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On the Stimulation of Viral DNA Polymerase Activity by Nonionic Detergent†

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ABSTRACT: Nonionic detergents stimulate purified RNA-directed DNA polymerase (reverse transcriptase) activity from various RNA tumor viruses ranging from avian to primate species. The stimulatory effect of the nonionic detergent is dependent on the type and amount of template-primer. The greatest stimulation is obtained when high concentrations of $(dT)_{12-18} \cdot (rA)_n$ or activated salmon sperm DNA are used as template-primers. Little stimulation is obtained with viral 70S RNA or with $(dT)_{12-18} \cdot (dA)_n$. The detergent stimulation appears to be specific for viral reverse transcriptase since this effect is not observed with purified bacterial DNA polymerase or with three known mammalian cellular DNA polymerases. This finding may, therefore, be a useful additional criterion for distinguishing viral reverse transcriptase isolated from cells from other cellular DNA polymerases. Nonionic detergent also has a stabilizing effect on viral DNA polymerase against thermal inactivation. This stabilizing effect is further enhanced by the presence of template-primer.

Reverse transcriptase of RNA tumor viruses is located in the cores of the virus structure (Gerwin *et al.,* 1970; Coffin and Temin, 1971). In order to detect the maximum activity of this enzyme, virus particles are usually disrupted with a predetermined optimal concentration of nonionic detergent (Baltimore, 1970; Temin and Mizutani, 1970; Scolnick *et al.,* 1970; Bishop *et al.,* 1971). Recently, nonionic detergents were shown to stimulate $(dT)_n$ synthesis with purified murine leukemia sarcoma virus reverse transcriptase using $(dT)_{12-18} \cdot (rA)_n$ as template-primer (Thompson *et al.,* 1972; Wu *et al.,* 1973; Wu and Gallo, 1974). Therefore, nonionic detergents are used not only to disrupt virus particles and solubilize viral reverse transcriptase but also to enhance the activity of the viral enzyme. The purpose of this study is to further elucidate the mechanisms of this enhancement of DNA polymerase activities by nonionic detergent.

Our results indicate that the stimulatory effect of nonionic detergents is dependent on the type and amount of template-primer. The largest stimulation is obtained when high concentrations of $(dT)_{12-18} \cdot (rA)_n$ or activated salmon sperm DNA are used as template-primers. Very little stimulation is observed with viral 70S RNA or with $(dT)_{12-18} \cdot (dA)_n$. The stimulation appears to be specific for viral reverse transcriptase since there is no effect on bacterial and mammalian cellular DNA polymerases. Studies on thermal inactivation of the enzyme show that nonionic detergents slightly stabilize the activity of the enzyme. The enzyme stability is further increased by the presence of template-primer.

Materials and Methods

Nonionic Detergents and Template-Primers. The nonionic detergents used in this study were obtained from the

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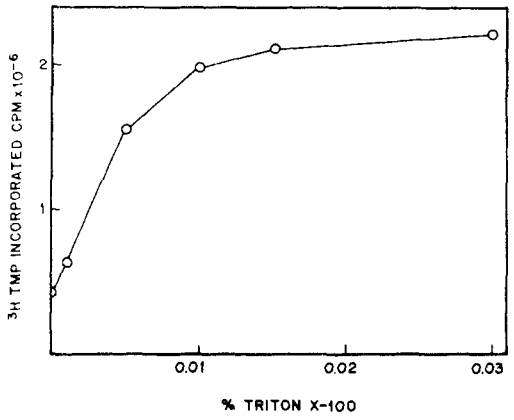

FIGURE 1: Triton X-100 stimulates RNA-directed DNA synthesis catalyzed by R-MuLV reverse transcriptase. The procedures of the RNA-directed DNA synthesis was as described under Materials and Methods. The reaction mixture included R-MuLV reverse transcriptase (4 μg/ml) and template-primer $(dT)_{12-18} \cdot (rA)_n$ (10 μg/ml). Other components of the assay mixture were described under Materials and Methods. Various concentrations of Triton X-100 were added as indicated.

following sources: Triton X-100 from Packard Instrument Co., Inc.; Tween-40 from J. T. Baker Chemical Co.; Triton N-101 from Sigma Chemical Co.; Nonidet P-40 from Shell Chemical Co.; and Sterox SL from Monsanto Co.; $(dT)_{12-18} \cdot (rA)_n$, $(dT)_m \cdot (rA)_n$, $(dG)_{12-18} \cdot (rC)_n$, and $d(AT)_n$ were purchased from Collaborative Research; $(dT)_{12-18} \cdot (dA)_n$ from Miles Laboratories, Inc.; and salmon sperm DNA from General Biochemical. Activated salmon sperm DNA (SSA)[1] was prepared by a partial DNase I digestion using a published procedure (Abrell and Gallo, 1973). 70S RNA from Rauscher leukemia virus (R-MuLV) was prepared as previously described (Bhattacharyya *et al.*, 1973).

