Some characteristics of a porcine retrovirus from a cell line derived from swine malignant lymphomas

Iwao Suzuka, Kiichi Sekiguchi and Michi Kodama

National Institute of Animal Health, Tsukuba Science City, Ibaraki 305, Japan

Received 15 January 1985

The characteristics of the enzyme of porcine retrovirus from a cell line derived from swine malignant lymphomas fit with the template specificity and divalent cation requirement for reverse transcriptase. It is further found that the virus particle contains a high molecular mass 70 S RNA as viral genome.

Template specificity *Divalent cation* *Viral RNA*

1. INTRODUCTION

In a preliminary report of some of our data [1], it has been shown that a cell line derived from swine malignant lymphomas (Shimozuma-1) produces spontaneously virus-like particles with type C appearance by electron microscopy, with densities of 1.15–1.16 g/cm^3 in a sucrose gradient and with reverse transcriptase activity measured by poly(rA):oligo(dT). This virus is referred to as porcine retrovirus Tsukuba-1, but the biochemical properties of its virus have not yet been demonstrated. In general, reverse transcriptase of RNA tumor viruses has a greater preference for poly(rA):oligo(dT) over poly(dA):oligo(dT) as template/primer [2]. However, poly(rA):oligo(dT) is also the effective template/primer for DNA polymerase α, β, and γ [3,4], whereas viral reverse transcriptase can use efficiently poly(rC):oligo(dG) or natural poly(A)RNA(mRNA) [5,6]. Therefore, appearance of an enzyme with the ability to use these templates confirms the existence of viral reverse transcriptase. On the other hand, since a number of retroviruses contain RNA of high molecular mass [7,8], genomic RNA should exist in the virus particle. Here, we describe some characteristics of a reverse transcriptase and viral RNA of porcine retrovirus.

2. MATERIALS AND METHODS

The virus particles were obtained by methods previously described [9] from the culture fluid of Shimozuma-1 cells derived from swine malignant lymphomas [1]. The culture fluid (1 l) was sedimented to a cushion of 20% glycerol in STE buffer [10 mM Tris-HCl (pH 7.6), 100 mM NaCl, 1 mM EDTA] and the pellet was subjected to isopycnic centrifugation in preformed gradients of 15–60% sucrose in STE buffer. The band of virus at densities of 1.15–1.16 g/cm^3 was pelleted, dissolved in an appropriate volume of STE buffer containing 50% (v/v) glycerol and stored at −20°C. The viral RNA was prepared as in [10]. The virus pellet was suspended in STE buffer containing 0.5% SDS, 0.01 M EDTA and 200 μg proteinase K, then incubated for 1 h at 37°C. After 2-fold phenolization, the RNA was layered onto a gradient of sucrose (15–30%) in TE buffer [10 mM Tris-HCl (pH 7.6), 1 mM EDTA] containing 0.1% SDS, and then centrifuged in an SW 50.1 rotor at 48000 rpm for 90 min at 20°C. The viral RNA was finally dissolved in sterilized water and stored in liquid nitrogen. Rat liver mRNA was isolated from rat liver polysomes as in [11], by the SDS-proteinase K-phenol procedure and oligo(dT)-cellulose chromatography [12]. Synthetic templates and primers, AMV reverse

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

transcriptase, [^{3}H]dGTP and [^{3}H]dTTP were obtained commercially.

3. RESULTS AND DISCUSSION

It has been shown that globin mRNA carrying oligo(dT) to the poly(A) strand of the 3′-end of mRNA is an excellent template for reverse transcriptase [15]. To demonstrate that the virus produced by Shimozuma-1 cells can use mRNA template for reverse transcriptase, we studied the utilization of rat liver mRNA by the viral enzyme. Table 1 shows that full activity is dependent on the presence of the template, primer, the virus particle and all 4 deoxynucleotide triphosphates, but is markedly inhibited by RNase. Actinomycin D partially reduced the reaction, suggesting that it did not proceed further than the DNA:RNA hybrid under the conditions used. The slight activity in the absence of mRNA is probably due to priming oligo(dT) hybridized to endogenous viral RNA in the virus particle. Such endogenous DNA synthesis was less efficient (not shown), in contrast with the exogenous templates. The viral enzyme responded highly to poly(rC):oligo(dG) as well as to poly(rA):oligo(dT), but was inactive with poly(dA):oligo(dT). Oligo(dG) alone gave no appreciable activity. It is clear from this table that the viral enzyme is a reverse transcriptase and distinct from cellular DNA polymerases [3,4].

