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DNA Polymerase Activity Associated with RNA Tumor Viruses

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Abstract. C-type RNA viruses, originating in mouse, cat, hamster, and viper, catalyze the synthesis of DNA from its constituent deoxyribonucleoside triphosphates. Both the rate and extent of the reaction were significantly enhanced by brief treatment of the intact virus with ether. A proportion of the newly synthesized DNA was associated with virions when intact virus was used as template; this was not the case with ether-treated virus. In both cases, DNA extracted from the reaction mixture sedimented slowly, at about 2–4 S.

Introduction. The recent demonstration of RNA-dependent DNA synthesis by intact murine and avian tumor viruses of the C-type[1,2] appears to provide striking support for the thesis originally advanced by Temin,[3] namely that these viruses multiply via a DNA intermediate. This finding promises to provide new experimental approaches to the yet unanswered questions regarding mechanism of replication and transmission of these viruses. The purpose of this communication is to show that this enzyme activity is also associated with C-type viruses of hamster,[4] cat,[5] and viper[6,7] origin and to describe certain features of the reaction.

Materials and Methods. Viruses: The AKR and Rauscher strains of murine leukemia virus were obtained from chronically infected cultures of rat tumor cells and mouse embryo fibroblasts, respectively. The hamster helper-virus (HaLV) was obtained from chronically infected embryo fibroblasts of the LSH inbred hamster strain.[4] The feline sarcoma virus (FSV) was obtained from beagle embryo cells transformed by the G.A. strain of FSV.[5] Feline leukemia virus (FLV) was obtained by terminal dilution of FSV beyond the focus-forming endpoint. The viper C-type virus was obtained from the chronically infected VSW cell line.[6] This virus is related to the C-type viruses in both morphological[6,7] and biophysical[7] properties.

Concentration and purification procedures: Supernatant fluids from the chronically infected cultures were clarified by centrifugation at 10,000 × *g* for 10 min; then viruses were pelleted at 100,000 × *g* for 1 hr. Pellets were resuspended in 0.1 M Tris buffer, pH 8.3, that contained 0.12 M NaCl, usually in 1/50–1/200th of the original volume. In some instances, virus was purified by centrifugation to equilibrium through 15–60% (w/v) sucrose gradients.

Enzyme assay: Virus suspensions were incubated with an equal volume of reaction mixture for DNA polymerase assays. The standard reaction mixture contained 0.1 M Tris buffer, pH 8.3; 6 mM Mg acetate; 0.12 M NaCl; 0.04 M dithiothreitol; 8 mM (each) of dATP, dGTP, and dCTP, and 6.0 μM [^{3}H]TTP (Schwarz, 4 Ci/mmol). After the desired incubation time, aliquots were removed and material insoluble in cold 10% trichloracetic acid was collected on Millipore filters. Radioactivity was determined in a Beckman LS 250 scintillation system.

Ether treatment: Virus preparations were shaken gently with an equal volume of anesthesia-grade ether for 10 min at room temperature. Ether was removed by aspiration and then evaporation with gentle agitation in a stream of N_2 gas.

Results. Species distribution of DNA polymerase: C-type viruses of mouse, hamster, cat, and viper origin were tested for DNA polymerase activity. All of these viruses stimulated the incorporation of [^{3}H]TTP into acid-insoluble material (Table 1). These preparations contained no RNA polymerase activity,

TABLE 1. *DNA polymerase activity in C-type virions of several species.*

Species of virus origin	Acid-insoluble [^{3}H]TTP cpm	Protein concentration mg/ml
Mouse—AKR	1,281	0.48
Rauscher	848	0.35
Hamster	1,691	0.40
Cat—Leukemia	441	0.05
Sarcoma	687	0.13
Viper	12,825	0.27

0.1 ml virus and 0.1 ml reaction mixture were incubated at 37°C for 2 hr before determination of acid-insoluble radioactivity. A reaction mixture blank similarly incubated yielded 103 cpm.

and enzyme activity was dependent on the presence of all four deoxyribonucleoside triphosphates. Of the viruses tested, the highest activity was repeatedly found with the viper virus. This would appear to be a result of greater intrinsic activity (stability) of the viper RNA-enzyme complex since total virus mass was not greater for this preparation than the mouse or hamster viruses.