DNA Polymerases. SOURCES. R-MuLV and Kirsten murine sarcoma virus (Ki-MuSV) reverse transcriptase was purified as previously described (Wu *et al.*, 1974). Briefly, virus particles were disrupted with Triton X-100 and the enzymes were purified through steps of phosphocellulose and DEAE-cellulose chromatography. The enzymes eluting from DEAE-cellulose were purified more than 300-fold and were 90% pure as judged by 0.1% SDS acrylamide gel electrophoresis. Reverse transcriptase from murine mammary tumor virus (MMTV) was purified by the same procedure. The reverse transcriptases of avian myeloblastosis virus (AMV) (Robert *et al.*, 1972) and type-C gibbon ape leukemia virus (Abrell and Gallo, 1973) were prepared as previously described. All of the reverse transcriptases contained ribonuclease H activity but not the other nuclease or phosphatase activities. The methods for preparing reverse transcriptase from fresh human leukemic blood cells was also previously described (Sarngadharan *et al.*, 1972; Gallo *et al.*, 1973a,b; Todaro and Gallo, 1973). The preparation of DNA polymerase I(α), II(β) (Smith and Gallo, 1972)[2] and III(γ) (Lewis *et al.*, 1974)[2] from RPMI 1788 (a human lymphoblastic cell line) has also been previously described. DNA polymerase α and β are the nomenclature for high molecular weight and low molecular weight DNA polymerases isolated from mammalian cells (Smith and Gallo, 1972) and DNA polymerase γ for "R"-DNA polymerase (Fridlender *et al.*, 1972). *Escherichia coli* and *Micrococcus luteus* DNA polymerases were purchased from General Biochemicals.

Table I: Effect of Nonionic Detergents on $(dT)_n$ Synthesis by Rauscher Leukemia Virus DNA Polymerase.

Nonionic Detergent (0.05%)	Enzyme Activity[a] (cpm × 10^{-5})	Stimulation by Detergent
None	6.5	1
Triton X-100	21.9	3.4
Triton N-101	21.4	3.3
NP-40	21.6	3.3
Tween-40	22.4	3.4
Sterox SL	22.7	3.5

[a] The procedure for measuring $(dT)_n$ synthesis was the same as described under Materials and Methods; 4 μg/ml of R-MuLV DNA polymerase was used in each assay.

ASSAYS. The procedures for assaying DNA polymerase activity are described elsewhere (Wu and Gallo, 1974). Standard assays contained in 50 μl: 50 mM Tris-HCl (pH 7.9), 1 mM dithiothreitol, 0.2 mM EDTA, 60 mM KCl, 1 mM $Mn(OAc)_2$ (or 10 mM $Mg(OAc)_2$ as indicated), 5.4 μM [^{3}H]TTP (13,000 cpm/pmol, Schwarz/Mann), an indicated amount of enzyme, and 10 μg/ml of $(dT)_{12-18} \cdot (rA)_n$. When other template-primers were used, the appropriate deoxyribonucleoside triphosphates were used as indicated in each experiment. The concentrations of nonradioactive deoxyribonucleoside triphosphates were 0.1 mM. When $(dG)_{12-18} \cdot (rC)_n$ was used as template-primer, the concentration of [^{3}H]dGTP was 6.4 μM (11,000 cpm/pmol, Schwarz/Mann). The concentration of the nonionic detergent is described in each experiment. The divalent cations used in the assay were based on the preference of each enzyme which is indicated in each experiment. Reaction mixtures were incubated at 37° (or 30° only when $(dT)_{12-18} \cdot (rA)_n$ was used) for 30 min. Reactions were stopped by the addition of ice cold 10% trichloroacetic acid containing 0.02 M sodium pyrophosphate. The acid precipitable radioactivity was then determined.

Results

Nonionic Detergent Stimulates RNA-Directed DNA Synthesis. Several nonionic detergents were previously shown to stimulate DNA synthesis by crude reverse transcriptase extracted from M-MuSV infected cells (Thompson *et al.*, 1972). Since the crude enzyme preparations used in that study still contained low concentrations of nonionic detergent, a quantitative measurement of this stimulation was not possible. To overcome this difficulty, purified DNA polymerases (free of nonionic detergent) were used to study the effect of these compounds on DNA polymerase activity. Figure 1 shows that DNA synthesis is stimulated by a relatively low concentration of Triton X-100. A plateau representing a fivefold stimulation was achieved with 0.02% detergent. Many other nonionic detergents have the similar stimulatory activity (Table I). Apparently, this stimulatory activity is not due to an inhibition of nuclease activity by

[1] Abbreviations used are: SSA, activated salmon sperm DNA; R-MuLV, Rauscher murine leukemia virus; Ki-MuSV, Kirsten murine sarcoma virus; M-MuSV, Moloney murine sarcoma virus; SDS, sodium dodecyl sulfate; MMTV, murine mammary tumor virus; AMV, avian myeloblastosis virus.