Table 1

Response of DNA synthesis by virus particles

Conditions	[^{3}H]dGMP or [^{3}H]dTMP incorporated (dpm/tube)
Complete	7457
− oligo(dT)	1197
− rat liver mRNA	1705
− virus particles	570
− dCTP	778
+ RNase A (10 μg/ml)	524
+ actinomycin D (50 μg/ml)	3394
+ poly(rC):oligo(dG)	66031
+ oligo(dG)	950
+ poly(rA):oligo(dT)	45320
+ poly(dA):oligo(dT)	850

The complete reaction mixture for DNA polymerase assay contained in 50 μl: 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 8 mM $MgCl_2$, 10 mM dithiothreitol, 0.025% Nonidet P-40, 0.5 mM each of dATP, dCTP, and dTTP, 0.05 mM [^{3}H]dGTP (1 μCi), 3 μg rat liver mRNA, 1 μg oligo(dT), appropriate amount of the virus suspension. Rat liver mRNA and oligo(dT) were annealed previously for 3 min at 60°C. The reaction mixture with synthetic template/primer contained: 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM $MgCl_2$, 0.05 mM [^{3}H]dGTP or [^{3}H]dTTP, 1 μg synthetic template or 0.5 μg primer/virus suspension. The reaction was carried out for 1 h at 37°C. Labeled products were precipitated with cold 10% trichloroacetic acid and the radioactivity was measured

Since the stimulation by Mn^{2+} is a characteristic of most viral reverse transcriptases [16,17], except that reverse transcriptase of bovine leukemia virus (BLV) has an absolute requirement for Mg^{2+} [18], it was of interest to study the dependence on divalent cation concentration of DNA synthesis by the virus particle. As shown in fig.1A, the mRNA-directed DNA synthesis occurred well either in Mn^{2+} or Mg^{2+}, but slightly preferred Mn^{2+} to Mg^{2+}. The reaction displayed a sharp optimum at 0.1 mM Mn^{2+}, whereas the optimal Mg^{2+} concentration varied from 6 to 10 mM. These results are similar to those reported for other type C viral reverse transcriptases [16,17]. In contrast, as shown by fig.1B, when poly(rC):oligo(dG) was used as template/primer, the preference switched from Mn^{2+} to Mg^{2+} and the optimum for Mg^{2+} was found to be between 1 and 4 mM. Although the reason for this reversal is not clear, differences in the properties of homo- and hetero-polymers presumably affect the divalent cation requirements. Such a reversal has also been observed with reverse transcriptase of human T-cell leukemia virus (HTLV) [19].

To demonstrate the nature of the product synthesized by the virus particle, Cs^2SO_4 density gradient analysis of the product was carried out as shown in fig.2. Most of the labeled products lie in an intermediate position between RNA and DNA densities (1.56 g/cm^3), suggesting that the product is a DNA:RNA hybrid. After alkaline treatment, the majority of the product shifted to the region of DNA density (not shown).

Retroviruses contain single-stranded 70 S RNA, which sediments at about 35 S after denaturation, and slow-sedimenting RNAs [7,8]. As shown in

