Vol. 90, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 12, 1979 Pages 864-870

# STUDIES ON THE PROCESSIVITY OF HIGHLY PURIFIED CALF THYMUS 8S and 7.3S DNA POLYMERASE α

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Received July 30,1979

SUMMARY Template-challenge experiments indicate no gross difference in processivity of the calf thymus DNA polymerase α A and C enzymes. Both enzymes appear to be distributive. Results showing the apparent processive nature of both enzymes on poly (dC). oligo $(dG)_{10}$ when challenged with poly (dA). oligo $(dT)_{10}$ are explicable by the failure of both enzymes to bind to the challenging template rather than by the presence of an initiation factor which preferentially binds to certain templates.

## INTRODUCTION

A great deal of interest has centred lately on the mode of action of highly purified DNA polymerases, especially on whether these enzymes act processively or distributively. E. coli DNA polymerase I(1,2) and calf thymus DNA polymerase α and β (2) have been shown to act distributively, that is, after incorporation of a few or only one deoxynucleoside monophosphate the DNA polymerase is released from the growing chain. It then has to rebind before elongation of that chain can continue. Although KB cell DNA polymerase β has been shown to act distributively under all conditions, when the conditions of synthesis are altered E.coli DNA polymerase I can become more processive and an increased number of deoxynucleoside monophosphates can be added to the growing chain before the enzyme is released (1, 3). Rat liver DNA polymerase α has been claimed to act processively and results showing the apparent distributive nature of the enzyme under certain conditions have been attributed to the presence of an initiation factor which preferentially binds to certain templates during template-challenge experiments (4).

0006-291X/79/190864-07$01.00/0

Previously we have shown that the calf thymus DNA polymerase α 7.3S, C enzyme, consisting of a 155,000 molecular weight polypeptide chain, and the 8S, A enzymes, consisting of this same polypeptide chain plus material of 50-70,000 molecular weight, show differences in the utilisation of synthetic template-initiator complexes (5). These differences may reflect differences in binding to and stabilisation of the complexes, differences in processivity and/or differences in the rate at which the internucleotide link can be made. The fact that A enzyme is more active than C enzyme on these complexes has led us to speculate that the 50-70,000 molecular weight material, which has no polymerase activity, may be able to alter the DNA polymerase subunit in one or more of the above processes. Differences in results obtained by other workers on the processive nature of these enzymes may be due to one group using enzyme equivalent to A enzyme (4) the other using C enzyme (2). Our attempts, using template-challenge experiments, to show differences in processivity between $A_2$ and C enzymes are reported here. $A_1$ enzyme gave similar results as $A_2$.

## MATERIALS AND METHODS

All materials were as described (6) except as mentioned below. Poly (dA), poly (dT), $(dT)_{10}$, $(dG)_{10}$ and $(A)_{10}$ were obtained from P.L. Biochemicals. Poly (dC) was obtained from a $(dC)_5$ initiator using calf thymus terminal transferase as described (7) and was a gift from Dr. I.R. Johnston.

DNA polymerase α C enzyme was prepared by the action of urea on $A_2$ enzyme and $A_2$ enzyme was reconstituted from C enzyme as described (5). DNA polymerase activity using activated DNA was assayed as previously (6), except that the buffer used was 50 mM tris HCl pH 7.8. 1 unit of DNA polymerase activity incorporates 1 nmol of [$^3$H] dTMP into an acid insoluble form in one hour at 37°C. Both enzymes had a specific activity in excess of 50,000 units/mg on this template.