[2] Recently, a group of concerned scientists has renamed cellular DNA polymerase I, II, and III, DNA polymerase α, β, and γ, respectively.

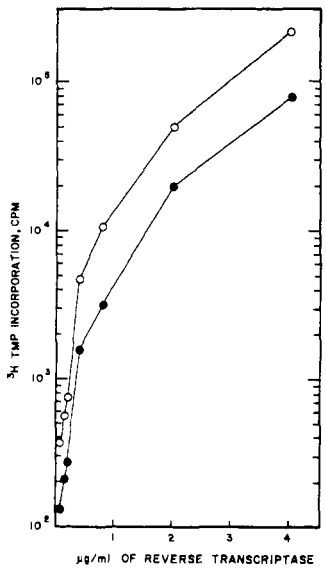

FIGURE 2: Effect of Triton X-100 on RNA-directed DNA synthesis with various concentrations of R-MuLV reverse transcriptase. $(dT)_n$ synthesis was carried out as described under Materials and Methods. The concentration of Triton X-100 in the reaction mixture was 0.05%. The concentrations of enzyme are indicated in the figure. (○) $(dT)_n$ synthesis in the presence of Triton X-100; (●) $(dT)_n$ synthesis in the absence of Triton X-100.

Table II: Effect of Type of Template-Primer on the Stimulation of DNA Synthesis by Triton X-100 using Rauscher Leukemia Virus DNA Polymerase.

Template–Primer	Substrate	Enzyme Activity (cpm × 10^{-3})[a] −Triton X-100	Enzyme Activity (cpm × 10^{-3})[a] +Triton X-100	Stimulation by Triton X-100
$(dT)_{12-18}\cdot(rA)_n$	[^{3}H]TTP	130.8	403	3.0
$(dT)_m\cdot(rA)_n$	[^{3}H]TTP	152.6	413	2.70
$(dT)_{12-18}\cdot(dA)_n$	[^{3}H]TTP	0.4	0.5	1.2
$(dG)_{12}\cdot(rC)_n$	[^{3}H]dGTP	7.7	20.8	2.7
$d(AT)_n$	[^{3}H]TTP dATP	10.6	18.1	1.7
Activated salmon sperm DNA	[^{3}H]TTP, dGTP dATP, dCTP	4.2	12.9	3.1
RLV 70S RNA	[^{3}H]TTP, dGTP dATP, dCTP	2.2	2.9	1.3
RLV 70S RNA + $(dT)_{12-18}$	[^{3}H]TTP, dGTP dATP, dCTP	32.1	112.2	3.5

[a] R-MuLV DNA polymerase activity with various template–primers was measured according to the procedure described under Materials and Methods. Triton X-100 concentration used for this experiment was 0.05% and amount of enzyme per reaction mixture of 50 μl was 0.2 μg. The concentration of synthetic template–primers was 10 μg/ml; activated salmon sperm DNA, 10 μg/ml; and 70S RNA, 20 μg/ml; 10 mM $Mn(OAc)_2$ was used for all assays.

nonionic detergent because the purified enzymes used in this study did not contain any detectable nuclease or phosphatase activities except ribonuclease H activity. We observed that nonionic detergent did not inhibit ribonuclease H activity (data not shown).

Table III: Effect of Triton X-100 on the Activity of Various DNA Polymerases.

Source of DNA Polymerase[a]	Stimulation by Triton X-100[b] With $(dT)_{12-18}\cdot(rA)_n$	Stimulation by Triton X-100[b] With SSA
Viral or viral related DNA polymerase		
Avian myeloblastosis virus	2.9 (Mg^{2+})	3.0 (Mg^{2+})
Raushcer leukemia virus	2.8 (Mn^{2+})	3.6 (Mn^{2+})
Kirsten murine sarcoma virus	2.3 (Mn^{2+})	3.1 (Mn^{2+})
Murine mammary tumor virus	2.6 (Mg^{2+})	2.3 (Mg^{2+})
Gibbon Ape leukemia virus	2.8 (Mn^{2+})	3.0 (Mn^{2+})
Human leukemic RNA-directed DNA polymerase (reverse transcriptase)	2.7 (Mn^{2+})	2.3 (Mn^{2+})
Mammalian cellular DNA polymerases		
Polymerase α from normal human blood lymphocytes	1.1 (Mn^{2+})	1.2 (Mg^{2+})
Polymerase β from normal human blood lymphocytes	1.0 (Mn^{2+})	1.0 (Mg^{2+})
Polymerase γ from normal human blood lymphocytes	1.1 (Mn^{2+})	0.95 (Mg^{2+})
Bacterial DNA Polymerase		
E. coli polymerase I	1.2 (Mg^{2+})	0.93 (Mg^{2+})
M. luteus polymerase	0.8 (Mg^{2+})	0.97 (Mg^{2+})

[a] See Materials and Methods for a description of different enzyme preparations. The amount of enzyme used in each experiment was based on the activity rather than protein concentration since each preparation had a different degree of purity, especially in the case of enzymes isolated from human cells. [b] The DNA polymerase assay was as described under Materials and Methods. The divalent cation used for each assay was indicated in each result in parentheses. The concentrations of magnesium acetate and manganese acetate were 10 and 0.5 mM, respectively.

