Suggestive Evidence for an Oncorna-Virus-Specific DNA Polymerase from C-Type Particles of Bovine Leukosis

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Typical C-type oncorna virus particles as shown by electron microscopy have been purified from the supernatant of cultured lymphocytes from bovine leukosis. In the purified C-particle fraction a DNA-polymerase activity was detected. Using several synthetic RNA- or DNA-homopolymers and 70S Friend virus RNA the template response of this bovine leukosis cell particle DNA polymerase was compared with those of feline leukaemia virus DNA polymerase and DNA polymerase from normal bovine lymphocytes. The DNA polymerase detected in the viral preparation of bovine leukosis is suggested to be an oncorna-virus-specific enzyme.

Introduction

The virions of known RNA tumor viruses [1–3] the simian foamy agent [4] and visna virus of sheep [5] contain a RNA-dependent DNA polymerase.

The viral RNA-DNA polymerase is capable of synthesizing DNA from viral RNA and from synthetic RNA-RNA or RNA-DNA templates [6]. DNA polymerase II from *E. coli* and some DNA polymerases purified from various embryonic or cultured cells, however, also catalyze the synthesis of poly (dT) in the presence of poly(rA) or poly(dA) and oligo (dT) as primers [6, 7]. The DNA polymerase from RNA tumor viruses responds very well to poly(rA) · $(dT)_{10}$ and very poorly to poly(dA) · $(dT)_{10}$, whereas various cellular DNA polymerases do not exhibit this specificity [6].

Furthermore, the cellular enzymes differ from the RNA directed DNA polymerase associated with RNA tumor viruses by their inability to transcribe heteropolymeric regions of RNA templates [7, 8]. In this paper we report on a DNA polymerase detected in a C-particle containing fraction obtained from the supernatant of cultured lymphocytes of leukotic cattle. This enzyme was compared in its template requirement with a DNA polymerase from feline leukaemia virus and a DNA polymerase isolated from the cytoplasmic extract of normal bovine lymphocytes.

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Material and Methods

Virus cultivation and purification

Bovine leukosis cell particles (BLCP) were prepared from cultured lymphocytes of highly leukaemic cattle described previously [8]. Feline leukaemia virus (FeLV) was propagated in cat embryo cells [10] and murine leukaemia virus, strain Friend, was multiplied in STU cells [11]. C-type particles from tissue culture supernatant were concentrated and purified according to the method of Bauer [12].

Preparation of DNA polymerases

DNA polymerase was liberated from purified BLCP of FeLV, respectively, by means of non-ionic detergents. Purified viruses (50 µg each) were suspended in 100 µl NDT buffer (0.08 M Tris-HCl, pH 7.9, 0.06 M KCl, 0.01 M dithiothreitol, 0.25% Nonidet P40) and incubated at 4 °C for 20 min.

DNA polymerase from normal bovine lymphocytes was prepared according to the method of Ackerman *et al.* [13].

Extraction of Friend virus RNA

Purified Friend virus was lysed with 0.5% SDS and twice extracted with phenol at 4 °C [14]. The RNA was purified by centrifugation in a continuous sucrose gradient (5 – 20% w/v sucrose in STE buffer (0.15 M NaCl, 0.01 M Tris-HCl, pH 7.4, 0.001 M EDTA).

The gradient was spun in a type SW 65 Beckman rotor and centrifuged for 60 min at 178,000 × *g*.

DNA-polymerase tests

DNA polymerase assays with synthetic templates were carried out following the method of Ross *et*

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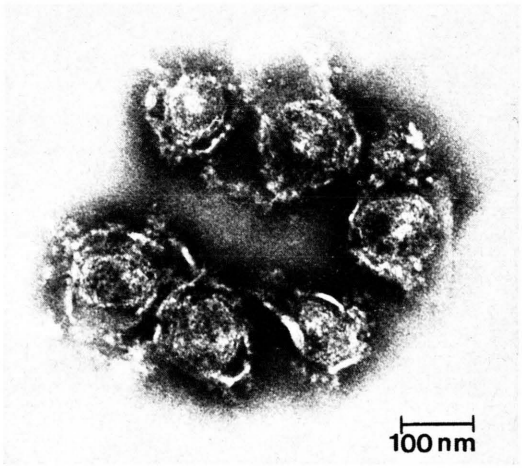

Fig. 2. Electron micrograph of bovine C-type particles after staining with PTA, pH 8. The outer envelope of particles is partially disintegrated and the structure of the inner core component is displayed. The particles were found in a fraction with a density of 1.16 g/ml after isopycnic sucrose density centrifugation.

al.[15]. The reaction mixtures contained in 100 µl 80 mM Tris-HCl, pH 7.8, 60 mM KCl, 6 mM magnesium acetate, 30 µM TTP, 5 µCi [^{3}H]TTP, 20 mM dithiothreitol, 2 µg FeLV-, 20 µg BLCP-crude polymerase or 10 µg polymerase from lymphocytes, 0.01 OD^{260} units poly(rA)·poly(dT), poly(dA)·poly(dT) or poly(rA)·$(dT)_{10}$, respectively.