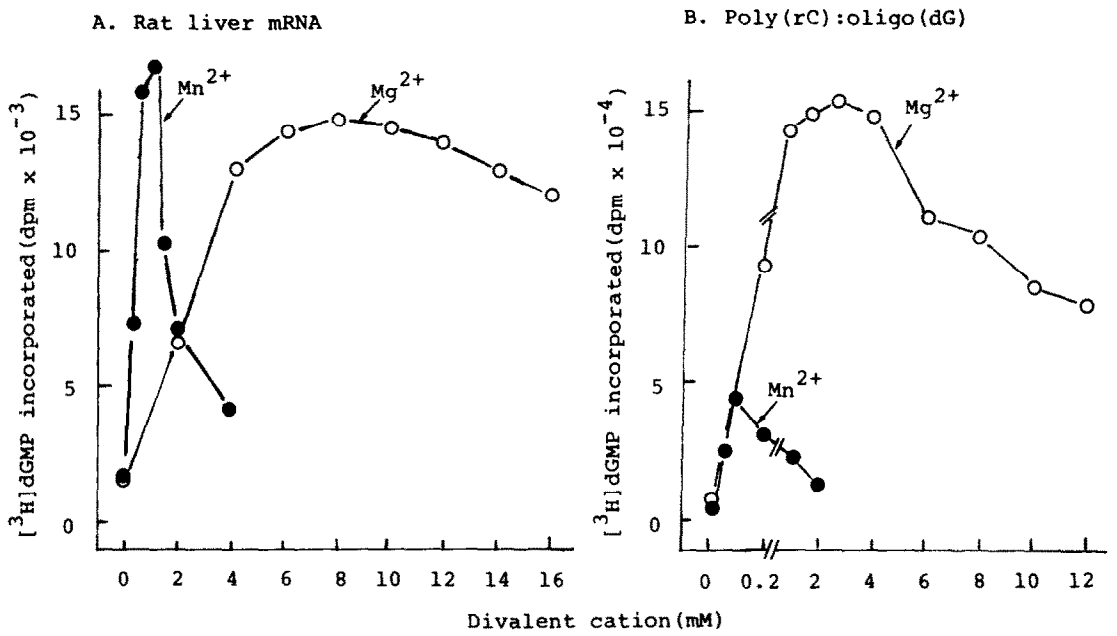

Fig.1. The effect of divalent cations upon DNA polymerase activity of the virus particle. The assay was the same as in table 1, except that divalent cations were added as indicated in the figure. Rat liver mRNA in (A) or poly(rC):oligo(dG) in (B) was used as template.

fig.3A, the RNA from the virus particles yielded two components upon sedimentation. The first component sedimented in the vicinity of the 70 S area and slow components in the vicinity of the 4–28 S areas. When the 70 S area was recentrifuged in a sucrose gradient and then agarose gel electrophoresis carried out, it can be seen in fig.3B and C that the viral RNA migrated in the 70 S position as single bands and was converted into about 35 S by treatment with dimethyl sulfoxide. Consequently, the size of the viral RNA was found to resemble that of the RNA genomes of other retroviruses [7,8]. Table 2 shows the DNA synthesis directed by the 70 S RNA with AMV reverse transcriptase. Evidently, the 70 S viral RNA is an active template primed by oligo(dT), indicating the presence of RNA-containing poly(A). The remaining activity without oligo(dT) may reflect priming

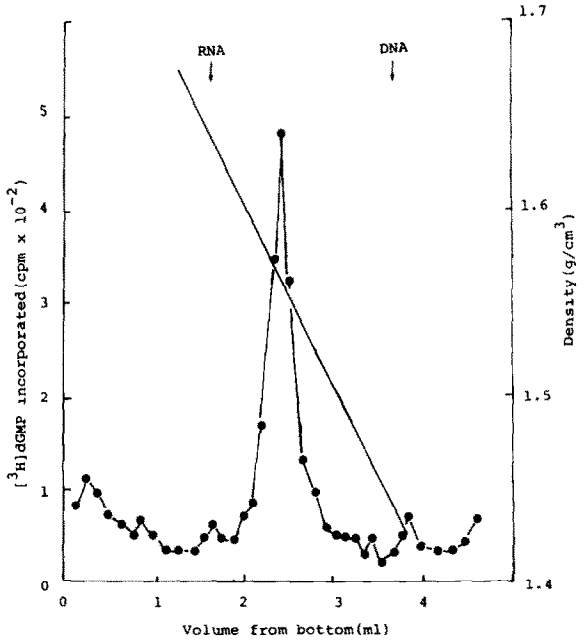

Fig.2. Cs_2SO_4 density gradient analysis of the product synthesized by the virus particle, in response to rat liver mRNA. The DNA synthesis was done as in table 1, except that the reaction was scaled up to 4-fold volume and contained 10 μg actinomycin D. The product was purified by the SDS-phenol procedure. The density gradient was performed by mixing the product with Cs_2SO_4 at a density of 1.15 g/cm^3 and centrifuging this mixture in an SW 50.1 rotor at 33000 rpm for 66 h at 15°C.