Nonionic detergents may prevent nonspecific loss of protein by adherence to various containers. To demonstrate that the observed stimulation was not solely due to this effect, the effect of Triton X-100 at various enzyme concentrations was measured. The results presented in Figure 2 indicate that the stimulation is nearly constant at all concentrations of enzyme. Although in some experiments, more stimulation is observed at very low concentrations of enzyme, this result suggests that nonspecific protective effect may play some role in the stimulation of enzyme activity, but it cannot account for the total stimulation observed especially when higher concentrations of enzymes were used.

The Stimulatory Effect of Triton X-100 Depends on the Template-Primer. The results shown in Table II demonstrate that the stimulatory effect of detergent is dependent on the template-primer. DNA synthesis is stimulated most with $(dT)_{12-18}\cdot(rA)_n$ and SSA, while there is minimal stimulation with $(dT)_{12-18}\cdot(dA)_n$ or 70S viral RNA. If $(dT)_{12-18}$ is first annealed to the 70S RNA, the polymerase activity is stimulated to the same degree as that of $(dT)_{12-18}\cdot(rA)_n$. This is probably due to the formation of

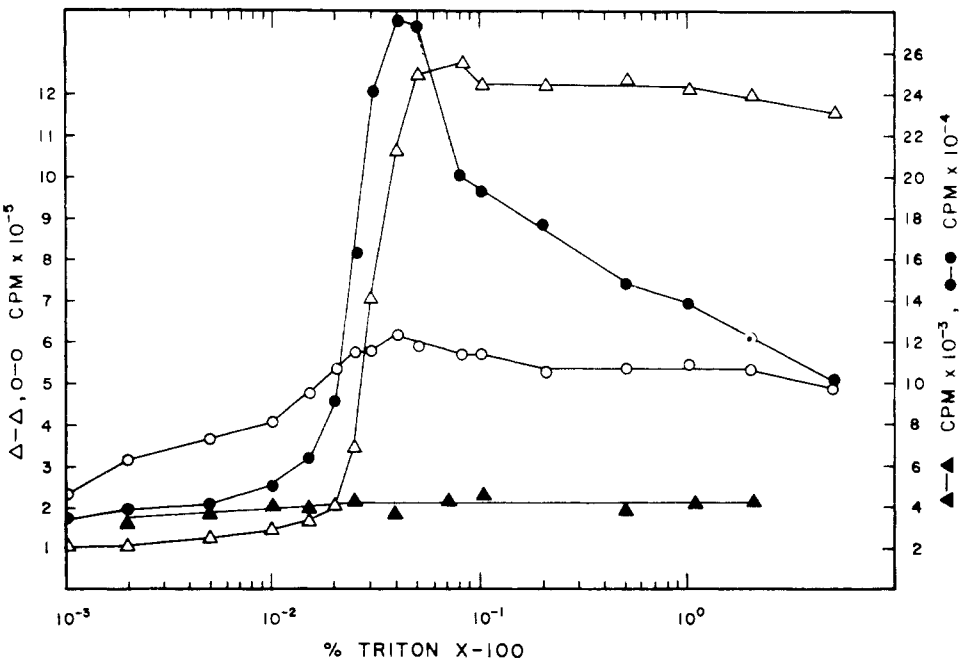

FIGURE 3: Effect of Triton X-100 on RNA-directed DNA synthesis with endogenous 70S RNA and with $(dT)_{12-18} \cdot (rA)_n$. RNA-directed DNA synthesis was carried out according to the procedure described under Materials and Methods using disrupted R-MuLV virus (1 μg in 50-μl reaction mixture) or purified DNA polymerase from the same virus (0.1 μg in 50-μl reaction mixture). Various concentrations of Triton X-100 were included in the reaction mixture as indicated. When $(dT)_{12-18} \cdot (rA)_n$ was used as template-primer, 5.6 μM [^{3}H]TTP (specific activity 13,000 cpm/pmol) was given as substrate. When endogenous template-primer was used (without addition of $(dT)_{12-18} \cdot (rA)_n$), 0.1 mM each of dCTP, dGTP, and dATP were also included. (●) Disrupted virus using endogenous template-primer; (△) disrupted virus using $(dT)_{12-18} \cdot (rA)_n$ as template-primer; (○) purified enzyme using $(dT_{12-18} \cdot (rA)_n$ as template-primer; and (▲) purified enzyme using purified R-MuLV 70S RNA.

