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# Characterization of the reverse transcriptase of a type C RNA virus produced by a human lymphoma cell line

**(RNA-dependent DNA nucleotidyltransferase/peptide maps)**

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**ABSTRACT** **The reverse transcriptase (RNA-dependent DNA nucleotidyltransferase) of the type C RNA virus produced by the human lymphoma cell line SU-DHL-1 was purified by ion-exchange chromatography of SU-DHL-1 culture fluids and repetitive affinity chromatography on poly(rC)-agarose, as were the polymerases of several other type C viruses. The DHL-1 enzyme used template-primers at levels expected of a viral reverse transcriptase, and sodium dodecyl sulfate gel electrophoretic analysis of radioiodinated DHL-1 enzyme revealed a peak at a position corresponding to those of several other type C viral reverse transcriptases (namely, at 72,000–78,000 daltons). The purified enzyme was partially neutralized by antibodies specific for the reverse transcriptase of simian sarcoma virus. Two-dimensional analysis on thin-layer cellulose plates of tryptic hydrolysates of the radioiodinated enzymes of several viruses revealed that six peptides are common to the polymerases of simian sarcoma virus, gibbon ape leukemia virus, baboon endogenous virus, and the DHL-1 virus, and that two to four peptides are unique to each of these enzymes. The DHL-1 viral reverse transcriptase appears to be most closely related structurally to the enzymes of simian sarcoma virus, gibbon ape leukemia virus, and baboon endogenous virus. However, the DHL-1 viral enzyme differed from any one or combination of the other subhuman primate viral enzymes by virtue of its unique peptides. The implications of these findings with respect to the probable origin of the DHL-1 virus are discussed.**

Type C RNA viruses have been detected in the culture fluids of a human histiocytic lymphoma cell line SU-DHL-1 (1) and in several other similar human lymphoma cell lines (2) previously established and characterized in this laboratory (3–5). The virus produced by the SU-DHL-1 cell line possesses the morphological, biochemical, and biological characteristics of a type C RNA virus (1). In electron micrographs of culture fluid pellets, the DHL-1 virus resembled other typical mammalian type C viruses in both size and morphology. Cocultivation of SU-DHL-1 cells with rat XC cells induced typical syncytial plaques, a biological phenomenon also observed with murine (6, 7) and subhuman primate (8, 9) type C RNA viruses. The polymerase activity associated with the virus banding at a density of 1.15 g/cm$^3$ in sucrose density gradients was partially inhibited by antibodies specific for the polymerases of simian sarcoma virus (SSV-1) and RD114 virus, but not that of murine leukemia virus (MuLV).

The partial inhibition of the DHL-1 viral polymerase by antibodies against SSV-1 or RD114 reverse transcriptase (RNA-dependent DNA nucleotidyltransferase) suggests that the DHL-1 virus is related to the subhuman primate viruses. This relationship was further studied by tryptic peptide analysis of the radiolabeled DHL-1 enzyme in comparison with several other viral reverse transcriptases. The purification and characterization of the DHL-1 enzyme and the preparation and analysis of these tryptic peptide maps are the subjects of this report.

## MATERIALS AND METHODS

**Cells.** Human lymphoma cell line SU-DHL-1 used in this investigation was established from the malignant pleural effusion of a patient with diffuse histiocytic lymphoma (3). That the cells in continuous cultures are derived from the neoplastic cell population was demonstrated by their aneuploid karyotypes and their heterotransplantability in the athymic *nude* mouse. The cell line has remained free of mycoplasma during its maintenance in this laboratory.

**Viruses.** The Moloney leukemia virus (M-MuLV) was propagated in rat cell line 78A-1 and purified on sucrose density gradients (10). Gibbon ape leukemia virus (GaLV) and SSV-1 grown on either NC37 or A204 cells, feline leukemia virus (FeLV) grown on FL-64 cells, RD114 endogenous feline virus propagated on RD cells, and baboon endogenous virus (BaEV) grown on BKCT cells were doubly banded in sucrose density gradients (supplied by Pfizer, through the Resources and Logistics Program of the National Cancer Institute).

