DNA POLYMERASE ACTIVITY IN HOMOGENATES OF CELLS INFECTED WITH MC29 VIRUS*

by

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SUMMARY

DNA polymerase activity was found in chick embryo cells (CEC) that were infected with a tumor virus, strain MC29 (myelocytomatosis) virus. The enzyme activity in homogenates of virus infected cells was greater than in homogenates from noninfected control cells.

DNA polymerase activity from noninfected CEC was not able to use the homopolymer duplex, poly dC:poly rG as a template. However, polymerase in cells infected with tumor virus was able to utilize this homopolymer duplex as template, as was the DNA polymerase associated with MC29 virus.

The results demonstrated that DNA polymerase activity found in MC29 virus was detectable in the infected-cell homogenate and was distinguished from the cell DNA polymerase by its ability to utilize dC:rG as template.

INTRODUCTION

The presence of a DNA polymerase has been demonstrated in many oncogenic viruses (1,2,3,4). RNA-directed DNA synthesis with the enzyme from tumor viruses was indicated by RNase inhibition of DNA synthesis and by the formation of DNA-RNA hybrids between the product and the virus RNA (1,2,3). DNA was shown to be a good template for DNA polymerase from oncogenic virus particles (5,6,7,8) and recently synthetic DNA-RNA and RNA-RNA duplexes were shown to stimulate DNA synthesis with the DNA polymerase from tumor viruses (9).

MC29 tumor virus causes transformation of chick embryo cells as early as 36-48 hours after infection (10,11). DNA polymerase can be measured in tissue culture supernate at 18-36 hours following infection (Weber, unpublished data). Our observations with MC29 tumor virus (12) encouraged us to examine virus-infected CEC for a unique DNA polymerase which should be present after infection with oncogenic virus. Utilizing a synthetic DNA:RNA hybrid it was possible to distinguish a new DNA polymerase activity in the infected CEC that was unlike the

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polymerase activity from noninfected CEC.

MATERIALS AND METHODS

Preparation of cells and virus: The preparation of chick embryo cultures (CEC) and infection with MC29 virus was previously described (11,12). Cells were initially seeded at a concentration of 5 x 10^6 cells per petri dish (20 x 100 mm). Twenty-four hours later the attached cells, chiefly fibroblasts, were washed with phosphate buffer-saline (PBS) and infected with 2.8 x 10^4 FFU/culture. Two days after infection, and on each succeeding days, the culture medium was changed. Five days after infection the medium was decanted and the cells washed with 4 ml PBS. One ml of medium was added to each culture and the cells scraped off with a rubber policeman. The cells were collected in two plate pools and centrifuged (3000 x g) for 10 minutes. The medium was decanted and the cell pellets frozen at -70°C.

MC29 virus was prepared for enzyme assay as was previously described (12) from 6 day infected CEC.

Preparation of cell extract: The frozen cells from two petri dishes were thawed, resuspended in 1.0 ml of Buffer 1 (0.01 M tris pH 8.3; 0.01 M KCl; 0.005 M $MgCl_2$; 0.005 M EDTA; 0.001 M GSH and 10% glycerol), and let stand in ice for 2 hrs. The cells were disrupted using a tight fitting Dounce homogenizer (about 30 strokes). Cell breakage was followed by microscopic examination during homogenation. Nuclei and cell debris were pelleted by centrifugation for 10 minutes at 1640 x g. The supernatant was decanted, made 0.25% Nonidet by adding an equal volume of 2X Nonidet stock (0.5% Nonidet Shell P40; 0.13 M dithiothretol) and allowed to stand overnight at 0°C before assaying for enzyme activity.

RESULT

Study of the DNA polymerase activity in homogenates from noninfected cells showed that the enzyme was present. DNA polymerase in the noninfected cells was stimulated slightly by DNA from salmon sperm, Cl. perfringens, B. subtilis, and calf thymus (Table 1). Template DNA from M. lysodeikticus and from avian myeloblastosis virus provided the greatest amount of DNA synthesis. No stimulation of enzyme activity was obtained with either of the hybrids from synthetic homopolymers (dC:rG and rI:rC).

A comparison of DNA synthesis by polymerase from infected cells is made in the same Table. The most important result demonstrated that, in contrast to the control cell preparations, the dC:rG hybrid gave a three fold stimulation of DNA synthesis. The template activity of the synthetic DNA:RNA hybrid

Table 1

DNA polymerase from MC29 virus infected cells

and from control cells

DNA source	Enzyme activity (cpm) from: Control Cells ^{3}H-dGMP	^{3}H-TMP	Infected Cells ^{3}H-dGMP	^{3}H-TMP	Virus ^{3}H-dGMP	^{3}H-TMP
dC:rG	70	56	1,837	212	10,172	153
M. lysodeikticus	750	90	3,921	1,698	4,396	2,926
Avian myeloblastosis virus	600	450	4,670	3,360	12,200	6,303
Salmon sperm	94	91	2,401	1,573	2,572	2,227
Cl. perfringens	236	247	2,613	2.767	5,356	4,019
B. subtilis	169	111	1,914	1,481	856	553
Calf thymus	166	102	2,127	1,777	5,427	4,731
Human leukemic lymphocytes	63	120	333	209	467	329
Human placenta	66	108	979	921	1,264	735
rI:rC	96	63	336	283	537	218
None	88	65	529	438	311	652

