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Purification and characterization of the DNA polymerase of human breast cancer particles

(reverse transcriptase/human malignancy/oncornavirus/synthetic templates)

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ABSTRACT Previous studies have identified human breast tumor particles possessing many of the features characteristic of RNA tumor viruses. In addition to the expected size (600 S) and density (1.16 g/ml) these include possession of an outer membrane and an inner one surrounding a "core" containing a DNA polymerase and a large-molecular-weight (70 S) RNA possessing detectable homology to the RNAs of the mouse mammary tumor virus (MMTV) and of the Mason-Pfizer monkey virus (MPMV).

We report here the purification and characterization of the DNA polymerase from the human breast cancer particles. Its key properties are very similar to those of the RNA-dependent DNA nucleotidyltransferase (reverse transcriptases) found in MMTV and MPMV. Thus, like these viral enzymes, the purified human breast cancer DNA polymerase exhibits the following three features that together distinguish the known viral reverse transcriptases from normal cellular DNA polymerases: (*i*) a strong preference for oligo(dT)·poly(rA) over oligo(dT)·poly(dA) as a template for the synthesis of poly(dT); (*ii*) the acceptance of the highly specific oligo(dG)·poly(rCm) as a template for the formation of poly(dG); (*iii*) the ability to use a viral RNA (AMV) as a template to fashion a faithful DNA complementary copy; and (*iv*) its preference for Mg^{++} over Mn^{++}.

In summary, the data described here on the enzyme of the human breast cancer particles add further evidence of similarities to the viral agents associated with the corresponding malignancies in the mouse and monkey models. To date, an enzyme with these properties has not been detected in normal breast tissues or in benign tumors of the breast.

The first molecular indications of virus-like components in human breast cancer emerged (1) from hybridizations between the RNAs of breast tumors and [^{3}H]DNA complementary to the RNA of the mouse mammary tumor virus (MMTV), findings that were independently confirmed (2). Another agent yielding a probe (3) that can detect homologous RNA in human breast malignancies is the Mason-Pfizer monkey virus (MPMV), an isolate from a spontaneous breast carcinoma of a rhesus monkey (4). Neither of these homologies were detected in the RNAs of normal tissues or of benign tumors (fibroadenomas) of the breast (1, 3).

To further delineate the nature of the viral-related RNA sequences found in breast malignancies, recourse was had to the "simultaneous detection test" of Schlom and Spiegelman (5). This experimental procedure was designed to detect the presence of a large (70 S or 35 S) RNA molecule complexed to a DNA polymerase (a reverse transcriptase, RNA-dependent DNA nucleotidyltransferase) that can use the RNA as a template to make a DNA complementary copy. It was found (6) that 79% of the malignant specimens examined by this test showed positive evidence of such RNA-enzyme complexes. In contrast, none of the control samples from normal tissue and benign tumors were positive. It was further established in the same study that the RNA-reverse transcriptase complexes identified in the malignant tissues were encapsulated in particles possessing a density between 1.16 and 1.19 g/ml, a value characteristic of intact animal RNA tumor viruses. Finally, it was shown (7) that these human tumor particles, like those found in mouse mammary tumors, can be converted by Sterox-SL treatment into "core particulates" with a density of 1.26 g/ml or greater and containing the diagnostic reverse transcriptase and 70S RNA. Recently, the presence of reverse transcriptase in human breast cancer tissue was established (8) by the use of the highly specific synthetic template, oligodeoxyguanylate·poly(2′-*O*-methylcytidylate) [oligo(dG)·poly(rCm)].

It is evident that the particles found in human breast cancers exhibit many of the features characteristic of the two animal oncornaviruses (MMTV and MPMV) associated with the corresponding disease in mice and monkeys. The potential implications of their presence stimulated further attempts at characterizing the components of the breast cancer particles. The reverse transcriptase they contain is particularly amenable to isolation since it can be easily detected during fractionation by a relatively sensitive assay of its activity. Indeed we have already demonstrated (9) the feasibility of this approach by purifying to homogeneity the reverse transcriptase from particles found in the spleens of leukemic patients.