**Polymerase Assays.** Assays for viral reverse transcriptase were performed at pH 8.1 and 30°C with 0.25 $A_{260}$ unit of template to 0.025 $A_{260}$ unit of primer per ml (11). The utilization of 70S RNA was measured by the addition of enzyme to a reaction mixture containing 60 mm Tris·HCl (pH 8.3), 8 mM $MgCl_2$, 80 mM KCl, 60 $\mu$M each dGTP, dATP, and dCTP, 15 $\mu$M [$^3$H]dTTP (20 Ci/mol; 1 Ci = 3.7 × 10$^{10}$ becquerels), and 50 $\mu$g of M-MuLV 70S RNA per ml with or without 0.25 $A_{260}$ unit of $(dT)_{12-18}$ per ml. Enzymes were compared at concentrations demonstrating similar levels of poly(rA)·oligo(dT) utilization.

**Ion-Exchange Chromatography of SU-DHL-1 Culture Fluid.** SU-DHL-1 culture fluids, clarified by centrifugation at 12,000 rpm for 20 min at 4°C, were concentrated with DEAE-cellulose (Whatman DE-52) treated with 1 M $K_2HPO_4$. Viral material was eluted from columns with linear KCl gradients. Fractions (0.5 ml) were collected and stored in 50% (vol/vol) glycerol at −20°C.

**Antibody to the Reverse Transcriptase of SSV-1.** Rabbit immunoglobulin raised against the purified reverse transcriptase of SSV-1 was generously provided by Robert C. Gallo (National Cancer Institute).

**Reagents.** Iodinated MuLV glycoprotein was supplied by Maria Lung of this laboratory. Synthetic polynucleotides were purchased from P-L Biochemicals; bovine serum albumin, rabbit muscle phosphorylase A, *N*-tosylphenylalanine chloromethyl ketone (TPCK)-trypsin and myoglobin were from

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Abbreviations: SSV-1, simian sarcoma virus; MuLV, murine leukemia virus; GaLV, gibbon ape leukemia virus; BaEV, baboon endogenous virus; FeLV, feline leukemia virus; MPMV, Mason–Pfizer mammary virus.

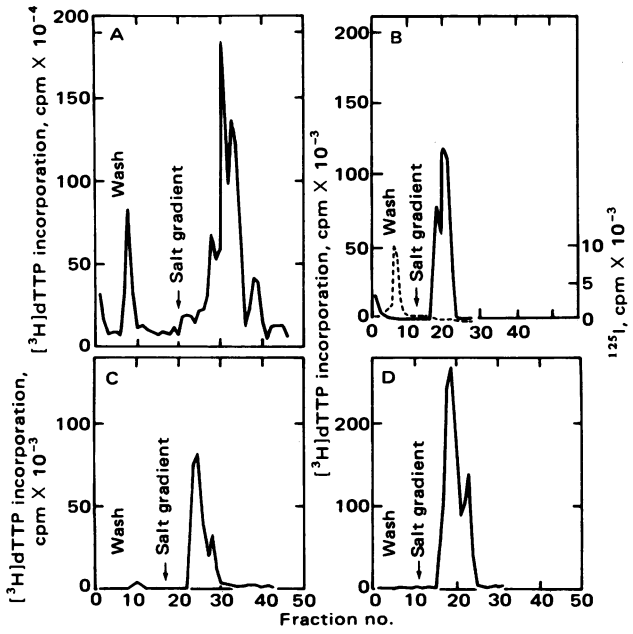


FIG. 1. Affinity chromatography of the polymerases of (*A*) SSV-1, (*B*) MuLV, (*C*) DHL-1 virus, and (*D*) GaLV. The virus harvested from 20 liters of DHL-1 culture fluid and preparations of the other viruses containing 20–50 mg of viral protein were dialyzed against TNE buffer (0.1 M Tris·HCl, pH 7.8/0.15 M NaCl/1 mM EDTA) and concentrated by using polyethylene glycol. The viruses were lysed by freeze-thawing and the core-associated material was separated by centrifugation at 100,000 × *g* for 90 min at 4°C in an SW 50.1 rotor. The core-associated polymerase was liberated by the addition of 0.5 M KCl/1.0% Triton X-100 to the pellets, and the material was clarified by subsequent centrifugation. After incubation at 4°C for 90 min, the clarified lysates were cycled at least five times through 5- to 10-ml columns of poly(rC)-agarose equilibrated with TNE buffer (12). The reverse transcriptases were eluted from the affinity columns with 20- to 40-ml, linear KCl (0–0.7 M) gradients. Fractions (0.5 ml) were collected and stored in 50% (vol/vol) glycerol at −20°C. Aliquots (50 μl) of each of the fractions were assayed for exogenous polymerase activity. Purified radioiodinated MuLV glycoprotein was added to the MuLV lysate prior to the chromatography.