Effect of ether on enzyme activity: In testing several virus preparations it was noted that freshly prepared, sucrose purified, virus was frequently inactive or showed low activity, while stored preparations (−70°C) were almost uniformly active. For this reason, several mild treatments were tried in an effort to enhance enzyme activity. A very gentle ether extraction was noticeably effective in increasing activity with all the C-type viruses (Table 2). Both the ki-

TABLE 2. *Effect of ether treatment on DNA polymerase activity of C-type viruses.*

Virus	Acid-insoluble [^{3}H]TTP cpm
Feline sarcoma virus	
Intact	50
Ether	800
Rauscher leukemia virus	
Intact	12
Ether	147
Viper C-type virus	
Intact	470
Ether	2418

0.2 ml of virus, 0.1 ml of ether or distilled water, and 0.2 ml of DNA polymerase reaction mixture were incubated for 45 min at 37°C. The ether was not removed for these experiments. Total acid-insoluble radioactivity was determined and blank values of approximately 100 cpm subtracted. The results shown for the viper virus have been regularly reproduced; however, some irregularity has been noted with other C-type viruses.

netics of [^{3}H]TTP incorporation and the extent of reaction were markedly stimulated (Fig. 1), as shown for the viper virus. In the absence of ether, the reac-

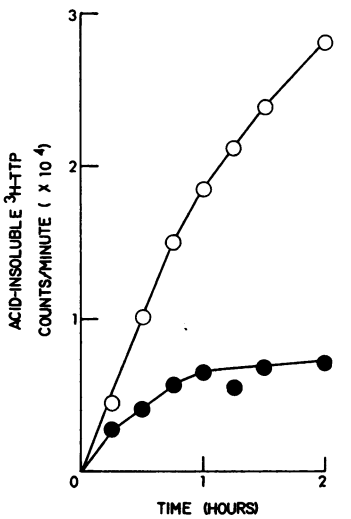

FIG. 1.—Effect of ether on DNA polymerase activity of viper C-type virus. 0.5 ml of intact and ether-treated viper C-type virus were incubated separately with 0.5 ml of reaction mixture for DNA polymerase assays. At the indicated times, 0.1 ml aliquots were removed for determination of acid-insoluble [³H]TTP. Intact virus: ●——●, ether-treated virus: O——O.

tion appears to reach a plateau after 1 hr of incubation, while ether-treated preparations showed appoximately linear kinetics for up to 2 hr. This treatment presumably removes cellular lipids from the virus surface allowing enhanced substrate penetration and/or product accumulation.

The mild ether treatment did not noticeably affect the integrity of viper virions since upon sedimentation through sucrose gradients a distinct opalescent band was still present at the characteristic buoyant density of the virus (1.16–1.17 g/cm³). DNA polymerase activity was associated only with the virus-containing fractions and no evidence of a subviral, enzymatically active, structure was found (Fig. 2). The enhancing effect of ether was clearly retained in the banded virus preparations.