It is the purpose of the present paper to describe the properties of the reverse transcriptase purified from the human breast cancer particles. We find that the human enzyme is very similar in its distinctive properties to the known animal reverse transcriptases.

MATERIALS AND METHODS

Subcellular Fractionation of Breast Tumor Tissue. Depending on the amounts of material available, between 9 and 30 g of tumor were thawed, minced, suspended in 4 volumes of cold 5% sucrose (wt/vol)-TNE (0.01 M Tris·HCl at pH 8.0, 0.15 M NaCl, 3 mM EDTA) and blended in a Silverson homogenizer. The homogenate was centrifuged at 4000 × *g* and then 10,000 × *g* to remove nuclei and mitochondria, respectively. Trypsin (Worthington Biochemicals) was added to the postmitochondrial supernatant to a final concentration of 0.5 mg/ml. After incubation at 20° for 10 min, proteolytic activity was inhibited by the addition of lima bean trypsin inhibitor (1-fold excess, Worthington Biochemicals) and Trasylol (100 kallikrein inactivator units/ml, Mobay Chemical Corp). The sample was layered over discontinuous sucrose gradients composed of 6 ml of 50% sucrose (wt/vol)-TNE and 8 ml of 25% sucrose (wt/vol)-TNE. After centrifugation at 25,000 rpm for

Abbreviations: MMTV, murine mammary tumor virus; MPMV, Mason-Pfizer monkey virus; oligo(dG)·poly(rCm), oligodeoxyguanylate·poly(2′-*O*-methylcytidylate); TNE buffer, buffer containing 0.01 M Tris-HCl at pH 8.0, 0.15 M NaCl, 3 mM EDTA; DTT, dithiothreitol; solution A, solution containing 2 mM DTT, 1 mM EDTA, 0.02% Triton X-100 (wt/vol), 10% glycerol (vol/vol); AMV, avian myeloblastosis virus.

90 min at 4° in a Spinco SW-27 rotor, material at the 25/50% interface was collected, diluted with TNE, and layered over linear 20–50% sucrose-TNE gradients. The samples were centrifuged as above for 16 hr and the different density regions collected. The density region (1.16–1.19 g/cm^3) in which RNA tumor viruses localize was pooled, diluted, and centrifuged as above for 90 min. The resulting pellets were resuspended in approximately 0.6 ml of 0.1 M Tris·HCl at pH 8.0.

Six lots of tumors were processed for enzyme in the manner described. Four of these (A, B, C, and D) yielded enough material to characterize the enzyme. One preparation (A), a metastatic liver tumor, came from a single patient; all the others being pooled material from a number of different individuals.

Polyacrylamide Agarose Gel Filtration. The resuspended pellet was solubilized and disrupted at 0° for 15 min by the addition of KCl (to 0.4 M), DTT (dithiothreitol, to 0.01 M) and Triton X-100 (to 0.6%, vol/vol). The sample, approximately 0.9 ml, was applied to a 0.9 × 50 cm column of polyacrylamide agarose gel (Ultrogel AcA44, LKB) equilibrated with 0.3 M potassium phosphate at pH 8.0 in solution A (2 mM DTT, 1 mM EDTA, 0.02% Triton X-100, and 10% glycerol). The column was eluted with the same buffer at a flow rate of about 2 ml/hr. Fractions (0.5 ml) were assayed for DNA polymerase and terminal transferase activities as described below.