Sigma; goat IgG was from Antibodies, Inc.; and radioisotopes were from New England Nuclear.

## RESULTS

**Purification and Characterization of DHL-1 Viral Polymerase.** The virus in 20 liters of SU-DHL-1 culture fluid was concentrated by ion-exchange chromatography as described in *Materials and Methods*. The reverse transcriptase of the DHL-1 virus and preparations of other type C viruses and of Mason–Pfizer mammary virus (MPMV) were prepared by affinity chromatography on poly(rC)-agarose as described in the legend to Fig. 1. In each case, most of the polymerase activity eluted at approximately 0.25–0.4 M KCl (Fig. 1).

The affinity chromatographed DHL-1 enzyme was tested for its ability to utilize various template-primer and divalent cation combinations relative to purified MuLV reverse transcriptase. Utilization of poly(rA)·oligo(dT) was greater than utilization of poly(dA)·oligo(dT) with $Mn^{2+}$ as the preferred cation (Table 1). Moreover, the enzyme utilized poly(rCm)·oligo(dG), though at a level less than that of MuLV reverse transcriptase. The utilization of MuLV 70S RNA was stimulated 3-fold by the addition of oligo(dT).

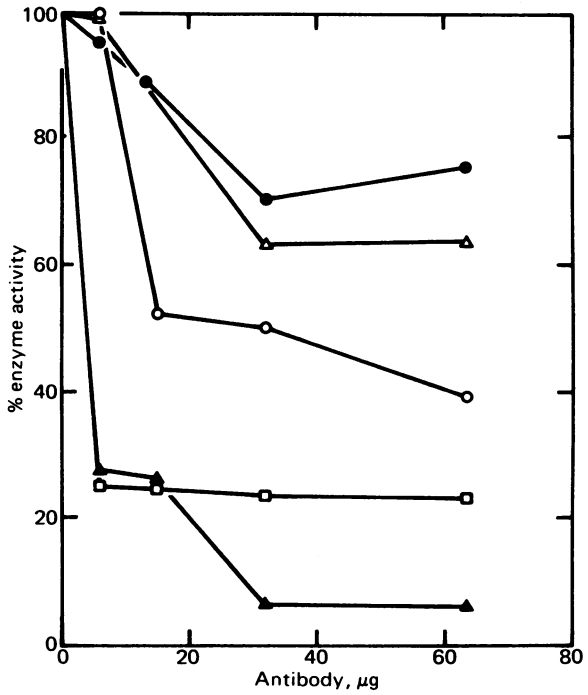


FIG. 2. Enzymatic inhibition of the purified DHL-1 polymerase by antibody specific for SSV-1 reverse transcriptase. Fifty-microliter aliquots of the viral polymerases chromatographed on poly(rC)-agarose were assayed for poly(rA)·oligo(dT)-stimulated activity in the presence of various concentrations of rabbit antibody specific for the reverse transcriptase of SSV-1. The percent enzymatic activity was calculated upon the basis of exogenous polymerase activities assayed in the presence of normal rabbit IgG at similar concentrations. Enzyme activities were between 0.1 and 1.0 pmol of [3H]dTMP incorporated per hr. ●, BaEV; △, RD114; ○, DHL-1; □, GaLV; ▲, SSV-1.

When the DHL-1 enzyme was incubated with an antibody raised against the purified reverse transcriptase of SSV-1, enzyme activity was partially inhibited (Fig. 2). The polymerase of GaLV was not inhibited to the same extent as the SSV-1 enzyme, suggesting that these enzymes could be distinguished on the basis of neutralization with this antibody. The relative extents of inhibition suggested that the DHL-1 enzyme is antigenically more closely related to SSV-1 reverse transcriptase than to the polymerases of BaEV or RD114.

