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INHIBITION OF DNA SYNTHESIS IN THE ADENOVIRUS DNA REPLICATION COMPLEX BY APHIDICOLIN AND 2',3'-DIDEOXYTHYMIDINE TRIPHOSPHATE

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SUMMARY

DNA synthesis in the adenovirus DNA replication complex, containing host DNA polymerases-α and -γ, was inhibited completely by aphidicolin and by 2',3'-dideoxythymidine triphosphate (ddTTP). Double reciprocal plots of DNA polymerase activity in the replication complex against each dNTP gave a straight line although the complex contained two species of DNA polymerase. Inhibition by aphidicolin of DNA polymerase activity was competitive with dTTP but that of purified DNA polymerase-α isolated from adenovirus infected KB cells was competitive with dCTP. The above results suggest that DNA polymerases-α and -γ are integrated in the replication complex to behave as a single enzyme.

INTRODUCTION

When human cells are infected with adenoviruses, there is a gradual decline in cellular DNA synthesis, followed by replication of viral DNA (1). Several adenovirus DNA replication systems that are capable of elongating or initiating viral DNA chain in vitro have been obtained from virus infected cells (2-7). It is suggested that DNA synthesis in these systems depends on cellular DNA polymerases-α and -γ (2-7), and new DNA polymerases specified by the virus are not involved (8).

Experiments using ddTTP, a nucleotide analogue which inhibits DNA polymerases-β and -γ but not DNA polymerase-α, have indicated that DNA polymerase-γ plays a major role in adenovirus DNA chain elongation (3,9). On the other hand, aphidicolin which inhibits only DNA polymerase-α prevents DNA synthesis in the adenovirus DNA replication complex, suggesting that DNA polymerase-α is also involved in virus DNA synthesis (10).

We investigated kinetics of inhibition by aphidicolin on DNA synthesis in the adenovirus DNA replication complex and found that the mode of inhibition

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by aphidicolin on DNA synthesis in the replication complex was different from that of purified DNA polymerase-α from adenovirus infected KB cells.

MATERIALS AND METHODS

Tritiated dTTP (43 Ci/mmole) and [^{3}H]dCTP (17 Ci/mmole) were purchased from New England Nuclear, Boston. [^{3}H]dATP (24 Ci/mmole) and [^{3}H]dGTP (12 Ci/mmole) were from Radiochemical Centre, Amersham. Unlabeled deoxyribonucleoside triphosphates were products of Yamasa, Chiba. ddTTP was from P-L Biochemicals. Aphidicolin was prepared as described (11). Activated DNA was prepared from calf thymus DNA by the method of Fansler & Loeb (12). DNA polymerase-α obtained from the whole extract of adenovirus infected KB cells by purification with columns of DEAE cellulose, phosphocellulose, DEAE cellulose, DEAE Sephadex and single stranded DNA cellulose (M. Arens, unpublished), was kindly supplied from Dr. M. Arens, St. Louis University.

Peparation of adenovirus 2 DNA replication complex

The adenovirus DNA replication complex was prepared as described (2). KB cells were infected with adenovirus 2 at 100 PFU/cell. Nuclei were isolated 18 h after infection and suspended in 50 mM Tris-HCl (pH 7.8) containing 25 mM KCl, 2 mM Na_2HPO_4 and 2 mM dithiothreitol. Sodium heparin was then added at a final concentration of 1 mg/ml and the nuclear suspension was placed in an ice bath for 30 min. After centrifugation at 45,000 g for 30 min at 4°C, the supernatant was dialyzed against 50 mM Tris-HCl (pH 7.8) containing 25 mM KCl, 20 % glycerol, 3 mM dithiothreitol and 1 mM EDTA. The dialysate was fractionated with a Bio-Gel A50m column. The active fraction was used as the replication complex.

Assays of DNA polymerase activity

The standard reaction mixture (90 µl) for the determination of endogenous DNA polymerase activity in the replication complex contained 50 mM Tris-HCl (pH 7.9), 3 mM 2-mercaptoethanol. 20 mM $MgCl_2$, four dNTP's (1 µCi of [^{3}H]dNTP and 90 µM each of the other three dNTP's) and 20 µl (about 20 µg protein) of the replication complex unless otherwise stated. After incubation at 37°C for 30 min, an aliquot (80 µl) was transferred onto a Whatman 3MM paper disc. The acid-insoluble radioactivity on the dried paper disc was measured in 10 ml of toluene-based scintillation liquid.

The reaction mixture (90 µl) for ddTTP inhibition contained as described above except that the concentration of dTTP was kept at 90 µM and [^{3}H]dCTP was used.

