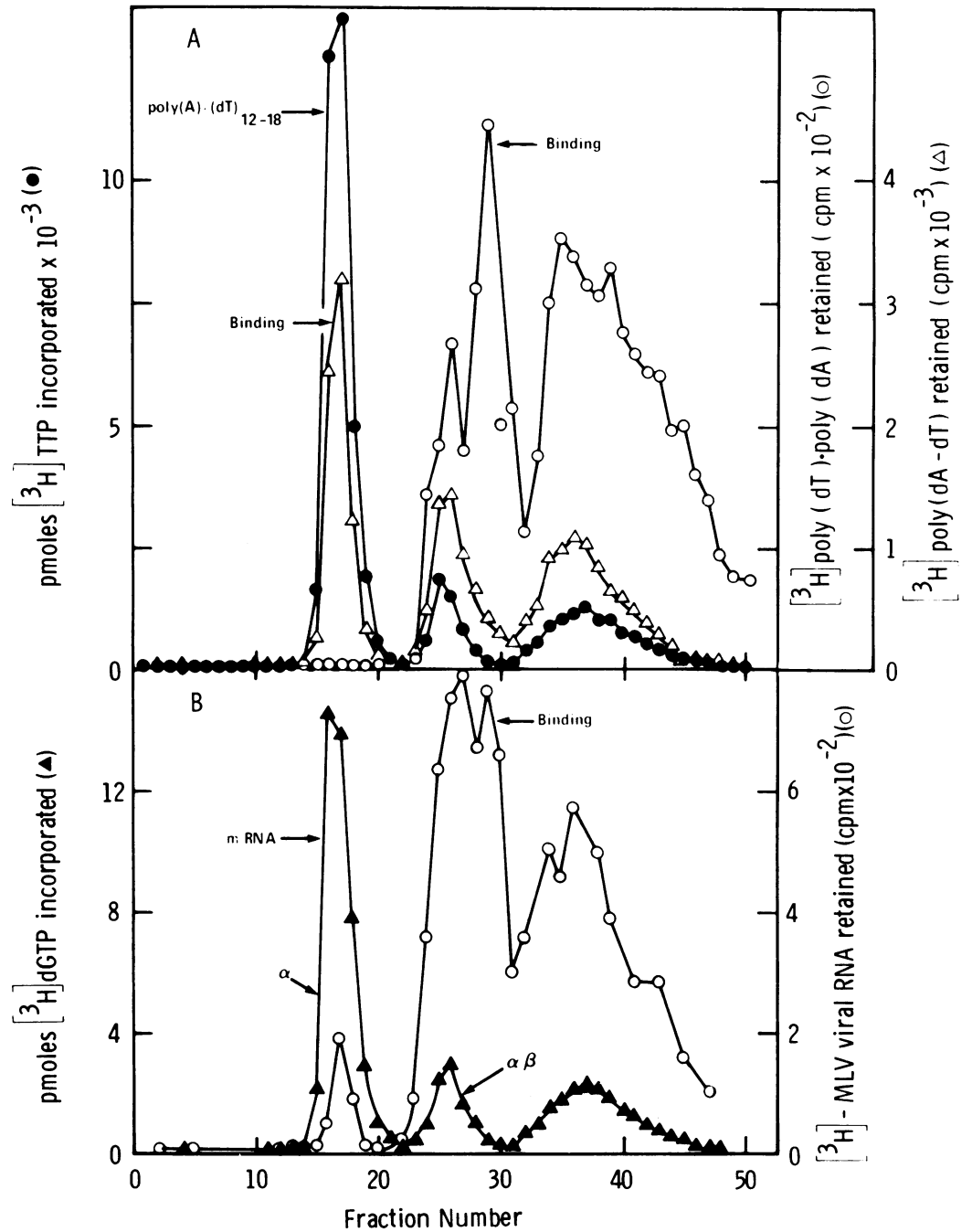

FIG. 6. *Binding properties of the β subunit. αβ DNA polymerase (160 μg) was dissociated with 30% dimethyl sulfoxide and 0.5 M KCl and then chromatographed on phosphocellulose. Samples were assayed from the indicated fractions as follows: (A) 20 μl for 15 min with poly(A)·(dT)$_{12-18}$ (●) for polymerase activity; 10 μl for 3-min binding assays using [^{3}H]poly(dT)·poly(dA) (○) and [^{3}H]poly(dA-dT) (△); (B) 10 μl for 15 min using rabbit globin mRNA (0.65 μg) with 0.5 μg of (dT)$_{12-18}$ (▲) for polymerase activity; 5 μl for binding of denatured ^{3}H-labeled MLV 60–70S RNA (2,900 counts/min per assay) (○). No DNA synthesis was observed with mRNA without (dT)$_{12-18}$ present with either α or αβ DNA polymerase.*

tivity when compared with the α subunit. No definitive conclusion can be made until the β subunit is purified to homogeneity. Efforts to purify the dissociated β subunit completely free of the α subunit have been unsuccessful.

The possibility exists that the β subunit was more easily inactivated by dimethyl sulfoxide treatment than the α subunit. But this seems unlikely when one examines the catalytic properties of the dissociated enzyme species. The third peak of polymerase activity (peak III), which had a higher ratio of β to α, possessed all the catalytic properties of αβ. In particular, peak III polymerase possessed the ability to synthesize viral DNA from AMV 60-70S RNA, in contrast to the limited synthesis by α DNA polymerase. In addition, both αβ and peak III are able to bind to various nucleic acids more avidly than α. It is not known whether α, β, or both subunits are necessary for the specific binding of αβ to the tryptophan tRNA primer found associated with avian leukosis virus 35S RNA (10).