The peak polymerase fractions from the affinity purified

Table 1. Utilization of various templates and primers by DHL-1 and MuLV reverse transcriptases

| Enzyme | Poly(rA)·oligo(dT) $Mn^{2+}$ | Poly(rA)·oligo(dT) $Mg^{2+}$ | Poly(dA)·oligo(dT) $Mn^{2+}$ | Poly(dA)·oligo(dT) $Mg^{2+}$ | Poly(rCm)·oligo(dG) | 70S RNA | 70S RNA + oligo(dT) | Poly(rA) | Oligo(dT) |
|---|---|---|---|---|---|---|---|---|---|
| MuLV polymerase | 30.5 | 0.01 | 0.35 | 0.07 | 1.91 | 0.014 | 0.050 | 0.001 | 0.001 |
| DHL-1 extracellular polymerase | 36.1 | 1.20 | 0.25 | 0.08 | 0.13 | 0.03 | 0.09 | 0.001 | 0.001 |

Values are pmol of [3H]dTMP or [3H]GMP incorporated measured at 60 min.

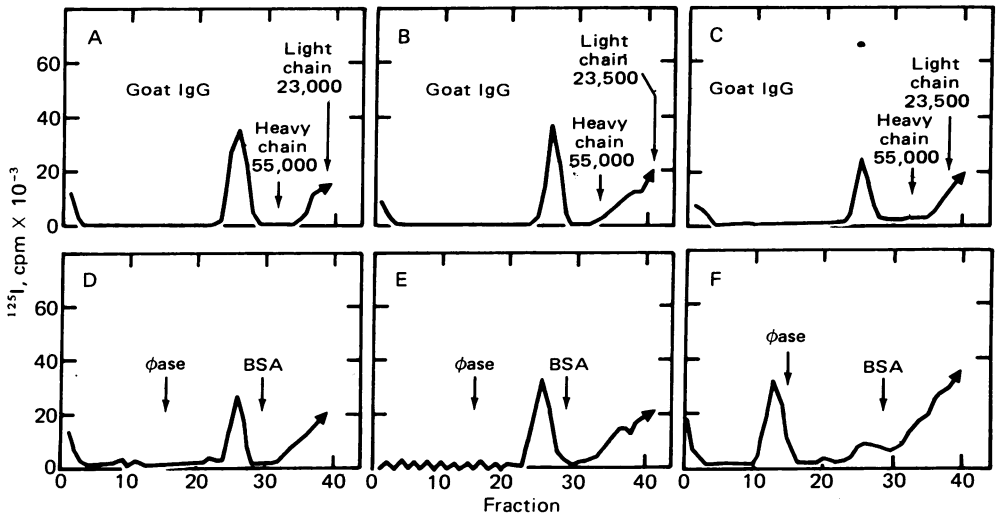


FIG. 3. Analytical sodium dodecyl sulfate/polyacrylamide gel electrophoresis of the purified reverse transcriptases of (*A*) GaLV, (*B*) SSV-1, (*C*) DHL-1, (*D*) BaEV, (*E*) FeLV, and (*F*) MPMV. Peak fractions of reverse transcriptase activity obtained from affinity chromatography of viral lysates were pooled, dialyzed against TNE buffer, and concentrated with polyethylene glycol. The samples were then iodinated by the chloramine T method (13), 5 μg of myoglobin was added as carrier, and the labeled proteins were analyzed by electrophoresis on 10-cm 5% sodium dodecyl sulfate/acrylamide disc gels (14). Each fraction measured in a Searle gamma counter contained two 1-mm gel slices. The molecular weight of each of the peaks was estimated from its extent of migration relative to that of $^{125}$I-labeled goat IgG, bovine serum albumin (BSA), and phosphorylase A (ϕase) run on a parallel gel.

samples were iodinated and electrophoresed on 5% sodium dodecyl sulfate/polyacrylamide disc gels. All of the enzymes except that of MPMV migrated to a position characteristic of a 72,000- to 78,000-dalton molecule (Fig. 3). The material obtained from MPMV migrated to a position characteristic of a 105,000- to 110,000-dalton molecule, in agreement with a previous report (15).