The reaction mixture (90 µl) for the assay of purified DNA polymerase-α contained 50 mM Tris-HCl (pH 7.9), 3 mM 2-mercaptoethanol, 20 mM $MgCl_2$, 50 µg of bovine serum albumin, 10 µg of activated DNA, 30 µM each of dATP, dTTP and dGTP, and various concentrations of [^{3}H]dCTP.

Aphidicolin which was dissolved in dimethylsulfoxide to provide a final concentration of 0.1 % (W/V) was added at the indicated concentration.

RESULTS AND DISCUSSION

Adenovirus DNA synthesis in the replication complex was inhibited by ddTTP 73 % at a ratio [ddTTP]/[dTTP] = 1 (Fig. 1). Similar results have been demonstrated by van der Vliet et al. (9) and Abbound et al. (3). However, DNA polymerase activities found in the replication complex are composed of activities of DNA polymerases-α and -γ in the ratio of 3-4 to 1 (13). Therefore, the inhibition cannot be ascribed only to that of DNA polymerase-γ activity in the replication complex.

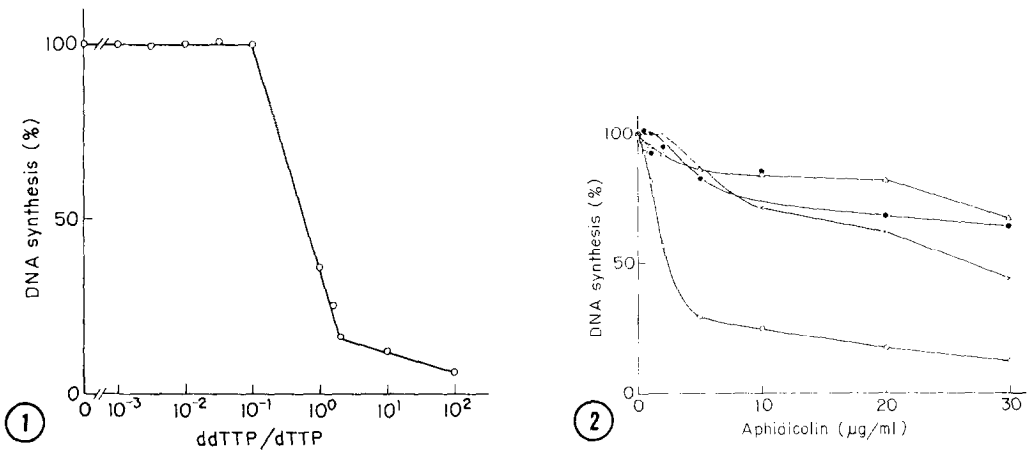
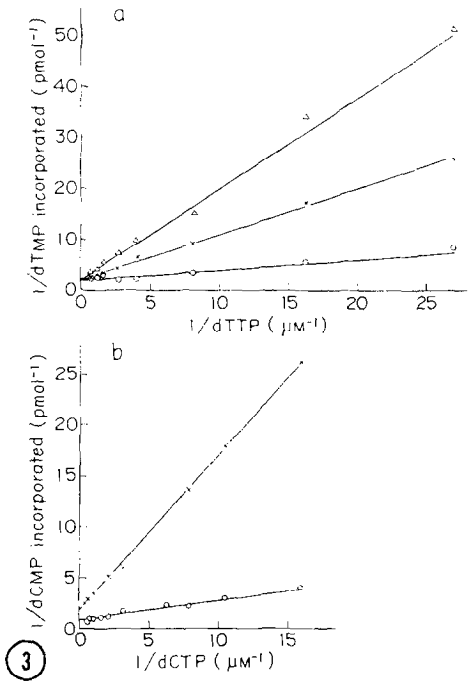


Fig. 1: Effect of ddTTP on DNA synthesis in the adenovirus replication complex. The reaction was carried out as described in MATERIALS AND METHODS (100 % = 14,016 c.p.m.).

Fig. 2: Effect of aphidicolin on DNA synthesis in the adenovirus replication complex. The reaction was carried out as described in MATERIALS AND METHODS. (o), Incorporation of [^{3}H]dTMP (100 % = 19,267 c.p.m.); (×), Incorporation of [^{3}H]dCMP (100 % = 15,265 c.p.m.); (•), Incorporation of [^{3}H]dGMP (100 % = 13,436 c.p.m.); (Δ), Incorporation of [^{3}H]dAMP (100 % = 16,053 c.p.m.).