oligo(dT) · $(A)_m$ duplexes in the viral RNA, since R-MuLV 70S RNA contains $(rA)_n$ sequence (Lai and Duesberg, 1972; Gillespie *et al.*, 1972; Green and Cartas, 1972). DNA synthesis was also substantially stimulated by Triton, when $(dT)_m \cdot (rA)_n$ and $(dG)_{12-18} \cdot (rC)_n$ were used as template-primers. The stimulation of DNA synthesis by Triton X-100 is not limited to reverse transcriptase of R-MuLV. As shown in Table III, the activity of reverse transcriptase from avian, mouse, and primate type-C virus were all stimulated by Triton X-100 when either $(dT)_{12-18} \cdot (rA)_n$ or SSA was used as the template-primer. Furthermore, the reverse transcriptase activity isolated from the cytoplasmic pellet fraction of human leukemic leukocytes was also enhanced by Triton X-100. In contrast, the activities of DNA polymerase purified from prokaryotes (*E. coli* and *M. luteus*) and from human cells (DNA polymerase α, β [Smith and Gallo, 1972], and γ [Fridlender *et al.*, 1972; Lewis *et al.*, 1974a]) were not affected by Triton X-100. This finding suggests that the stimulation of polymerase activity by nonionic detergent may provide another useful distinguishing feature between the virus-related reverse transcriptase and cellular DNA polymerases.

Triton X-100 Concentration and Viral DNA Polymerase Activity. There is a narrow range of Triton X-100 concentration for maximal endogenous DNA polymerase activity from detergent disrupted viruses (Scolnick *et al.*, 1970; Bishop *et al.*, 1971). Higher concentrations of Triton are inhibitory (Figure 3). As shown in Figure 3, when crude endogenous viral polymerase activity was measured (no template-primer added) DNA synthesis reached a peak at about 0.04% Triton X-100 and then the activity gradually decreased with increasing concentration of Triton. The concentration of Triton X-100 for optimal endogenous viral polymerase activity varies from types and batches of RNA tumor virus. It ranges from 0.015 to 0.05%. If $(dT)_{12-18} \cdot (rA)_n$ was added to the reaction mixture DNA polymerase activity reached a plateau at 0.05% Triton X-100 with disrupted virions as the source of polymerase and at 0.04% Triton X-100 with the purified enzyme. In both cases, DNA polymerase activity remained relatively constant up to about 2% Triton and then gradually declined. In some experiments, the decline in polymerase activity in the presence of $(dT)_{12-18} \cdot (rA)_n$ occurred at a lower concentration of Triton X-100 (0.1–0.5%), but this decrease was usually very small (about 10–20% of maximum activity). We have also observed that R-MuLV DNA polymerase is able to synthesize DNA with purified 70S RNA at a Triton concentration as high as 2%. Since an association of reverse transcriptase with template-primer at such a high concentration of Triton X-100 is still possible, inhibition of endogenous DNA polymerase activities by higher concentrations of Triton was most likely due to release of nucleases following detergent treatment. In fact, a degradation of 70S RNA in higher detergent concentrations was observed (Bishop *et al.*, 1971).

Effect of Template-Primer Concentration on Stimulation of DNA Synthesis by Triton X-100. The effect of the template-primer concentration on the stimulatory activity of detergent is shown in Figure 4. In the absence of Triton X-100, DNA synthesis reached a maximum at a $(dT)_{12-18} \cdot (rA)_n$ concentration of 20 μg/ml. Higher concentrations of template-primer were slightly inhibitory, but in the presence of Triton X-100, the rate of DNA synthesis continuously increased up to a concentration of $(dT)_{12-18} \cdot (rA)_n$ of 200 μg/ml. Stimulation with 5 μg/ml of $(dT)_{12-18} \cdot (rA)_n$ was about fourfold while at 200 μg/ml it was about 50-fold. Apparently, Triton somehow makes the template-primer more available to the enzyme. To test if the prevention of the collapse of $(rA)_n$ is a cause of this Triton effect, we studied the effect of Triton on purified viral