**Two-Dimensional Analysis of Tryptic Hydrolysates of Various Reverse Transcriptases.** Gel slices containing the radiolabeled material were pooled and processed for tryptic

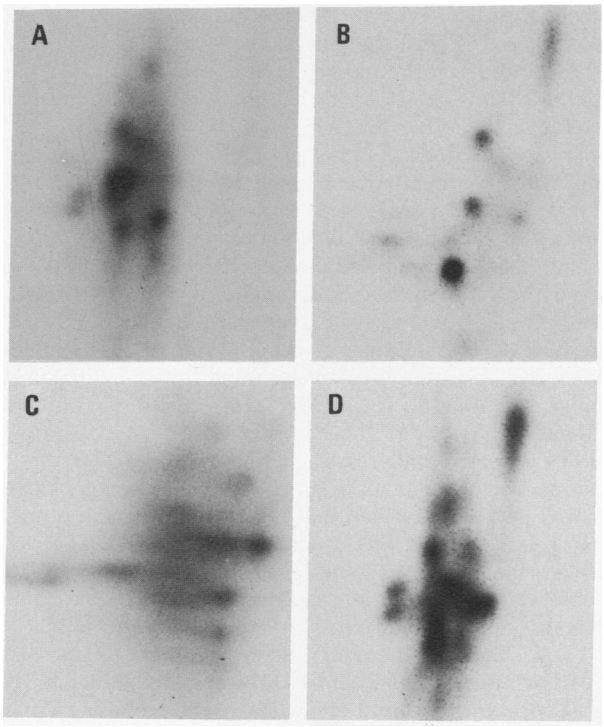


FIG. 4. Two-dimensional analysis of the tryptic hydrolysates of the reverse transcriptases of (*A*) SSV-1, (*B*) GaLV, (*C*) DHL-1 virus, and (*D*) BaEV. The polymerase fractions isolated by affinity chromatography were analyzed by tryptic peptide mapping. Two-dimensional electrophoretic and chromatographic analysis of tryptic hydrolysates on thin-layer cellulose plates was performed by the method of Elder *et al.* (16). The maps were compared on the basis of the relative mobility ($R_F$) of peptides in both dimensions relative to the migration of dye markers.

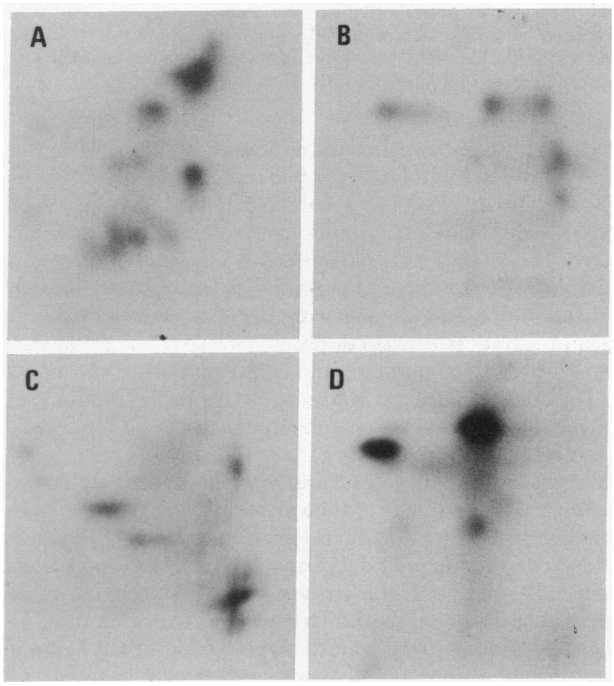


FIG. 5. Two-dimensional analysis of the tryptic hydrolysates of the reverse transcriptase of (*A*) RD114, (*B*) MuLV, (*C*) FeLV, and (*D*) MPMV. The purified reverse transcriptases of MuLV, RD114, FeLV, and MPMV, electrophoresed on sodium dodecyl sulfate/polyacrylamide gels, were subjected to digestion with trypsin and the hydrolysates were analyzed in two dimensions on thin-layer cellulose plates as described in the legend to Fig. 4.