Phosphocellulose Chromatography. The peak fractions from the Ultrogel column were pooled (3 ml) and Trasylol was added to a concentration of 100 kallikrein inactivator units/ml. The sample was dialyzed against 0.01 M potassium phosphate at pH 7.2, in solution A until the phosphate concentration was less than 0.02 M, and then was loaded onto a 0.9 × 10 cm phosphocellulose column (Whatman P-11) equilibrated with the same buffer. The column was washed with 30 ml of the 0.01 M phosphate buffer and the enzyme activity was eluted with a 120 ml linear gradient of 0.01–0.5 M potassium phosphate buffer at pH 7.2 in solution A, at a flow rate of 14 ml/hr. Fractions (1.2 ml) were assayed for both DNA polymerase and terminal transferase activities. The fractions containing the main peak of polymerase activity were pooled and Trasylol was added to 100 kallikrein inactivator units/ml. This enzyme fraction was concentrated by dialysis at 0° against Aquacide 11-A (Calbiochem).

Glycerol Gradient Centrifugation. For estimation of molecular weight, the concentrated phosphocellulose enzyme was diluted 3-fold with 0.1 M potassium phosphate at pH 8.0, and layered on a linear 10–30% (vol/vol) glycerol gradient containing 0.1 M potassium phosphate at pH 8.0, 2 mM DTT, and 0.02% Triton X-100. Centrifugation was at 48,000 rpm for 12 hr at 1° in a Spinco SW-50.1 rotor. Fractions were collected from the bottom of the gradient and assayed for reverse transcriptase activity with oligo(dG)·poly(rC) as template. Bovine serum albumin served as a sedimentation marker in a parallel gradient.

DNA Polymerase Assays. Assay mixtures for polymerase activity with synthetic polymer templates contained (in 100 μl): 5 μmol Tris·HCl at pH 8.0, 0.5 μmol of $MgCl_2$, 0.1 μmol of DTT, and the following combinations of polymer and dNTPs: 0.4 μg of oligo(dG)·poly(rC) or oligo(dG)·poly(rCm), 0.02 μmol of dCTP and 1.0 nmol of [^{3}H]dGTP (4000 cpm/pmol); 0.4 μg of oligo(dT)·poly(rA) or oligo(dT)·poly(dA), 0.02 μmol of dATP and 1.0 nmol of [^{3}H]dTTP (4000 cpm/pmol). In reactions with oligo(dG)·poly(rCm), $MnCl_2$ (0.02 μmol) replaced $MgCl_2$.

Assays with avian myeloblastosis virus (AMV-RNA) contained (in 100 μl): 5 μmol Tris·HCl at pH 8.0, 0.8 μmol of $MgCl_2$, 0.1 μmol of DTT, 10 μg of actinomycin D (Sigma Corp.), 5 μg of distamycin A (Calbiochem), 2 μg of AMV 70S RNA, 0.1 μg of oligo$(dT)_{12-18}$, 0.1 μmol each of dATP, dGTP, dTTP, and 5 nmol of [^{3}H]dCTP (1.5 × 10^4 cpm/pmol).

All reactions were incubated at 36° for 15–30 min and were terminated by the addition of 0.5 ml of cold 0.067 M sodium pyrophosphate/1 M sodium phosphate at pH 7.2, and then 0.5 ml of cold 80% trichloroacetic acid. Acid-insoluble radioactivity was collected on membrane filters and measured in a scintillation counter.

Terminal deoxynucleotidyl transferase activity was measured by the polymerization of [^{3}H]dGTP in the absence of a complementary polymer template. Reactions were carried out as described above except that polymer dNTP combination was replaced with 0.4 μg of oligo$(dG)_{10-18}$ plus 0.02 μmol of dCTP and 1.0 nmol of [^{3}H]dGTP.

All synthetic oligo- and polynucleotides were obtained from Collaborative Research, Inc. and tritiated dGTP, dTTP, and dCTP were obtained from New England Nuclear. AMV-70S RNA was isolated from the purified virus as previously described (11).

Hybridization Reactions. Procedures for the hybridization reactions and their analysis with S_1 nuclease have been reported elsewhere (11). Conditions for Cs_2SO_4 equilibrium density centrifugation (1) were modified by the addition of 0.02% sodium *N*-lauroyl sarcosinate and 20 μg each of *E. coli* DNA and RNA to the gradients.