Complete elimination of proteolytic cleavage of the β subunit during treatment of αβ with dimethyl sulfoxide was difficult. Using Sephadex G-100-purified enzyme and maintaining a high concentration of KCl during dissociation were reasonably successful. Apparently, dimethyl sulfoxide treatment renders the β subunit highly susceptible to proteolysis in contrast to the α polypeptide.

The subunit structure of AMV reverse transcriptase in isolated virions before disruption or in transformed cells is not known. Treatment of virions with phenyl-methylsulfonyl-fluoride, a protease inhibitor, prior to isolation of virion cores, increased the ratio of β to α in the core structures approximately fourfold, with an apparent increase also in DNA polymerase and RNase H activity compared to the usual core preparations not treated with protease inhibitor (7). These data are in contrast to the finding reported here that the β subunit may have limited polymerase and nuclease activity. It was recently reported that a low level of reverse transcriptase protein, detected by radioimmunoassay, was found in uninfected chicken embryo cells (9). Possibly, β or ββ dimer is the inactive precursor found in uninfected as well as in infected chicken embryo cells and is cleaved by a protease upon maturation of the virion from infected cells.

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LITERATURE CITED

1. **Duesberg, P. H., and N. S. Robinson.** 1966. Nucleic acid and proteins isolated from the Rauscher mouse leukemia virus (MLV). Proc. Natl. Acad. Sci. U.S.A. **55**:219-227.
2. **Gibson, W., and I. M. Verma.** 1974. Studies on the reverse transcriptase of RNA tumor viruses. Structural relatedness of two subunits of avian RNA tumor viruses. Proc. Natl. Acad. Sci. U.S.A. **71**:4991-4994.
3. **Grandgenett, D. P., G. F. Gerard, and M. Green.** 1973. A single subunit from avian myeloblastosis virus with both RNA-directed DNA polymerase and ribonuclease H activity. Proc. Natl. Acad. Sci. U.S.A. **70**:230-234.
4. **Grandgenett, D. P., and M. Green.** 1974. Different mode of action of ribonuclease H in purified α and αβ RNA-directed DNA polymerase from avian myeloblastosis virus. J. Biol. Chem. **249**:5148-5152.
5. **Grandgenett, D. P.** 1975. Correlation of the binding properties of α and αβ DNA polymerase of avian myeloblastosis virus with their different mode of ribonuclease H activity. *In* M. M. Goulian, P. C. Hanawalt, and C. F. Fox (ed.), DNA synthesis and its regulation. W. A. Benjamin, Inc., Menlo Park, Calif.
6. **Kacian, D. L., K. F. Watson, A. Burny, and S. Spiegelman.** 1971. Purification of the DNA polymerase of avian myeloblastosis virus. Biochim. Biophys. Acta **246**:365-383.
7. **Mölling, K.** 1974. Reverse transcriptase and RNase H: present in a murine virus and in both subunits of an avian virus. Cold Spring Harbor Symp. Quant. Biol. **39**:969-973.
8. **Panet, A., I. M. Verma, and D. Baltimore.** 1974. Role of the subunits of the avian RNA tumor virus reverse transcriptase. Cold Spring Harbor Symp. Quant. Biol. **39**:919-923.
9. **Panet, A., D. Baltimore, and T. Hanafusa.** 1975. Quantitation of avian RNA tumor virus reverse transcriptase by radioimmunoassay. J. Virol. **16**:146-152.
10. **Panet, A., W. A. Haseltine, D. Baltimore, G. Peters, F. Harada, and J. E. Dahlberg.** 1975. Specific binding of tryptophan transfer RNA to avian myeloblastosis virus RNA-dependent DNA polymerase (reverse transcriptase). Proc. Natl. Acad. Sci. U.S.A. **72**:2535-2539.
11. **Rho, H., D. P. Grandgenett, and M. Green.** 1975. Sequence relatedness between the subunits of avian myeloblastosis virus reverse transcriptase. J. Biol. Chem. **250**:5278-5280.
12. **Riggin, C. H., P. M. C. Bondurant, and W. M. Mitchell.** 1974. Differences between murine leukemia virus and murine sarcoma virus: effect of virion age and multiplicity of infection on viral RNA. Intervirology **2**:209-221.
13. **Verma, I. M., W. S. Mason, S. D. Drost, and D. Baltimore.** 1974. DNA polymerase activity from two temperature-sensitive mutants of Rous sarcoma virus is thermolabile. Nature (London) New Biol. **251**:27-31.
14. **Verma, I. M.** 1975. Studies on reverse transcription of RNA tumor viruses. I. Localization of thermolabile DNA polymerase and RNase H activities. J. Virol. **15**:121-126.
15. **Wells, R. D., R. M. Flügel, J. E. Larson, P. F. Schendel, and R. W. Sweet.** 1972. Comparison of some reactions catalyzed by deoxyribonucleic acid polymerase from avian myeloblastosis virus, *Escherichia coli*, and *Micrococcus luteus*. Biochemistry **11**:621-629.