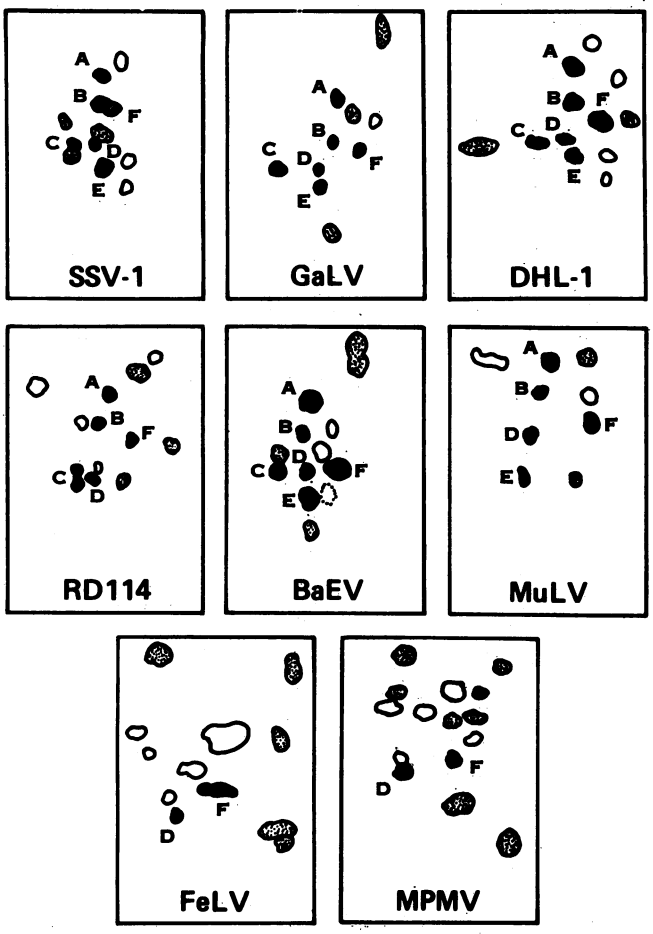


FIG. 6. Comparison of the tryptic peptide maps of the reverse transcriptases of SSV-1, GaLV, and DHL-1 virus. See legends of Figs. 4 and 5. The tryptic peptide maps of the viral reverse transcriptases were compared on the basis of peptides of similar $R_F$ values. Peptides shared with all other enzymes (●), shared with at least one other enzyme (○), and unique peptides (⊖).

peptide analysis as described in the legend to Fig. 4. After the separation of the hydrolysates in two dimensions on thin-layer cellulose plates, the maps were detected by fluorography (Figs. 4 and 5). A duplicate experiment involving a different preparation of the DHL-1 enzyme iodinated separately revealed a similar pattern of iodinated peptides (data not shown). Even when the iodinated enzymes were digested with a different trypsin preparation or when duplicate samples of a hydrolysate were compared, the numbers and configurations of the iodinated peptides remained consistent relative to the positions of the dyes used to monitor the electrophoretic and chromatographic steps.

In order to compare the various maps as objectively as possible, the maps obtained for the various reverse transcriptases were analyzed by measuring the relative mobility ($R_F$) values of the various iodinated peptides in both the first and second dimensions relative to the positions of the dye markers. In comparisons of the maps of different viral enzymes, those iodinated peptides with $R_F$ values varying by less than 1 mm in either direction were scored as common.

Five iodinated peptides (A, B, C, D, and E) formed a configuration that appeared in several of the maps (Fig. 6). Iodinated peptide F was also found in the maps of all of the enzymes, but it occupied a variable position diagonally downward and to the right of iodinated peptide B. The presence or absence in each map of iodinated peptides A–E served as a means of classifying the various enzymes. The SSV-1, GaLV, DHL-1, and BaEV reverse transcriptases contained all of the peptides A–F. RD114 viral reverse transcriptase lacked E, the MuLV preparation lacked C, and the FeLV and MPMV polymerases both lacked A, B, C, and E. The murine, subhuman primate, and putative human viral enzymes are distinguishable from enzymes such as FeLV or MPMV reverse transcriptase by the presence of at least the E iodinated peptide. Only the D iodinated peptide appears in all maps and, therefore, may represent a common amino acid sequence of related structural or enzymatic function. The variable position of the F iodinated peptide is intriguing and suggests that its sequence in different enzymes may tolerate limited amino acid substitution. To test whether the iodinated peptides in one map may actually correspond to the apparently common iodinated peptides of another map, we removed various iodinated peptides from the thin-layer plates, combined them, and re-examined them by two-dimensional analysis. The limited amounts of materials available necessitated this approach instead of that of hydrolysate mixing experiments. When the A iodinated peptides of DHL-1 and SSV-1 or the E iodinated peptides of BaEV and GaLV were thus reanalyzed together, only one iodinated peptide was observed in the combined map in each instance (data not shown). This result supports the validity of using $R_F$ values as a means of comparing iodinated peptides in different maps.