RESULTS

The data described below are based on the results of independent enzyme isolations from four different tumor collections (labeled A through D). The fractionation and the properties of the breast tumor polymerase did not vary significantly from one preparation to another and hence the results on each one of the four need not be reported in complete detail.

Polymerase preparation A was isolated from a metastatic lesion in the liver of a patient with breast cancer. Nine grams of tumor were homogenized and the particulate material, banding at a density of 1.16–1.19 g/ml, was collected as described in *Materials and Methods*. The recovered pellet was solubilized by the addition of Triton X-100 and then fractionated through a polyacrylamide/agarose gel (Ultrogel ACA-44) column. Each column fraction was assayed for (*i*) DNA polymerase with oligo$(dG)_{12-18}$·poly(rC) as a template and (*ii*) for terminal transferase, by using oligo$(dG)_{12-18}$ as a primer. Fig. 1A shows the fraction profile of these activities and their positions relative to bovine serum albumin and ovalbumin which were prerun on the same column as molecular weight markers. Three peaks (two major and one minor) of DNA polymerase activity are observed and it is evident that the first major peak also contains terminal transferase. The two major peaks are found (Table 1) to contain 90% of the applied polymerase activity and 3% of the protein as measured by the fluorescamine procedure (12). To examine the reality of the separation of the polymerase activities observed in Fig. 1A, the two major peaks were pooled, dialyzed to reduce the phosphate buffer concentration, and then chromatographed through a phosphocellulose column with a linear (0.01–0.5 M) phosphate gradient with results as shown in Fig. 1B. We see that the polymerase activities again are resolved into two major peaks, one eluting at 0.08 M phosphate and the other at 0.18 M. It will be noted that again the second major polymerase peak is devoid of terminal transferase activity. The latter splits into two peaks, one associated with the first DNA polymerase activity at 0.08 M phosphate, and another eluting by itself at 0.23 M phosphate.