Assays using synthetic template-initiator complexes were carried out at 30°C in 0.13 ml and multiples of this volume. 0.13 ml contained 1 mM

dithiothreiol, 62.5 μg bovine serum albumin, 10 mM $MgGl_2$, 1 μg of the indicated template-initiator complexes, enzyme protein and the relevant [$^3$H] and [$^{14}$C] deoxynucleoside triphosphate at 0.1 mM and 12-15 cpm/pmol for [$^3$H] and 3-5 cpm/pmol for [$^{14}$C]. These assays were carried out at either pH 6.4 in 20 mM sodium-potassium phosphate, or at pH 7.8 in 50 mM tris HCl. The template-initiator complexes were prepared and assays processed as described (6). Template-challenge experiments were carried out in multiples of 0.13 ml and samples were withdrawn at intervals as described in the relevant figure legends. The complexes used were:- poly (dA). oligo $(dT)_{10}$ (A:T = 20:1), poly (dT). oligo $(A)_{10}$ (T:A = 1:1) and poly (dC). oligo $(dG)_{10}$ (C:G = 5:1). The base ratios used are at or close to the optimum for both A and C enzymes.

## RESULTS AND DISCUSSION

Preliminary template competition experiments were carried out to ascertain the effects of several of the template-initiator complexes on the utilisation of others by highly purified A and C enzymes. An example is shown in Table 1. Although the incorporation of dGMP is negligible both poly (dC) and poly (dC). oligo $(dG)_{10}$ inhibited the incorporation of dTMP, using poly (dA). oligo $(dT)_{10}$ as template-initiator, by both A and C enzymes. Further experiments at pH 7.8, at which pH poly (dC). oligo $(dG)_{10}$ does support the incorporation of dGMP, showed that poly (dA). oligo $(dT)_{10}$ was not used to support the incorporation of dTMP and had no effect on dGMP incorporation. Also at pH 7.8, when activated calf thymus DNA was being utilised, addition of poly (dC). oligo $(dG)_{10}$, poly (dC). poly (dT). oligo $(A)_{10}$ or poly (dT) reduced the activity of both A and C enzymes on this template. Neither poly (dA) nor poly (dA). oligo $(dT)_{10}$ had any effect on the utilisation of activated calf thymus DNA.

When time courses of A and C enzymes on poly (dA). oligo $(dT)_{10}$ were carried out at pH 6.4, addition of poly (dC). oligo $(dG)_{10}$ to the incubation immediately inhibited the incorporation of dTMP by both enzymes (Fig. 1). Similar results were obtained in the absence of dGTP or when poly (dC) was added. The reciprocal experiment at pH 6.4,allowing synthesis to

TABLE 1 PRELIMINARY TEMPLATE COMPETITION EXPERIMENT

| Templates present | pmol [$^3$H] dTMP incorporated | | pmol [$^{14}$C] dGMP incorporated | |
|---|---|---|---|---|
| | by A | by C enzyme | by A | by C enzyme |
| poly(dA).oligo(dT)$_{10}$ | 180 | 134.5 | 0 | 0 |
| poly(dC).oligo(dG)$_{10}$ | 0 | 0 | 2 | 2.5 |
| poly (dC) | 0 | 0 | 0 | 0 |
| poly(dA).oligo(dT)$_{10}$ and poly(dC).oligo(dG)$_{10}$ | 25.5 | 37 | 4 | 2 |
| poly(dA).oligo(dT)$_{10}$ and poly (dC) | 29 | 36.5 | 0 | 0 |

Assays were for 10 min. at pH 6.4 as described in MATERIALS AND METHODS. On activated DNA A incorporated 132 pmol [$^3$H] dTMP and 93 pmol [$^{14}$C] dGMP and C incorporated 332 pmol [$^3$H] dTMP and 222 pmol [$^{14}$C] dGMP in the same time.

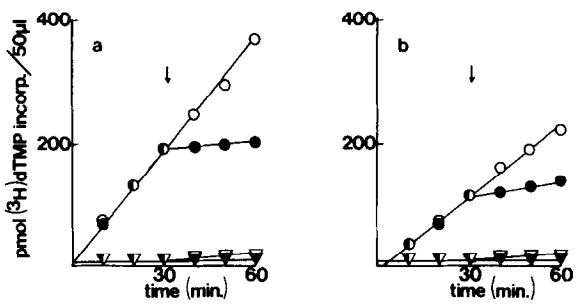


Figure 1 Template challenge experiment showing the effect of addition of poly (dC). oligo (dG)$_{10}$ on ongoing poly (dA) replication at pH 6.4. Each incubation contained 0.39 ml and 50 µl samples were withdrawn at 10 min. intervals and processed as described (6). The mix contained [$^3$H] dTTP and [$^{14}$C] dGTP. The arrow shows the time of addition of the challenging template. (a) 2 units of A enzyme (b) 5 units of C enzyme per incubation

O-O 3 µg poly (dA). oligo (dT)$_{10}$ added at 0 min., sterile glass distilled water added after 30 min.

