A kinetic study of rat recombinant DNA polymerase β : detection of a slow (hysteretic) transition in polymerase activity and inhibition by butylphenyl-deoxyguanosine triphosphate

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ABSTRACT

We have identified and characterized a distinct non-linearity in the time course of the reaction of mammalian DNA polymerase β with synthetic polynucleotides. Nucleotide incorporation is biphasic; an initial burst of activity decays exponentially to a lower steady-state velocity. This slow transition in polymerase activity is not due to substrate depletion, abortive complex formation, or enzyme inactivation. The data are consistent with description of the β -polymerase as a hysteretic enzyme, a finding which provides a potential explanation for the non-hyperbolic kinetics which have been reported previously for this polymerase.

We have also found, in contrast to some previous data, that the nucleotide analogue, N²-(*p*-n-butylphenyl)-2'-deoxyguanosine-5'-triphosphate (BuPdGTP), is an inhibitor of the β -polymerase. When poly(dC)·oligo(dG) is used as template·primer, inhibition of the initial velocity is competitive with dGTP with a K_i of 1.25 μ M. On activated DNA, however, β -polymerase displays sensitivity to BuPdGTP which overlaps with that previously reported for DNA polymerase δ ; 100 μ M BuPdGTP is required to inhibit the initial velocity of a dGTP-deficient, truncated assay. Finally, we demonstrate that, in addition to its inhibition of initial velocity, BuPdGTP also modulates both the rate constant of the slow transition in polymerase activity, and the steady-state velocity of the β -polymerase.

INTRODUCTION

The recent surge of interest in mammalian DNA polymerase δ (for a review see 1) has led to the frequent use of BuPdGTP (2–7) to distinguish polymerase δ activity from that of polymerase α , the other aphidicolin-sensitive mammalian DNA polymerase. A K_i for polymerase α on activated DNA of less than 10 nM has been reported (8), whereas BuPdGTP concentrations of 100 μ M and greater are typically required to inhibit polymerase δ activity (2–7). It is generally believed that only the aphidicolin-sensitive DNA polymerases, α and δ , are inhibited by BuPdGTP. We have recently identified in permeable human cells, however, a form of DNA repair synthesis which is resistant to aphidicolin, yet is significantly inhibited by 100 μ M BuPdGTP (DiGiuseppe, J.A. and Dresler, S.L., manuscript in preparation). Published data regarding the BuPdGTP sensitivity of DNA polymerase β , the major aphidicolin-resistant nuclear DNA polymerase are equivocal; the enzyme has been reported both to be resistant (8,9), and to be slightly sensitive (20% inhibition at 1 μ M), to the drug (10). To resolve this conflict, we undertook an investigation of the susceptibility of rat recombinant DNA polymerase β to inhibition by BuPdGTP. During the course of these studies, we detected and investigated the basis for non-linearity in the time course of the homopolymer-directed β -polymerase reaction.

MATERIALS AND METHODS

Reagents

Rat DNA polymerase β was the generous gift of Dr. Samuel H. Wilson and was purified from an overproducing strain of *E. coli* by a modification of a procedure (11), which will be published shortly. BuPdGTP was prepared as described (12). Poly(dC)₃₂₉ and oligo(dG)₁₂₋₁₈ were from Pharmacia. Poly(dA)₁₀₀₀ was from Midland Certified Reagent Co., Midland, TX. Oligo(dT)₁₂₋₁₈ was from Sigma. Oligomers were annealed to polymer in 10 mM Tris (pH 7.6) at a ratio of 1:10 (expressed as nucleotide) for poly(dC)·oligo(dG) and 1:2 for poly(dA)·oligo(dT), by heating to 50°C for 10 min followed by cooling to room temperature for 60 min (13). Therefore, in each case, polymer was in nucleotide excess over the oligomer. Calf-thymus DNA was activated with DNase I as described (14).