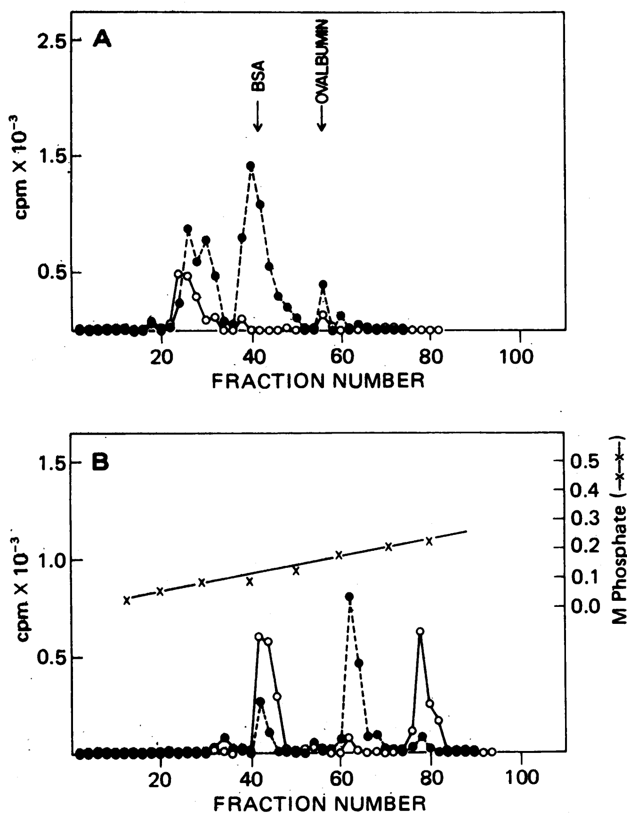

FIG. 1. Purification of the breast tumor polymerase. (A) Gel filtration. Breast tumor (sample A) was fractioned for particulate material of density 1.16–1.18 g/cm^3. This sample was solubilized and filtered through a polyacrylamide/agarose column as described in *Materials and Methods.* Aliquots of each column fraction (25 μl) were assayed for polymerase activity with oligo(dG)·poly(rC) (●- - -●) and for terminal transferase activity with oligo(dG)(○—○). (BSA, bovine-serum albumin.) (B) Phosphocellulose chromatography fractions containing polymerase activity from the Ultrogel column were pooled, dialyzed, and chromatographed on a phosphocellulose column (*Materials and Methods*). The column fractions were assayed as in (A).

The second (0.18 M phosphate) peak of polymerase activity observed on the phosphocellulose column is not found in normal tissues (three samples of breast tissue and three samples from spleens) or in benign fibroadenomas of the breast (three pools of three to four fibroadenomas each) and appears to be the DNA polymerase unique to breast cancer tissue. The fractions composing peak 2 of the phosphocellulose column are found to contain 65% of the applied DNA polymerase activity and 8% of the protein. These fractions are pooled and concentrated as described in *Materials and Methods* to yield the breast tumor

Table 1. Purification of DNA polymerase from a human breast tumor

Stage of purification	Total protein (mg)	Total activity (pmol)	Specific activity (pmol/mg)
Viral density region	8.3	168	20
Polyacrylamide agarose	0.25	148	5.6×10^2
Phosphocellulose	0.02	96	4.8×10^3

Polymerase was purified from tumor sample A as detailed in the *text.* All reactions contained oligo(dG)·poly(rC) as template and were incubated for 30 min at 36°. Total activity was calculated as pmol of [^{3}H]dCMP polymerized.

Table 2. Synthetic polynucleotides as templates for the breast tumor polymerase

		pmol [^{3}H]dNMP polymerized			
Template	[^{3}H]-dNTP	Prep. A	Prep. B	Prep. C	Prep. D
Oligo(dT)·poly(rA)	dTTP	0.472	0.445	0.221	0.495
Oligo(dT)·poly(dA)	dTTP	0.044	0.021	0.062	0.071
Oligo(dG)·poly(rC)	dGTP	0.534	0.444	0.180	0.532
Oligo(dG)·poly(rCm)	dGTP	0.502	0.410	N.T.	N.T.
Oligo(dG)	dGTP	0.010	0.008	N.T.	N.T.

Polymerase reactions were performed with the indicated template as described in *Materials and Methods.* Incubation was at 36° for 15 min (prep. C) or 30 min (preps. A, B, and D). Polymerase prep. A was isolated from a single tumor (9 g), B from a pool of three tumors (14 g), C from a pool of four tumors (32 g), and D from a pool of five tumors (61 g). N.T., not tested.

polymerase for further characterization. Table 1 summarizes the yields of activity and protein at each of the three steps in a typical purification.

Evidence That the Breast Cancer Polymerase Is a Reverse Transcriptase. There are several useful criteria which distinguish the reverse transcriptases of the RNA tumor viruses from normal mammalian DNA polymerases. The viral reverse transcriptases (13) show a preference for oligo(dT)·poly(rA) over oligo(dT)·poly(dA) and they also accept oligo(dG)·poly(rC) and oligo(dG)·poly(rCm) as excellent templates for the synthesis of poly(dG). Another, and more useful characteristic, is the ability of a reverse transcriptase to use a heteropolymeric RNA to direct the synthesis of a faithful complementary DNA as demonstrated by proper hybridization of the DNA product to the template used in the synthesis.