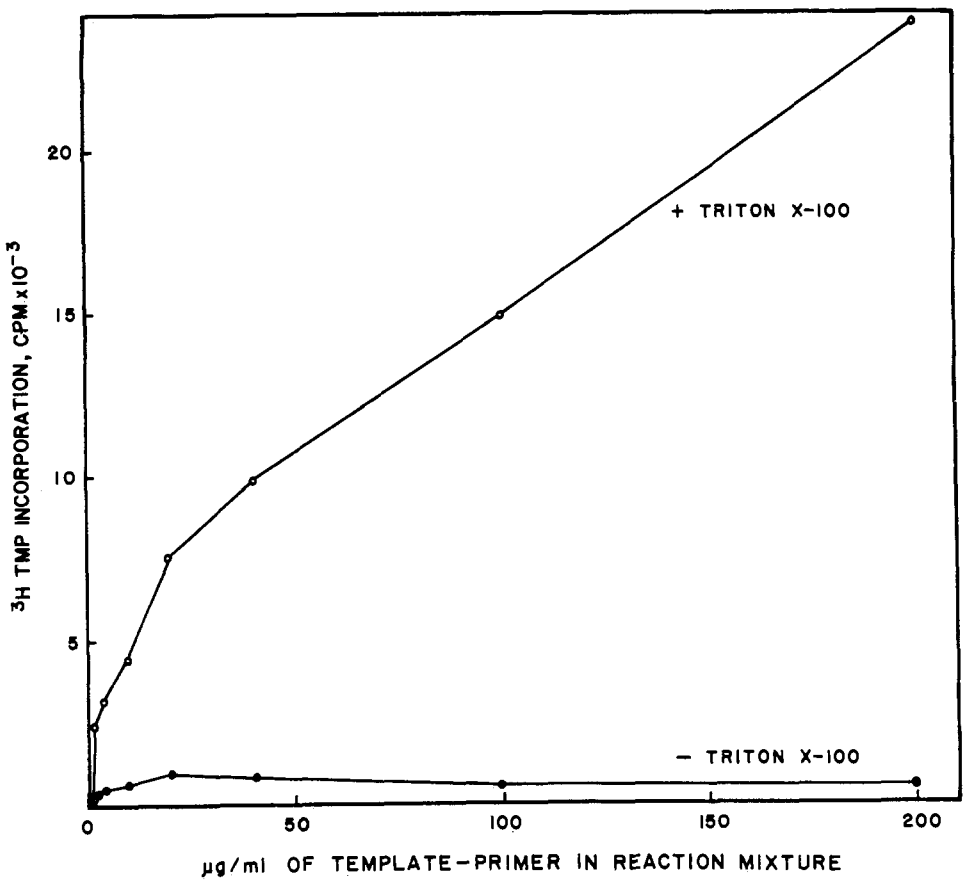

FIGURE 4: Effect of concentration of $(dT)_{12-18} \cdot (rA)_n$ on DNA synthesis in the presence and absence of Triton X-100. The procedures of the $(dT)_n$ synthesis were as described under Materials and Methods. Purified R-MuLV DNA polymerase was used at a concentration of 2 μg/ml. Concentration of Triton X-100 when present was 0.05%. The $(dT)_{12-18} \cdot (rA)_n$ concentrations are as indicated. (○) $(dT)_n$ synthesis in the presence of Triton X-100; (●) $(dT)_n$ synthesis in the absence of Triton X-100.

DNA polymerase activity with various concentrations of KCl since high concentrations of KCl favor the collapse of nucleic acids. The results shown in Figure 5 demonstrate that the relative stimulation of DNA synthesis by Triton X-100 was greatest with higher concentrations of KCl, although absolute activity has decreased. At 0.2 M KCl in the absence of Triton X-100, DNA polymerase activity is inhibited, but some of the activity was recovered by the addition of detergent. These findings are in keeping with the concept that the stimulation by Triton X-100 may at least in part be due to the prevention of (or to the decrease in) the collapse of the template-primer.

Kinetics of DNA Synthesis in the Presence and in the Absence of Triton X-100. The kinetics of $(dT)_n$ synthesis catalyzed by viral DNA polymerase are analyzed in the presence and absence of Triton X-100 (Figure 6). The results show that the nonionic detergent enhances the rate of DNA synthesis both early and late after initiation of $(dT)_n$ synthesis. When detergent is added 30 min after initiation of $(dT)_n$ synthesis, the rate of $(dT)_n$ synthesis increases and the slope of the curve becomes similar to that obtained with Triton added from the start of initiation of $(dT)_n$ synthesis. The determination of the size of these $(dT)_n$ products showed them to be heterogeneous under all conditions tested, and there were no detectable differences between the products from treated and nontreated samples (data not shown). Therefore, the stimulation of DNA synthesis by nonionic detergent is not due to elongation of $(dT)_n$.

Figure 6 also shows the kinetics of $(dT)_n$ synthesis using $(dT)_{12-18} \cdot (dA)_n$. As illustrated in Figure 6 and as previously reported (Robert *et al.*, 1972; Gallo *et al.*, 1973b) $(dT)_{12-18} \cdot (dA)_n$ is an extremely poor template-primer for the DNA polymerases of type-C viruses. The preferences for $(dT)_{12-18} \cdot (rA)_n$ as template-primer over $(dT)_{12-18} \cdot (dA)_n$ increases as the duration of $(dT)_n$ synthesis in-