Immunological tests: Attempts to relate the polymerase activity to one of

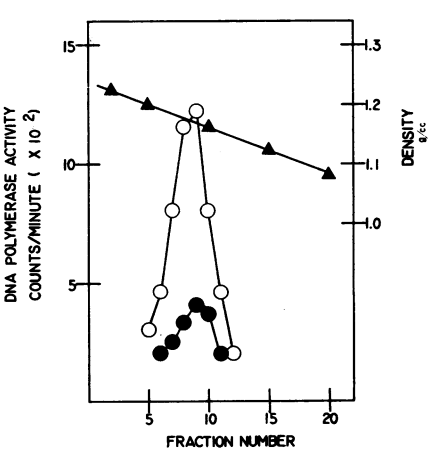

FIG. 2.—One-ml aliquots of a viper C-type virus pellet were treated with ether or buffer for 10 min at room temperature. The preparations were then centrifuged through 10 ml of a 15–60% (w/v) sucrose density gradient using the Spinco SW 41 rotor (90 min at 40,000 rpm). Twenty-three fractions of equal volume were collected from the bottom of the gradient tubes, and DNA polymerase activity was determined on 0.05 ml aliquots. Incubation was for 1 hr at 37°C. Density: ▲—▲, was calculated from the refractive index using sucrose solutions of known concentration as standard. Intact virus: ●—●, Ether-treated virus O—O. The activity peaks correspond to absorbance peaks at 280 nm and were found in both cases at ~1.165 g/cm³. All fractions other than those shown contained only background activity. The specific aetivity (cpm/mg protein) of the ether-treated peak fraction was 11 times higher than the corresponding nontreated fraction.

the known viral antigens have thus far been unsuccessful. Similar incorporation was achieved in the presence or absence of antiserum to the viral envelope (mouse and viper viruses) and to the internal, group-specific, antigen (mouse viruses). Because the DNA polymerase activity is restricted to whole virions it is not certain that the group-specific antibody could penetrate to the polymerase site. Alternatively, the enzyme site could be independent of the antigenic site, or the antigenicity of polymerized group-specific antigen could be different than that of the subunit form. Because of these many alternatives, we do not feel that the immunological approach will be meaningful until the components of the enzyme reaction can be separately reconstituted. We should also mention that mixtures of highly purified group-specific antigen and viral 70S-RNA have not shown evidence of enzyme activity. The possibility still remains that the group-specific antigen could be part of an internal structural complex of the virus necessary for enzyme activity.

Relative size of newly synthesized DNA: Using intact viper C-type virus as template, the sedimentation properties of newly synthesized DNA were compared directly and after extraction by the SDS-phenol method. In unextracted preparations, approximately 15–20% of the DNA cosedimented with intact virus particles; the remainder appeared to be of relatively low molecular weight. After extraction, all of the DNA sedimented slowly. Since this experiment was performed with preparations in the plateau region (1.5 hr) of the kinetic curve, it appeared likely that failure of DNA to dissociate from the virus was responsible for inhibition of the reaction. This assumption was supported by results using ether-treated virus as template. In this case there was no firm association of the DNA product with whole virions. Before and after extraction with SDS–phenol, the DNA sedimented to the same position in sucrose gradients. While precise determinations have not yet been made, we estimate the sedimentation coefficient of newly synthesized DNA to be in the 2–4S range.

Discussion. The original finding of DNA-synthesizing activity associated with the RNA-containing C-type viruses[1,2] can readily be confirmed, and extended to species encompassing three classes of vertebrates. We assume that this activity is relevant to the life cycle of this family of viruses, although details remain to be determined. We have shown additionally that gentle extraction with ether enhances the *in vitro* DNA yield and prolongs the extent of reaction, presumably by allowing release of the product from virions. It was found that similar ether treatment renders the RNA of viper virus susceptible to ribonuclease digestion;[7] however, this now appears to be due to increased permeability of the viral protein-envelope, or perhaps due to intimate association of the viral RNA with envelope protein, as opposed to disruption of the virion and release of RNA. Whether this is true of other C-type viruses treated gently with ether remains to be tested.

The size of DNA synthesized using C-type virus as template is relatively small, perhaps 1% or less of the viral RNA. This could mean that only a small portion of viral RNA functions as template or that the DNA is synthesized in relatively short fragments. The latter possibility is consistent with the mode of DNA replication which occurs in bacterial viruses,[8] bacteria,[8] and animal cells.[9]

This suggests that if a DNA copy of viral RNA is a functional intermediate in virus synthesis (as suggested by Temin[3]), then the DNA fragments could be joined by intracellular polynucleotide ligase.[10] The polymerase activity is apparently not a constituent of the viral envelope.

Abbreviation used: SDS, sodium dodecyl sulfate.

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