The responses of four of the breast cancer polymerases to the synthetic polyribonucleotides are summarized in Table 2. The results show a pattern of activities completely consistent with that obtained with reverse transcriptases isolated from authentic animal RNA tumor viruses (13). Thus, in all cases, oligo(dT)·poly(rA) is superior to oligo(dT)·poly(dA) for the synthesis of poly(dT). Further, both oligo(dG)·poly(rC) and oligo(dG)·poly(rCm) were excellent templates for the formation of poly(dG). It should be noted that in addition to the four breast tumor enzyme preparations described here, many others (more than 50) obtained from additional patients in an ongoing effort have been examined in the same way at different stages of purity by using various methods of tissue fractionation, and they all exhibited the response pattern described in Table 2.

The operational definition of a reverse transcriptase requires the demonstration that it can use a heteropolymeric RNA to make a DNA transcript. The response (Table 3) of the breast cancer polymerase to the RNA of the avian myeloblastosis virus (AMV) is that expected from the synthesis of a heteropolymeric DNA. Leaving out any one, or all, of the required three unlabeled deoxyribosidetriphosphates leads to the same virtual disappearance of synthetic activity as occurs on omission of the RNA template.

The most telling test of a putative reverse transcriptase reaction comes from an examination of the fidelity of the DNA transcript. This requires isolation of the [^{3}H]DNA product and challenging it in annealing reactions with the RNA template used in the synthesis. A 2-ml reaction, leading to the synthesis of approximately 3 ng [^{3}H]DNA at 2×10^7 cpm/μg, was run for 15 min with the DNA polymerase preparation A and AMV-RNA as the template. The [^{3}H]DNA product was puri-

Table 3. Deoxynucleoside triphosphate and RNA requirements of the breast tumor DNA polymerase

Reaction	pmol [^{3}H]dCMP polymerized A	B
Complete	0.200	0.170
−dATP	0.013	0.011
−dGTP	0.025	0.022
−dTTP	0.012	0.005
−dATP, dGTP, dTTP	0.008	0.011
−RNA	0.026	0.016

Polymerase preparations A and B were assayed for RNA-dependent DNA synthesis with AMV-RNA as the template. The reactions were incubated at 36° for 30 min under the conditions given in *Materials and Methods*.

fied and recovered by the usual procedures (11) and then annealed with AMV and Rauscher leukemia virus 70S RNAs. The reaction product was examined by separation in Cs_2SO_4 gradients and by resistance to S_1 nuclease. Both methods yielded less than 5% of the total product annealing to the unrelated Rauscher leukemia virus-RNA and between 80 and 85% hybridizing to AMV-RNA, the template used to direct the synthesis. These results suggest, therefore, that the [^{3}H]DNA is a single-stranded complement of the AMV-RNA. A more informative examination of the [^{3}H]DNA product is provided by a kinetic study of the annealing reaction. Fig. 2 compares the annealing kinetics of two [^{3}H]DNA products to AMV-RNA; one product was synthesized under the direction of AMV-RNA by AMV reverse transcriptase and the other synthesized by the human breast cancer polymerase instructed by the same template. It is evident that the kinetics of annealing to AMV-RNA of the two DNA products are indistinguishable.

Other Properties of the Breast Cancer Polymerase. Variations in temperature and pH were examined for their effects on the activities of several preparations of the breast cancer polymerase using oligo(dG)·poly(rC) and oligo(dT)·poly(rA) as templates. The maximal rate of polymerization occurred at 37° in 5 mM $MgCl_2$ over a pH range of 7.0–8.5.