## DISCUSSION

The polymerase of the DHL-1 virus demonstrated several of the attributes of a viral reverse transcriptase. The enzyme utilized all of the template-primer combinations, including 70S RNA, at levels similar to those for MuLV reverse transcriptase. Moreover, the DHL-1 polymerase was partially neutralized by antibody raised against the purified reverse transcriptase of SSV-1 virus; the degree of cross reaction with DHL-1 enzyme was greater than with BaEV or RD114 polymerases, but less than with GaLV. When the purified DHL-1 enzyme was analyzed by sodium dodecyl sulfate gel electrophoresis, the major peak of material migrated as a single polypeptide with a size of 72,000–78,000 daltons (Fig. 3), in agreement with published size estimates for other type C viral polymerases (15, 17).

The number of $^{125}$I-labeled peptides detected in the various tryptic hydrolysates of these peaks analyzed in two dimensions varied between 9 and 12 for the lower molecular weight enzymes. This number is similar to that observed for the $\alpha$ subunit of avian myeloblastosis virus reverse transcriptase iodinated in a similar manner (18). If the peaks were contaminated with additional polypeptides, the numbers of iodinated peptides would be expected to increase accordingly.

Comparison of the maps in terms of the numbers of shared iodinated peptides revealed that the DHL-1 enzyme is most closely related to the SSV-1 and GaLV enzymes (Fig. 6). However, the DHL-1 enzyme revealed two unique peptides not observed in any of the other maps. Although the BaEV polymerase appears to be closely related structurally to that of SSV-1 and GaLV and, therefore, also to that of the DHL-1 virus, the polymerases of BaEV and RD114 are less similar to the DHL-1 viral enzyme immunologically on the basis of neutralization data obtained with specific antibodies (2). Based upon these tryptic digest map studies, the order of relatedness between the DHL-1 viral polymerase and the other enzymes analyzed appears to be SSV-1, GaLV, BaEV, RD114, MuLV, FeLV, and MPMV.

Recently, in as yet unpublished studies in collaboration with Ronald Levy, we have prepared a mouse myeloma–lymphocyte ("hybridoma") antibody against the DHL-1 viral reverse

transcriptase. The fact that this monoclonal antibody crossreacts with the other subhuman primate viral polymerases in enzyme inhibition assays supports the viral origin of the DHL-1 enzyme. In addition, the tryptic peptide map of the monoclonal antibody-immunoprecipitated DHL-1 polymerase shares the general configuration and numbers of iodinated peptides observed in the map of the enzyme purified by affinity chromatography, supporting the validity of the maps generated by the methodology outlined here.

The enzymatic, immunological, and structural characteristics of the DHL-1 viral polymerase establish its viral origin. Its unique iodinated peptides suggest that the DHL-1 polymerase is not identical with any subhuman primate virus polymerase or any mixture of two such polymerases. Thus, the possibility that the virus in DHL-1 culture fluids is a subhuman primate viral contaminant appears to be excluded. Instead, the DHL-1 enzyme resembles the subhuman primate viral polymerases structurally and antigenically to a degree compatible with expectation for a type C RNA virus of putative human origin. Nucleic acid hybridization analysis will be required to establish whether the DHL-1 virus is indeed of endogenous human origin. In that event, the tryptic peptide map of the DHL-1 viral reverse transcriptase would constitute a prototype pattern to which the enzymes from other putative human viral isolates could be compared.

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