The polymerase assay with 70S Friend virus RNA was done according to Goodman and Spiegelman[6]. 100 µl mixture contained 60 mM Tris-HCl, pH 8.3, 8 mM $MgCl_2$, 80 mM KCl, 60 µM dATP, dCTP, dGTP, each, 15 µM TTP, 5 µCi [^{3}H]TTP, 2 µg FeLV-, 20 µg BLCP-crude polymerase or 10 µg polymerase from lymphocytes and 10 µg 70S Friend virus RNA. The reaction mixture was incubated at 37 °C for 60 min and stopped by addition of 5% cold trichloroacetic acid. The acid-insoluble material was praecipitated on membrane filters and counted in a liquid scintillation counter.

Electron microscopy

Drops of virus preparations were mounted on carbon coated grids and allowed to settle. The drops were removed with filter paper, then the specimens were fixed for 5 min with 2% OsO_4, washed with STE buffer solution and then stained with 2% phosphotungstic acid (PTA) pH 7.0. The grids were screened at 40,000 fold magnification in a Siemens 101 electronmicroscope.

Material

Poly(rA)·poly(dT), poly(rA)·$(dT)_{10}$, poly(dA)·poly(dT). dATP, dCTP, dGTP and dTTP were purchased from Boehringer, Mannheim, Germany. Poly dT was a product of RL Biochemical Inc., Milwaukee, Wisc., USA. Oligo $(dT)_{10-12}$ was obtained from Collaborative Research Inc., USA. [^{3}H]TTP, specific activity 16.2 Ci/mmol, was purchased from Radiochemical Centre, Amersham, England.

Results

Figure 1 shows the density profile of BLCP-polymerase. This enzyme activity was associated with C-particles which were demonstrated by electron microscopic examination (Fig. 2 *). The main enzymatic activity was found in a region corresponding to a density of 1.16 g/ml.

Because of the poor endogenous activity of the BLCP preparation the incorporation of [^{3}H]TTP in an acid-insoluble material was tested using 3 different synthetic templates and 70S viral RNA from Friend virus. The results are shown in Fig. 3 and Table I. Figure 3 illustrates the kinetics of [^{3}H]TTP incorporation into an acid-insoluble product using poly(rA)·$(dT)_{10}$, poly(rA)·poly(dT) and poly

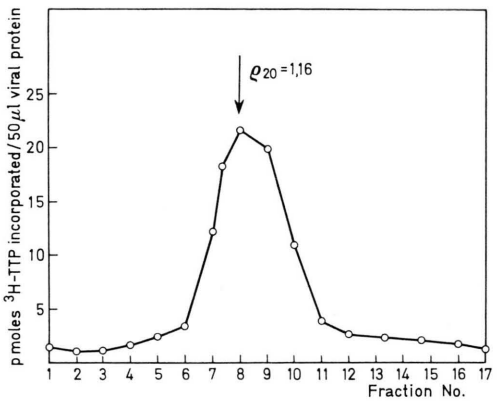

Fig. 1. Distribution of bovine C-type virus associated polymerase activity after isopycnic centrifugation. 300 µg bovine C-type virus in 500 µl STE-buffer was layered on a linear 5.0 ml gradient of 5—60% sucrose in STE-buffer and centrifuged for 4 h at 50.000 rpm in the Spinco SW 65 rotor. 50 µl of each fraction of the gradient were incubated for 60 min in a reaction mixture with poly(rA)·$(dT)_{10}$. The ordinate represents the amount of [^{3}H]TTP incorporated in an acid-insoluble material. The main polymerase activity was found to be in a fraction corresponding to a sucrose density of 1.16 g/ml.

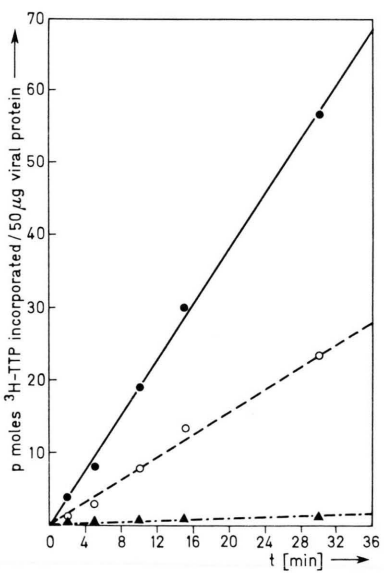

Fig. 3. Kinetics of incorporation of [^{3}H]TTP by bovine C-type particles associated DNA polymerase with 3 synthetic templates. ●—● Poly(rA)·$(dT)_{10}$; ○—○ poly(rA)·poly(dT); ▲—▲ poly(dA)·poly(dT).

* Fig. 2 see Table on page 72 a.