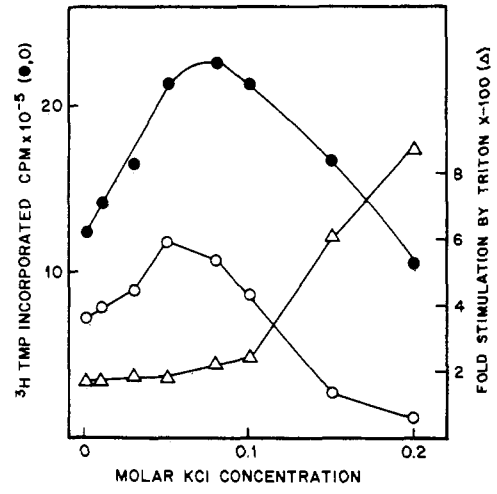

FIGURE 5: Effect of KCl concentration on the stimulatory effect of Triton X-100. $(dT)_n$ synthesis was carried out as described under Materials and Methods except that the KCl concentration was as indicated in the figure. R-MuLV reverse transcriptase at a concentration of 2 μg/ml was assayed in the presence of 0.5 mM $Mn(OAc)_2$. Triton concentration was 0.05%. (●) $(dT)_n$ synthesis in the presence of Triton X-100; (○) $(dT)_n$ synthesis in the absence of Triton X-100; (△) fold stimulation by Triton X-100.

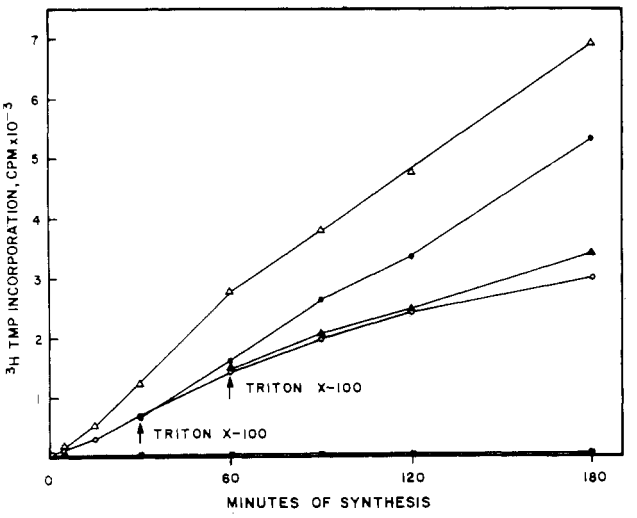

FIGURE 6: Effect of Triton X-100 on the kinetics of $(dT)_n$ synthesis. The procedure of $(dT)_n$ synthesis catalyzed by R-MuLV reverse transcriptase was as described under Materials and Methods. The arrow indicates the addition of Triton X-100 (0.05%) to the reaction mixture. The template-primer concentrations were 20 μg/ml both for $(dT)_{12-18} \cdot (rA)_n$ and $(dT)_{12-18} \cdot (dA)_n$. Symbols: for $(dT)_n$ synthesis directed by $(dT)_{12-18} \cdot (rA)_n$, (○) no Triton added; (△) Triton X-100 added at zero time; (●) Triton added at 30 min after initiation; (▲) Triton added at 60 min after initiation; for $(dT)_n$ synthesis directed by $(dT)_{12-18} \cdot (dA)_n$, (□) Triton added at zero time; (■) no Triton added.

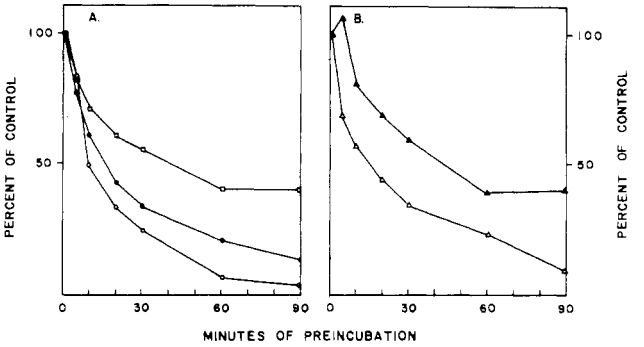

FIGURE 7: Effect of Triton X-100, $(dT)_{12-18} \cdot (rA)_n$, and bovine serum albumin on the thermal inactivation of RNA-directed DNA polymerases. Purified R-MuLV reverse transcriptase (20 μg/incubation mixture) was incubated in the reaction buffer containing 50 mM Tris-HCl (pH 7.9), 1 mM dithiothreitol, 0.2 mM EDTA, 60 mM KCl, and 1 mM $Mn(OAc)_2$ at 37° either alone or in the presence of Triton X-100 (0.05%), bovine serum albumin (50 μg/ml), and/or $(dT)_{12-18} \cdot (rA)_n$ (10 μg/ml). At various times after incubation, samples were drawn to the tubes containing the complementary components required for $(dT)_n$ synthesis (see Materials and Methods). The reaction was then carried out for another 30 min. Symbols: (A) preincubations were carried out in the absence of $(dT)_{12-18} \cdot (rA)_n$ (○) DNA polymerase alone; (●) DNA polymerase and 0.05% Triton X-100; (□) DNA polymerase and bovine serum albumin; (B) preincubations were carried out in the presence of $(dT)_{12-18} \cdot (rA)_n$ (△) DNA polymerases alone; (▲) DNA polymerase and 0.05% Triton X-100.

creases, and this is exaggerated even further when Triton X-100 is included in the reaction mixture.