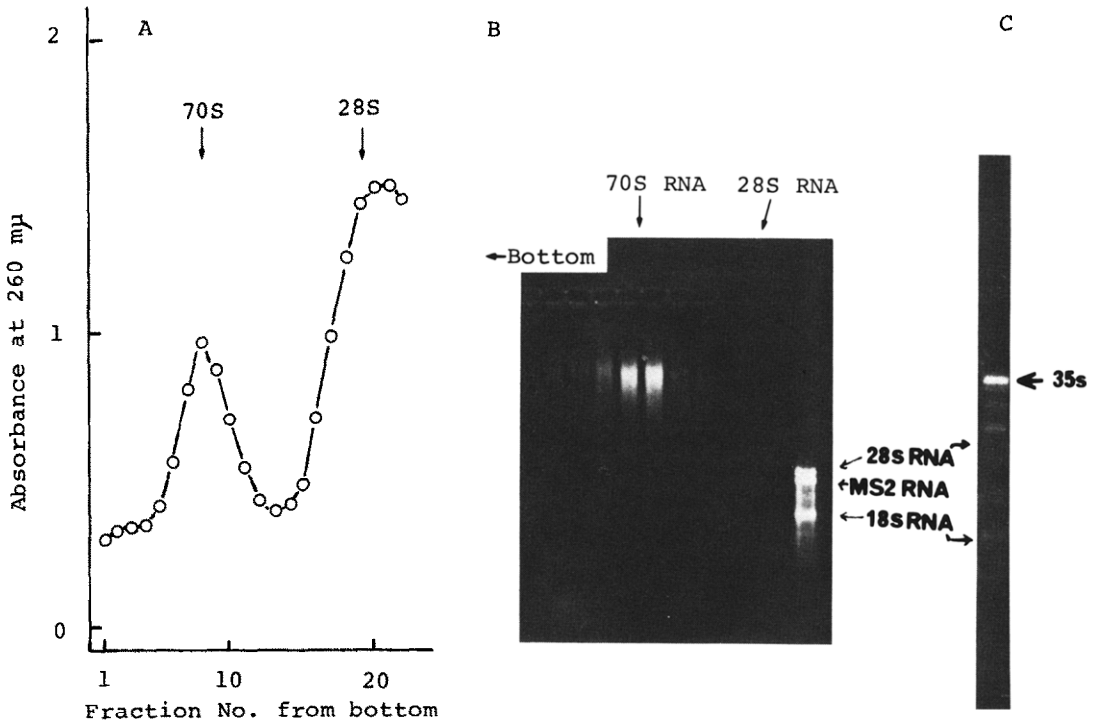

Fig.3. Sedimentation analysis and agarose gel electrophoresis of RNA from the virus particle. (A) The sucrose gradient centrifugation was performed as described in section 2. The absorbance of each fraction was measured at 260 nm. (B) 70 S area from (A) was sedimented as in (A), and fractions were electrophoresed in 30 mM Tris-acetic acid (pH 7.8), 2 mM EDTA buffer [13] on 0.8% agarose gel. (C) 70 S RNA from (B) was electrophoresed in 10 mM phosphate buffer after denaturation with 1 M glyoxal and 50% (v/v) dimethyl sulfoxide for 1 h at 50°C [14]. Ribosomal 28 S and 18 S RNAs and MS2 RNA were used as markers.

by trace oligonucleotides in the RNA preparation. RNase destroyed the template activity.

In summary, the virus particle from Shimozuma-1 cells had the following properties: (i) Its enzymatic properties correspond to those of viral reverse transcriptase. (ii) Its enzymatic product was a DNA:RNA hybrid. (iii) It contained viral RNA of 70 S which converted to 35 S. (iv) Its viral RNA had template activity primed by oligo(dT). (v) It showed type C appearance by electron microscopy. (vi) Its density in a sucrose gradient was 1.15–1.16 g/cm^3. From these observations, we can conclude that the swine cell line (Shimozuma-1) produces a new porcine retrovirus. Direct involvement of the porcine retrovirus in leukemogenesis remains obscure. Since recent reports show some similarities between HTLV and BLV with respect to the genetic structure of their proviral DNA [20–22], these findings will be informative in the genetic characterization of viral DNA of lymphoma-derived porcine retrovirus and in the understanding of leukemogenesis by this virus.