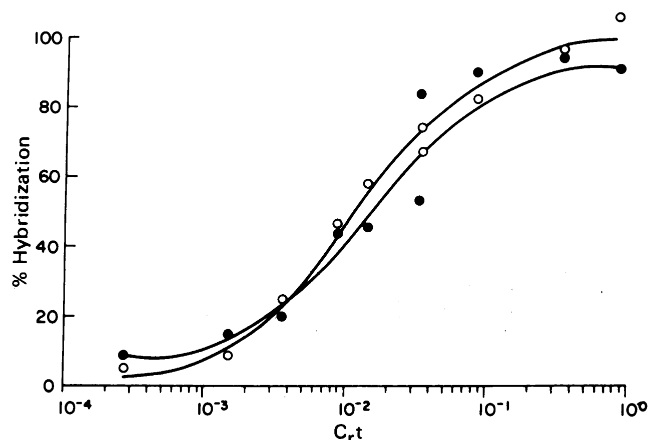

FIG. 2. Hybridization kinetics of breast polymerase AMV-cDNA. [^{3}H]DNA synthesized from AMV-70S RNA with the human polymerase (O) or with AMV DNA polymerase (●) was annealed with excess AMV-RNA to C_rt of 0.8 mol of nucleotide·liter^{-1} per sec at 50% in 0.60 M sodium chloride, 0.06 M sodium citrate at pH 6.5, 0.5% sodium dodecyl sulfate, and 42% formamide. Each curve is a composite of two annealing reactions of RNA concentrations of 7.3 × 10^{-3} and 1.8 × 10^{-1} μg/ml. At the indicated C_rt [initial concentration of total RNA (moles of nucleotide/liter) × time (seconds)] values, aliquots (740 cpm) were analyzed with S_1 nuclease. In the absence of added RNA, the background annealing of the human and AMV polymerase cDNAs was 10% and 4%, respectively.

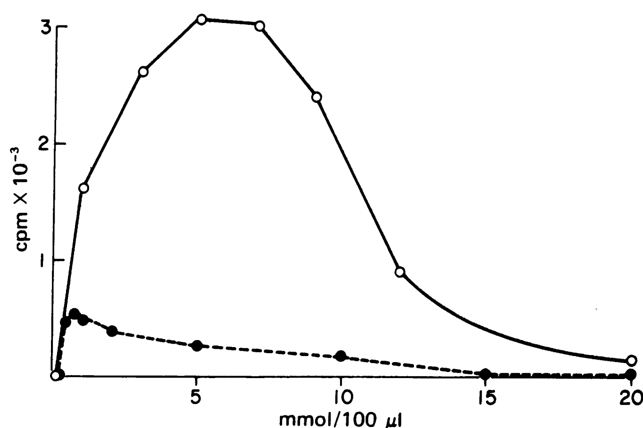

FIG. 3. Titration of divalent metal ion. The activity of the breast tumor polymerase was measured as a function of the Mg^{++} (O—O) or Mn^{++} (●- - -●) concentration with oligo(dT)·poly(rA) as the template. These assays were performed with enzyme preparation B.

The divalent ion requirements of reverse transcriptases are of some interest since they serve to divide the viruses into different groups. Thus, all six strains of MMTV from a variety of sources contain a reverse transcriptase showing a strong preference for Mg^{++} as compared with Mn^{++} (14). The same holds true for the MPMV, the bromodeoxyuridine-induced guinea pig virus (15), and the bovine leukemia virus (16). In contrast, the reverse transcriptases of the murine leukemia and sarcoma viruses (17, 18), the simian associated virus (18) and the hamster leukemia virus (19) function much more effectively in the presence of Mn^{++}. The cation requirement is examined for the human breast cancer enzyme in Fig. 3 from which it is evident that Mn^{++}, at its optimum concentration, yields only about one-seventh of the activity attainable with Mg^{++}. Thus, the human breast cancer enzyme shows a divalent metal ion requirement resembling the MMTV/MPMV virus group described above.

The molecular size of the breast cancer enzyme was estimated by sedimentation through a linear (10–30% vol/vol) glycerol gradient. The enzyme was located by assaying fractions with AMV-RNA as template with results as described in Fig. 4. The enzyme activity sediments between 5 and 6S, slightly faster than the bovine serum albumin marker, thereby giving a molecular weight estimate of about 70,000. This result is also consistent with the relative elution positions of these same proteins on Ultrogel (Fig. 1A). A number of enzyme preparations from different breast tumor sources yielded identical sedimentation values.