Chick embryo cell cultures were infected with strain MC29 (myelocytomatosis) virus. The cells were collected for enzyme preparation 5 days after infection and corresponding noninfected control cells were collected at the same time. Enzyme activity was measured by the incorporation of ^{3}H-dGMP and ^{3}H-TMP into acid insoluble radioactive material. The enzyme fraction was added at a level of 50 µg of protein for each assay both in the infected and control preparations. ^{3}H-dGTP was added to the reaction at a specific activity of 2000 cpm/pmole dGTP and ^{3}H-TTP was added at a specific activity of 2600 cpm/pmole TTP. DNA was added at 2 µg/0.1 ml assay volume. Reaction mixtures were the same as in Fig. 1 and incubated 37°C for 60 min.

revealed the presence of a second DNA polymerase activity appearing in the cell after infection with MC29 tumor virus and provided a method of assay for this new DNA polymerase activity in the presence of polymerase enzyme normally found in cells.

It seems unlikely that the synthetic DNA:RNA hybrid functioned by stimulating endogenous DNA synthesis since the ^{3}H-TMP did not, and would not be expected to, incorporate into the DNA product when dC:rG was used as template. It was apparent that DNA polymerase activity in the infected cell preparation greatly exceeded the activity found in the noninfected cell preparation with all effective DNA templates.

Homopolymer duplex, rI:rC, did not stimulate DNA synthesis by the enzyme from virus-infected cells (Table 1). In fact, a slight inhibition of the incorpo-

ration of ^{3}H-dGMP and ^{3}H-TMP may have been brought about by the homopolymeric complex. The homopolymer hybrid dC:rG appeared to depress the incorporation of ^{3}H-TMP relative to the incorporation of labeled substrate in the absence of template.

The virus DNA polymerase also utilized the dC:rG template and incorporated only ^{3}H-dGMP into polymer product. Thus the appearance of the new polymerase activity found in the cell extract was identified as an activity similar to that found in the MC29 virus.

The relative amounts of dGMP and TMP incorporated into DNA by polymerase were very close to the expected values when M. lysodeikticus was used as a primer. The mole fraction (dGMP/dGMP + TMP) of labeled substrate incorporated with M. lysodeikticus template was 0.70 which is reasonably close to 0.71, the mole fraction of (G + C) in the template molecule (13). On the other hand, similar calculations on products from the other DNA templates produced values greater than the mole fraction of (G + C) in the template molecule. This may reflect that the product of early synthesis has a high G content, or that copy was from selected regions in the template which are high in G. Studies with calf thymus DNA template have confirmed that labeled product-DNA had a higher

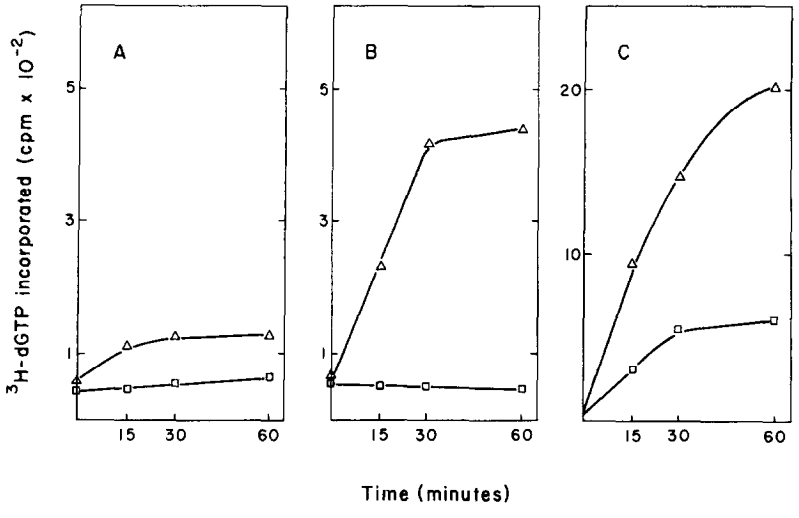

Figure 1. Kinetics of ^{3}HdGTP incorporation by DNA polymerase from MC29 infected cells (triangles) and noninfected cells (squares). The cell homogenates in A, B, C were not detergent treated. 50 µg of cellular protein were used per 0.1 ml assay which contained 4 µmoles Tris-HCl (pH 8.3); 0.8 µmoles $MgCl_2$; 6 µmoles NaCl; 0.37 µmoles reduced glutathione; 0.02 µmoles unlabeled deoxyribonucleoside triphosphates and ^{3}HdGTP at a specific activity of 2000 cpm/pmole. Fig. A shows the incorporation of ^{3}H-dGTP with no added primer. B indicates that activity when dC:rG is added (2µg/assay), and C when M. lysodeikticus DNA (2µg/assay) is added. For comparison with the results in Fig. 2 the homogenates were allowed to stand 24 hrs at 0°C prior to assay.

buoyant density (greater G content) than the bulk of the calf thymus DNA, suggesting that only a part of the DNA was being copied (12).