Table 2

Template activity of 70 S viral RNA

Conditions	[^{3}H]dGMP incorporated (dpm/tube)
Complete	14082
−70 S viral RNA	314
−oligo(dT)	1879
+RNase A (10 μg/ml)	215

The DNA synthesis was carried out as in table 1, except that the complete reaction mixture contained 0.5 μg of 70 S viral RNA from fig.3A, 0.5 μg oligo(dT) and 3 units AMV reverse transcriptase instead of the virus particle

ACKNOWLEDGEMENT

We are grateful to Dr K. Shimotohno, National Cancer Center Research Institute for encouragement during the course of this work.

REFERENCES

[1] Kodama, M., Sekiguchi, K., Kubo, M., Mitani, K., Osada, M., Sonoda, A., Kashiwazaki, M. and Saito, T. (1981) Abstract, 92nd Meeting Japanese Society of Veterinary Science, p.126.
[2] Baltimore, D. and Smoler, D. (1971) Proc. Natl. Acad. Sci. USA 68, 1507–1511.
[3] Weissbach, A., Bolden, A., Muller, R., Hanafusa, H. and Hanafusa, T. (1972) J. Virol. 10, 321–327.
[4] Brun, G., Rougeon, F., Lamber, M. and Chapevill, F. (1974) Eur. J. Biochem. 41, 241–251.
[5] Abrell, J.W. and Gallo, R.C. (1973) J. Virol. 12, 431–439.
[6] Lewis, B.J., Abrell, J.W., Smith, R.G. and Gallo, R.C. (1974) Science 183, 867–869.
[7] Duesberg, P.H. (1968) Proc. Natl. Acad. Sci. USA 60, 1511–1518.
[8] Erikson, R.L. (1969) Virology 37, 124–131.
[9] Verma, I.M. and Baltimore, D. (1974) Methods Enzymol. 25, 125–130.
[10] Hayward, W.S. (1977) J. Virol. 24, 47–63.
[11] Ramsey, J.C. and Steel, W.J. (1976) Biochemistry 15, 1704–1712.
[12] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408–1412.
[13] Maniates, T., Fritsch, E.F. and Sambrook, J. (1982) in: Molecular Cloning, pp.150–172, Cold Spring Harbor Lab., NY.
[14] McMaster, G.K. and Carmichael, G.G. (1977) Proc. Natl. Acad. Sci. USA 74, 4835–4838.
[15] Ross, J., Aviv, H., Scolinck, E. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 264–268.
[16] Scolnick, E., Rands, E., Aaronson, S.A. and Todaro, G.J. (1970) Proc. Natl. Acad. Sci. USA 67, 1789–1796.
[17] Faras, A.J., Tayler, J.M., Mcdonnel, J.P., Levinson, W.E. and Bishop, J.M. (1972) Biochemistry 11, 2334–2342.
[18] Gilden, R.V., Long, C.W., Hanson, M., Toni, R., Charman, H.P. and Oroszlan, S. (1975) J. Gen. Viol. 29, 305–314.
[19] Rho, H.M., Poiesz, B., Ruscetti, F.W. and Gallo, R.C. (1982) Virology 112, 355–360.
[20] Seiki, M., Hattori, S. and Yoshida, M. (1982) Proc. Natl. Acad. Sci. USA 79, 6899–6902.
[21] Sodroski, J., Trus, M., Perkins, D., Patarca, R., Wong-Staal, F., Gelmann, E., Gallo, R. and Haseltine, A.W. (1984) Proc. Natl. Acad. Sci. USA 81, 4617–4621.
[22] Sagata, N., Yasunaga, T., Ozawa, Y., Tsuzuku-Kawamura, J. and Ikawa, Y. (1984) Proc. Natl. Acad. Sci. USA 81, 4741–4754.