●-● 3 µg poly (dA). oligo (dT)$_{10}$ added at 0 min., 2 µg poly (dC). oligo (dG)$_{10}$ added after 30 min.

▼-▼ 3 µg poly (dC). oligo (dG)$_{10}$ added at 0 min., sterile glass distilled water added after 30 min.

▽-▽ 3 µg poly (dC). oligo (dG)$_{10}$ added at 0 min., 2 µg poly (dA). oligo (dT)$_{10}$ added after 30 min.

Incorporation of [$^{14}$C] dGMP was negligible and is not shown.

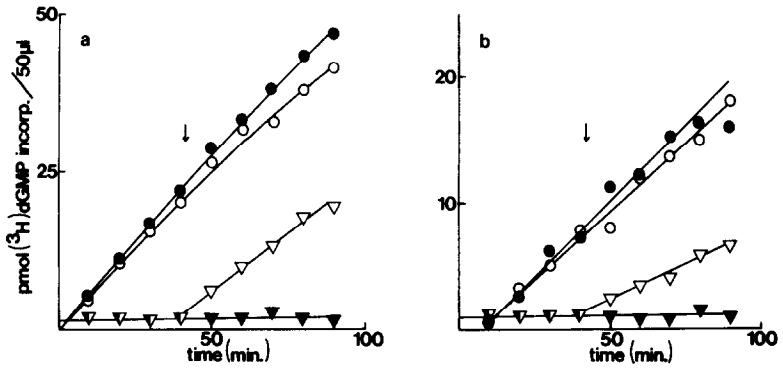


Figure 2 Template challenge experiment showing the effect of addition of poly (dA). oligo $(dT)_{10}$ on ongoing poly (dC) replication at pH 7.8. Each incubation contained 0.52 ml and 50 µl samples were withdrawn at 10 min. intervals and processed as described (6). The mix contained [$^3$H] dGTP and [$^{14}$C] dTTP. The arrow shows the time of addition of the challenging template (a) 1.45 units of A enzyme (b) 2.6 units of C enzyme per incubation.

O-O 4 µg poly (dC). oligo $(dG)_{10}$ added at 0 min., sterile glass distilled water added after 40 min.

●-● 4 µg poly (dC). oligo $(dG)_{10}$ added at 0 min., 2 µg poly (dA). oligo $(dT)_{10}$ added after 40 min.

▼-▼ 4 µg poly (dA). oligo $(dT)_{10}$ added at 0 min., sterile glass distilled water added after 40 min.

▽-▽ 4 µg poly (dA). oligo $(dT)_{10}$ added at 0 min., 2 µg poly (dC). oligo $(dG)_{10}$ added after 40 min.

Incorporation of [$^{14}$C] dTMP was negligible and is not shown.

begin on poly (dC). oligo $(dG)_{10}$ then challenging with poly (dA). oligo $(dT)_{10}$, showed that, although incorporation of dGMP was negligible, the presence of poly (dC). oligo $(dG)_{10}$ inhibited the incorporation of dTMP by both enzymes (Fig. 1). When the reciprocal experiment was carried out at pH 7.8, the addition of poly (dA). oligo $(dT)_{10}$ had no effect on incorporation of dGMP by either enzyme and no dTMP was incorporated (Fig. 2). The data in figures 1 and 2 and the preliminary template competition experiments would suggest that synthesis by A and C is distributive at pH 6.4 on poly (dA). oligo $(dT)_{10}$ and is processive at pH 7.8 on poly (dC). oligo $(dG)_{10}$. The preliminary template competition experiments would also tend to suggest that synthesis on activated DNA is distributive. These results are substantially in agreement with those of other workers (4). However, when synthesis on