Effects of aphidicolin on endogenous DNA polymerase activity in the replication complex are shown in Fig. 2. The reaction was carried out with [^{3}H]labeled dNTP without adding the carrier in the presence of excess of the other three dNTP's. The incorporation of dTMP was markedly inhibited by aphidicolin, that is, 50 % inhibition was observed at the concentration of 2.5 μg/ml of aphidicolin. On the contrary, the incorporation of the other three dNMP's was inhibited less than that of dTMP. More than 30 μg/ml of aphidicolin was required for 50 % inhibition. Longiaru et al. (10) have reported that almost all of DNA replication in the nuclear extract of adenovirus infected HeLa cells is inhibited by aphidicolin at a concentration similar to that required for inhibiting isolated DNA polymerase-α. This inhibition pattern corresponds to our result with [^{3}H]dTMP, suggesting that DNA polymerase-α is involved in the adenovirus DNA synthesis. Results of Fig. 2 suggest that aphidicolin inhibits DNA polymerase activity competitively with respect only to dTTP because inhibitory effect of aphidicolin increased at low concentrations of dTTP but not of the other three dNTP's. Fig. 3 shows Lineweaver-Burk plots of the inhibition by aphidicolin on DNA polymerase activity in the replication complex. Approximate Km values for dTTP and dCTP were calculated to be 0.12 and 0.2 μM, respectively. The mode of inhibition indicates that aphidicolin inhibits DNA polymerase activity in the replication complex competitively with respect to dTTP (Fig. 3a), but noncompetitively



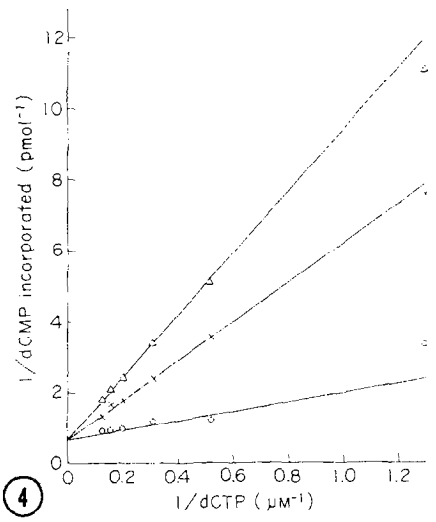


Fig. 3: Double reciprocal plots of the inhibition by aphidicolin of DNA polymerase activity in the adenovirus replication complex. The reaction mixture was as described in MATERIALS AND METHODS except that concentrations of dTTP (a) or dCTP (b) were changed at fixed concentrations of aphidicolin and that concentrations of the other three dNTP's were 20 μM. (a): (o), without aphidicolin; (×), 2 μg/ml of aphidicolin; (Δ), 4 μg/ml of aphidicolin. (b): (o), without aphidicolin; (×), 50 μg/ml of aphidicolin.

Fig. 4: Lineweaver-Burk plots of the inhibition by aphidicolin of DNA polymerase-α. The reaction was performed as described in MATERIALS AND METHODS. (o), without aphidicolin; (×), 0.4 μg/ml of aphidicolin; (Δ), 0.7 μg/ml of aphidicolin.

with dCTP (Fig. 3b), dATP and dGTP (data not shown). These results are surprising because the activity of DNA polymerase-α obtained from nuclei of sea urchin embryos (14), mouse myeloma cells (14) and HeLa cells (15) is inhibited competitively with dCTP and noncompetitively with the other three dNTP's. Therefore, we have examined the nature of aphidicolin inhibition on the activity of purified DNA polymerase-α obtained from adenovirus infected KB cells. As expected, aphidicolin competed with dCTP (Fig. 4), and did not compete with the other three dNTP's (data not shown), the Km value being approximately 2.1 μM.

From the above results, it seems that DNA polymerase activity of the replication complex does not come from a simple mixture of DNA polymerases-α and -γ. The followings support the highly organized form of these cellular DNA polymerases in the adenovirus replication complex. 1) The activity was completely inhibited by either ddTTP (Fig. 1) or aphidicolin (Fig. 2).

2) Inhibition by aphidicolin of DNA polymerase activity in the replication complex was competitive with dTTP (Fig. 3), but that of purified DNA polymerase-α was competitive with dCTP (14,15 and Fig. 4). 3) The apparent Km value for dTTP (0.12 μM, Fig. 3) of the activity in the replication complex was much lower than those of DNA polymerases-α (2 μM) and -γ (0.6 μM) dissociated from the replication complex with 0.5 M NaCl as described by Arens et al. (13). 4) Double reciprocal plots of the activity in the replication complex gave straight lines (Fig. 3), whereas those of DNA polymerase activities dissociated from the replication complex have been reported to give a biphasic curve (13).

As shown in this paper, the mode of aphidicolin inhibition on DNA synthesis in the replication complex was not coincident with that of purified DNA polymerase-α. It is general phenomena because such a different mode of inhibition from that of purified DNA polymerase-α was also observed in isolated nuclei from sea urchin embryos and HeLa cells as described in the following paper (15).

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