Table I. Characteristics of 70S Friend virus RNA directed DNA-synthesis by BLCP polymerase. DNA-polymerase assay was carried out as described in the Materials and Methods.

Additions	pmol [^{3}H]TTP incorporation
Complete [^{3}H]dTTP	1.20
Comple [^{3}H]dTTP + $(dT)_{10}$	10.70
Plus RNase *	0.08
Minus Friend 70S RNA	0.12
Minus dATP	0.12
Minus dCTP	0.08
Minus dGTP	0.07
Minus dATP, dGTP, dCTP	0.05

* The Friend virus RNA was preincubated with 50 μg/ml RNase at 37 °C for 30 min.

(dA) · poly(dT). The rate of synthesis is linear for more than 30 min. Furthermore, it can be seen that the enzyme responds very well to poly(rA) · $(dT)_{10}$, whereas poly(dA) · poly(dT) exhibits a poor template response.

Table I shows that the BLCP polymerase is also capable of using the 70S RNA from Friend virus as a template. In this case the presence of all four deoxyribonucleotide triphosphates in the incubation mixture is required. The pretreatment of the Friend virus RNA with pancreatic RNase led to a significant decrease of [^{3}H]TTP incorporation. This finding clearly demonstrates that BLCP polymerase is capable of transcribing heteropolymeric regions of the 70S Friend RNA template.

In Table II the enzyme activities of BLCP-, FeLV- and DNA-polymerases from bovine lymphocytes are compared using several homopolymers and 70S Friend RNA, as templates. Poly(rA) · $(dT)_{10}$ was shown to be an efficient template primer for FeLV- and for BLCP-polymerases. In contrast, the incorporation of [^{3}H]TTP was very low if poly(dA) · poly(dT) was used. However the DNA polymerase from bovine lymphocytes prefers poly(dA) · poly (dT). This latter enzyme did not respond to 70S Friend virus RNA, whereas BLCP- and FeLV-polymerases were able to respond to this template. From the results in Table II it is evident that the template requirements of the viral polymerases from BLCP and FeLV viral preparations are similar, but differ very strongly from the cellular DNA polymerase of normal bovine lymphocytes. Our attempts to differentiate the viral from the cellular enzyme by inhibition experiments with poly(U) [16] have failed, since the cellular polymerase of bovine lymphocites as well as FeLV and BLCP DNA polymerase were inhibited to the same extent.

Discussion

By the use of different templates we were able to distinguish the DNA polymerase from normal bovine lymphocytes and from that of viral C-particles isolated from cultured leukotic lymphocytes. It was clearly shown that the viral DNA polymerases (FeLV and BCLP) yielded a high rate of synthesis with poly(rA) · $(dT)_{10}$. These findings are in agreement with those of Goodman and Spiegelman [6] and Ross *et al.* [15]. We were also able to confirm the low response of cellular DNA polymerase to poly(rA) · $(dT)_{10}$. According to Rougeon *et al.* [7] and Temin and Baltimore [8] the most characteristic feature of the virus enzyme is the ability to copy heteropolymeric regions of RNA templates. In our experiments only the FeLV and BLCP DNA polymerases were able to transcribe heteropolymeric regions of 70S Friend RNA template, whereas DNA polymerase from normal lymphocytes did not respond. Our results suggest that the DNA polymerase found in BLCP has similar enzymatic characteristics as that of FeLV. This together with studies presented in an accompanying paper [18] and dealing with electron microscopic investigations, uridine pulse labelling experiments, haemagglutination and biophysical

Table II. Comparison of template response of polymerases from BLCP, FeLV and polymerase from normal bovine lymphocytes to Friend virus 70S RNA, poly(rA) · poly(dT), poly(rA) · $(dT)_{10}$ and poly(dA) · poly(dT).

Source of polymerase	pmol [^{3}H]TTP incorporated				
	Endogenous template	Friend 70S RNA	Poly(rA) · $(dT)_{10}$	Poly(rA) · poly(dT)	Poly(dA) · poly(dT)
BLCP	0.12	1.30	80.00	20.00	0.80
FeLV	0.40	3.00	156.00	56.00	1.20
normal bovine lymphocytes	0.09	0.08	1.60	NT	20.00

features of the C-particles isolated from cultured lymphocytes of bovine leukosis make it likely that BLCP's represent oncorna virus particles.

Compared to other oncorna viruses [2, 3] the endogenous template activity of BLCP associated DNA polymerase was very low. We explain this as being due to the fact that the BLCP particles are extremely labile and the virus RNA is very rapidly digested by the naturally occurring RNase.

Following the findings of Tuominen and Kenney [16] we tried to differentiate the BLCP polymerase from a normal cellular DNA polymerase by using poly(U) as an competitive DNA polymerase inhibitor. Contrary to Touminen and Kenney [16], however, in our experiments both the viral and the normal cellular DNA polymerase were inhibited to the same extent. This result is in agreement with the findings of Abrell *et al.* [17].

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