Fig. 1 summarizes a comparison of enzyme activity in noninfected cell cultures with enzyme activity in infected cell cultures. These cell homogenates had no detergent treatment and therefore represent enzyme from the cell that was free to participate in the reaction. The data showed that the noninfected cells contained a detectable polymerase (Fig. 1a) that is stimulated by M. lysodeikticus DNA template (Fig. 1c) but not by the synthetic hybrid dC:rG (Fig. 1b).

Addition of detergent to the cell homogenate stimulated enzyme activity of both the infected and noninfected cell homogenates (Fig. 2c,d) when DNA

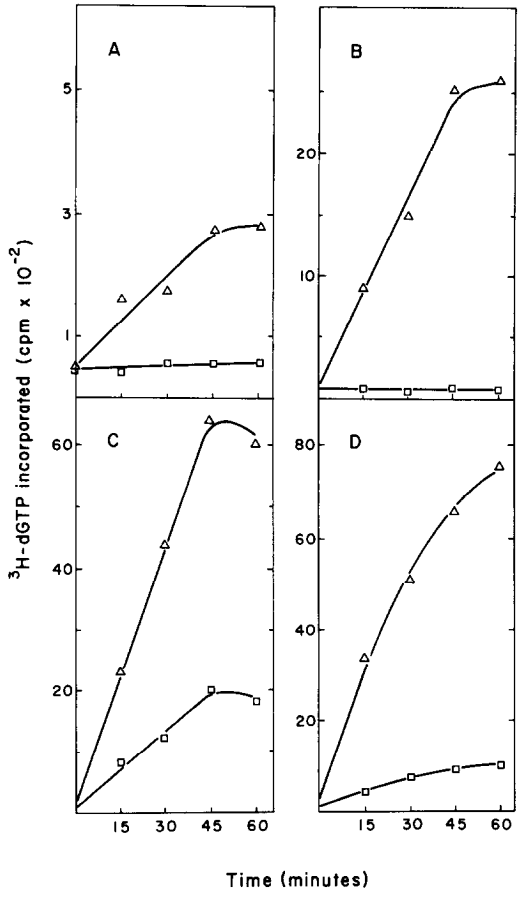

Figure 2. Kinetics of ^{3}H-dGTP incorporation by the DNA polymerase from detergent treated cell extracts in the absence of added DNA (A); with dC:rG (B); M. lysodeikticus DNA (C); and AMV DNA (D) added as primers. The assay conditions were the same as those cited in Fig. 1 with the exception that the cell supernatants were pretreated with equal volumes of 2X Nonidet stock and allowed to stand 24 hrs at 0°C before assaying for enzyme activity. Triangles designate enzyme activity from infected cells and the squares designate enzyme activity from non-infected cells.

template is added. The dC:rG hybrid served as template only in the infected cell homogenate (Fig. 2b). Apparently this offers a good method for distinguishing the DNA polymerase that results from tumor virus infection. DNA from AMV (6) was an excellent template for the DNA polymerase from MC29 infected cells (Fig. 2d). DNA from AMV also stimulated the DNA polymerase of the control cell homogenate but not to the level observed with the infected-cell enzyme.

DNA synthesis by enzymes from cell homogenates decreased rapidly after 45 minutes and it is likely that the cell homogenates contained sufficient nuclease activity to give these results. The enzyme from MC29 tumor virus has been observed to continue synthesis at a constant rate for 8 hours (Weber, unpublished data).

DISCUSSION

The results in this paper have shown that cells infected with MC29 tumor virus had a level of DNA polymerase greater than uninfected control cells. M. lysodeikticus DNA and DNA from AMV produced greater DNA synthesis in the infected cells than in the control cells (Fig. 2). Natural DNA template such as these will be useful in study of the DNA polymerase from infected cells when adequate fractionation procedures are available.

The DNA polymerase from infected cells utilized dC:rG template for synthesis, but the normal cell enzyme could not. Synthetic DNA:RNA hybrid was first observed by Spiegelman et al. to be an effective template for DNA synthesis with DNA polymerase from tumor viruses (9), and they suggested that this template could be used to advantage in detecting in cells DNA polymerase brought about by oncogenic virus infection. This hypothesis is supported by evidence presented in this paper, where it was possible to distinguish the polymerase activity in crude preparations from infected and noninfected cells. Indeed, it appears to be the most convincing method presently available for demonstrating the appearance of a new enzyme activity in the cell after infection with an oncogenic virus.

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