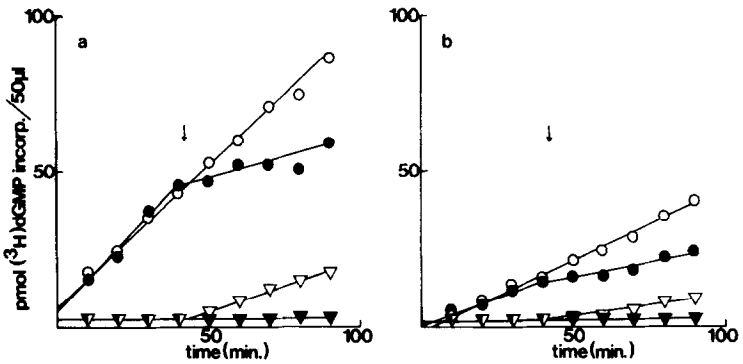


Figure 3 Template challenge experiment showing the effect of addition of poly (dT). oligo $(A)_{10}$ on ongoing poly (dC) replication at pH 7.8. Each incubation contained 0.52 ml and 50 µl samples were withdrawn at 10 min. intervals and processed as described (6). The mix contained [$^3$H] dGTP and unlabelled dATP. The arrow shows the time of addition of the challenging template (a) 1.5 units of A enzyme (b) 1.65 units of C enzyme per incubation.

O-O 4 µg poly (dC). oligo $(dG)_{10}$ added at 0 min., sterile glass distilled water added after 40 min.

●-● 4 µg poly (dC). oligo $(dG)_{10}$ added at 0 min., 2 µg poly (dT). oligo $(A)_{10}$ added after 40 min.

▼-▼ 4 µg poly (dT). oligo $(A)_{10}$ added at 0 min., sterile glass distilled water added after 40 min.

▽-▽ 4 µg poly (dT). oligo $(A)_{10}$ added at 0 min., 2 µg poly (dC). oligo $(dG)_{10}$ added after 40 min.

poly (dC). oligo $(dG)_{10}$ was challenged by the addition of poly (dT). oligo $(A)_{10}$ incorporation of dGMP was immediately inhibited (Fig. 3). This indicates that synthesis is not processive on poly (dC). oligo $(dG)_{10}$. Further template challenge experiments with [$^{14}$C]dATP present indicate that both A and C enzymes begin to incorporate [$^{14}$C]dAMP on the addition of poly (dT). oligo $(A)_{10}$ (data not shown). Also preliminary template binding experiments indicate that at pH 7.8 neither A nor C can bind to poly (dA). When synthesis was allowed to procede on poly (dT). oligo $(A)_{10}$ and was challenged by the addition of poly (dC). oligo $(dG)_{10}$ the results again showed both A and C to be acting in a distributive manner. Thus the difference in activity of calf thymus DNA polymerase α A and C enzymes on various template-initiator complexes are not due to any gross differences in

processivity, or lack of it, between these two enzymes. Also, we have no evidence that there is any initiation factor which binds preferentially to certain templates. The 50-70,000 material may contain certain factor(s) involved in initiation, but as A enzyme is more active than C on all the above templates (5), there may be no template preference for such a factor.

A dangerous assumption is usually made in this type of experiment that when a challenging template has no effect on incorporation on a challenged template that this is due to the DNA polymerase being processive on the challenged template, whereas the enzyme may be distributive on the challenged template, but fail to bind to the challenging template. Further experiments to demonstrate unequivocably the processive or distributive nature of a DNA polymerase should be carried out using a [$^{3}$H] or [$^{14}$C] initiator molecule and a [$^{14}$C] of [$^{3}$H]deoxynucleoside triphosphate as described by Chang (2).

ACKNOWLEDGEMENT

This work was supported by a grant from the Medical Research Council.

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