DISCUSSION

The immediate goal of the present investigation was to provide further information on the particles detected (1, 2) in human breast malignancies. These particles possess many of the features characteristic of the RNA tumor viruses including the following: (*i*) they sediment at about 600S and have a density of around 1.16 g/ml; (*ii*) removal of an outer membrane with detergent converts them to "core particulates" with densities of 1.26 g/ml or greater; (*iii*) the "cores" contain a 70S RNA that can dissociate into 35S subunits; (*iv*) the RNA of these particles shares some sequence homology with the RNA found in MMTV, the etiologic agent of murine mammary tumors and with the RNA in MPMV, the virus isolated from a spontaneous

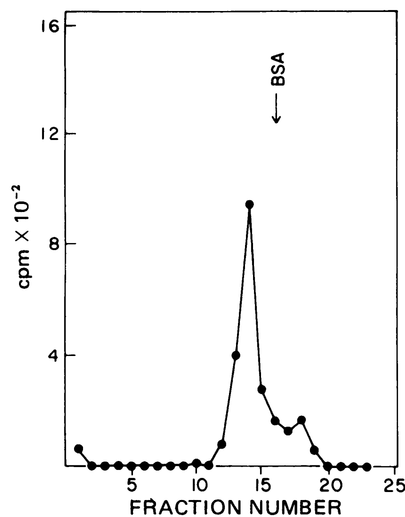

FIG. 4. Glycerol gradient centrifugation of the breast tumor polymerase. The enzyme activity purified from tumor sample B was sedimented on a 10–30% glycerol gradient *Materials and Methods.* A 25 μl aliquot of each gradient fraction was assayed for polymerase activity with oligo(dG)·poly(rC) as the template. The arrow marks the sedimentation position of bovine-serum albumin (BSA) in a parallel gradient. Similar results were obtained with enzyme preparation A.

breast tumor of a rhesus monkey; and finally, (*v*) the particles contain a reverse transcriptase which uses RNA as a template to fabricate a complementary DNA copy.

The data described here on the DNA polymerase purified from the human cancer breast particles add further evidence supporting similarities to the viral agents associated with similar malignancies in the mouse and monkey models. The properties of the purified polymerase are in complete accord with the published information on the various viral reverse transcriptases. Thus, like the animal viral enzymes, the purified human breast cancer DNA polymerase possesses the following features: (*i*) it has a sedimentation constant between 5 and 6S, corresponding to an approximate molecular weight of 70,000; (*ii*) it synthesizes poly(dT) about 10 times better in the presence of oligo(dT)·poly(rA) as compared with oligo(dT)·poly(dA); (*iii*) both oligo(dG)·poly(rC) and, more significantly, oligo(dG)·poly(rCm) are excellent templates for the synthesis of poly(dG); (*iv*) the human enzyme can accept a viral RNA (AMV-RNA) as a template to synthesize a [^{3}H]DNA product which by the hybridization test is as faithful a complementary copy as can be obtained by using the homologous avian reverse transcriptase; (*v*) the superiority of Mg^{++} to Mn^{++} for the detection of human polymerase activity mimics that observed with the enzymes of the MMTV and MPMV group.

The data described here stemmed from the examination of four enzyme preparations purified to the same stage—one from a metastatic tumor of the liver and three from pools of breast tumors. All yielded enzyme activities with the same biochemical and biophysical properties. We have examined normal tissues (5) and three pools (each containing between five and seven tumors) of the benign fibroadenomas by the same fractionation techniques described here. These nonmalignant samples yielded no enzyme activity with the properties of the reverse transcriptase purified from malignant tissue at any stage of purification.

The availability of the breast cancer enzyme in a comparatively pure state will permit us to further delineate the biochemical features that relate it to, and differentiate it from, the reverse transcriptases of animal viral origins. Because of its implications for potential clinical usefulness, it would be of immediate interest to focus on the immunological properties of the human enzyme with particular reference to possible cross reactivities with any of the known animal reverse transcriptases.

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