Effect of Triton X-100 on Thermal Inactivation of Viral DNA Polymerases. Viral DNA polymerases lose activity when incubated at 37° prior to the initiation of DNA synthesis (see Figure 7A). This thermal inactivation is partially overcome by the presence of some nonspecific proteins such as bovine serum albumin and by nonionic detergent (Figure 7A). However, the previously shown stimulation of DNA polymerase activity by Triton X-100 cannot be fully accounted for by this stabilization of the enzyme. The presence of template-primers also adds to the stability of the viral DNA polymerase, and in the presence of Triton X-100, this protective effect was further increased (Figure 7B).

Discussion

For a number of reasons nonionic detergents are included in the preparation and in the assay of viral reverse transcriptases. For example, detergents are useful for solubilizing the polymerase from virions, in stabilizing the enzyme, and as shown here for stimulating the enzyme activity. Our results indicate that in a purified enzyme system, the main effect of the nonionic detergent in stimulating RNA-directed DNA synthesis appears to be due to an increase in the utilization of the template-primer by the enzyme rather than stabilization of the enzyme. One interpretation of this effect is that nonionic detergents decrease the collapse of template-primer. In keeping with this interpretation are the observations that the viral DNA polymerase utilizes a higher concentration of template-primer more efficiently and that the rate of DNA synthesis rapidly increases in the presence of Triton X-100. This mechanism implies that the relative stimulation of DNA synthesis by Triton X-100 may be a function of the degree of aggregation (collapse) of the template-primer. In this respect, $(dT)_{12-18} \cdot (rA)_n$ has a high degree of aggregation (Kochetkou and Budouskii, 1971) and correspondingly a relatively large stimulation by Triton X-100 is found with this template-primer (see Table II). If this interpretation is correct, the minimal stimulation with viral 70S RNA suggests that there may be little aggregation of the non-poly(A) regions of this RNA.

The preference in using $(dT)_{12-18} \cdot (rA)_n$ as template over $(dT)_{12-18} \cdot (dA)_n$ by viral DNA polymerase has been one helpful criterion in distinguishing the viral enzyme from most cellular DNA polymerases (Goodman and Spiegelman, 1971; Robert *et al.*, 1972; Gallo *et al.*, 1972; Baltimore and Smoler, 1971; Gallo *et al.*, 1973a,b). A preference for $(dT)_{12-18} \cdot (dA)_n$ is usually found for cellular DNA polymerases, especially in the presence of magnesium ions (Smith and Gallo, 1972), although one cellular polymerase resembles the viral enzyme in this respect (Lewis *et al.*, 1974a). Our results show that under all conditions tested, purified R-MuLV reverse transcriptase cannot use $(dT)_{12-18} \cdot (dA)_n$ as template-primer. This is in agreement with previously published results (Wells *et al.*, 1972). Therefore, in the crude enzyme preparation, the use of Triton X-100 in the presence of high concentrations of template-primer will increase the contrast of DNA polymerase activity with $(dT)_{12-18} \cdot (rA)_n$ to that with $(dT)_{12-18} \cdot (dA)_n$ which reflects the enhancement of the detectability of viral DNA polymerase.

The observation that the detergent does not stimulate cellular DNA polymerase γ is of interest. One characteristic of DNA polymerase γ is its preference *in vitro* for utilizing the synthetic homopolymeric template-primer $(dT)_{12-18} \cdot (rA)_n$ over any DNA template, including activated salmon sperm DNA (Lewis *et al.*, 1974a,b). This property is similar to that of viral DNA polymerases and can lead to confusing these two different enzyme activities. At present these two enzymes when properly purified can be distinguished by size (Lewis *et al.*, 1974b; Gallo *et al.*, 1973b), by certain behavior in anion and cation exchange column chromatography (Lewis *et al.*, 1974a), by transcription of viral 70S RNA and $(dG)_{12-18} \cdot (rC)_n$ (Lewis *et al.*, 1974b;

Gallo *et al.*, 1973b; Todaro and Gallo, 1973; Gallagher *et al.*, 1974) (both being utilized by the viral polymerase but not by cellular DNA polymerase γ), and by immunological methods (Lewis *et al.*, 1974a; Gallagher *et al.*, 1974). The lack of stimulation of DNA polymerase γ activity by Triton X-100 adds another criterion to distinguish this enzyme from DNA polymerase of viral origin.

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