Assays for DNA Polymerase β Activity

Assays using synthetic polynucleotides contained, in a final volume of 50 μ l: 40 mM Tris·HCl (pH 7.6 at 37°C), 1 mM DTT, 50 mM NaCl, 400 μ g/ml nuclease-free BSA, 1% glycerol, 10 μ M EDTA and 0.5 mM MnCl₂ (free) along with 30 μ M of the indicated template·primer and [α -³²P]-labeled dNTP as indicated in the Figure Legends. The standard reaction contained 1.75 ng of β -polymerase. In cases where more enzyme was used, this fact is indicated in the Figure Legend. Unless noted otherwise, enzyme, which was added to the reaction mixture in an ice bath, was the last addition. Reactions were initiated by placement at 37°C, incubated for the indicated time, and terminated and processed as described (15). Incorporation of ³²P-dNMP into acid-insoluble material was quantitated by liquid scintillation counting. In the experiment using activated DNA (Figure 6), the truncated, dGTP-deficient assay (16) was employed under conditions determined to be linear throughout the assay. All reactions were performed in duplicate.

Data Analysis

Data were fitted to the appropriate function as described in the *Results* section using the 'Fit Function' procedure of the RS/1 software package (Bolt, Beranek, and Newman). Iterative fitting yielded individual functional parameters which were generally significant at $p < 0.01$ and always significant at $p < 0.05$. Straight lines were fitted by linear regression using the 'Fit Line' procedure of the same software package.

RESULTS

Evidence for a Slow Transition in the Poly(dC)·Oligo(dG)-Directed β -Polymerase Reaction

In control time course studies, we observed a characteristic non-linearity in the poly(dC)·oligo(dG)-directed β -polymerase reaction. As shown for one such time course in Figure 1, reaction velocity decays exponentially from an initial value to a lower, steady-state value. Product appearance predicted by such a model can be expressed as follows:

$$\text{(Eq. 1) } P = (v_{ss}t) - (v_{ss} - v_i)(1 - e^{-(k_{obs}t)})/k_{obs}$$

where: P = product at a given time

v_{ss} = steady-state velocity

t = time at which product is measured

v_i = initial velocity

and k_{obs} = apparent rate constant of the transition from v_i to v_{ss} .

Equation 1 has been used previously to describe the behavior of hysteretic enzymes, which are those capable of undergoing a transition in activity over the course of many catalytic cycles (see 17, 18 for reviews). Because enzyme hysteresis may produce kinetic cooperativity of the steady-state velocity of a monomeric enzyme (19), and there are several

Figure 1. Time course of β -polymerase reaction on poly(dC)·oligo(dG). Representative time course of incorporation with ^{32}P -dGTP at $4\ \mu\text{M}$. Values for v_i ($0.28\ \text{pmol/min}$), v_{ss} ($0.04\ \text{pmol/min}$), and k_{obs} ($0.14\ \text{min}^{-1}$) were determined by fitting Eq. 1 to the data. Values of v_i , v_{ss} , and k_{obs} from this experiment and from 5 other time course studies were used to construct Figures 2 and 3.

examples of unexplained cooperativity in studies of polymerase β in the literature (20–22), we examined the time-dependent change in β -polymerase activity in more detail.

To do so, we studied the time courses of six reactions, each performed at a different dGTP concentration, with poly(dC)·oligo(dG) as template·primer. Curves described by

Figure 2. Dependence of the initial velocity, v_i , and τ , the reciprocal of k_{obs} , on dGTP concentration for the poly(dC)·oligo(dG)-directed polymerase β reaction. Panel A: Initial velocities were determined by fitting Eq. 1 to data from time course studies (including that shown in Figure 1) of polymerase β reactions conducted at six different dGTP concentrations (●), or by assaying reactions under identical conditions at 1-min time points (■). Values of apparent K_m for dGTP determined by the two methods were $29.6\ \mu\text{M}$ and $27\ \mu\text{M}$, respectively. V_{max} values were $2.31\ \text{pmol/min}$ and $1.98\ \text{pmol/min}$, respectively. Panel B: Values of τ ($1/k_{\text{obs}}$) were determined by fitting Eq. 1 to data from six time course studies as described for Panel A. τ_{max} was $27.3\ \text{min}$; τ extrapolated to 0 dGTP concentration was $1.03\ \text{min}$.

Figure 3. Variation in v_{ss} with dGTP concentration. v_{ss} was determined by fitting Eq. 1 to data from six time courses as described for Figure 2A. Panel A: The curve shown resulted from fitting the Hill equation directly to the time course data; $n_{app} = 1.53$, $v_{ss(max)} = 1.03$ pmol/min. Panel B: Hill plot of data from Panel A; $n_{app} = 1.49$, $v = v_{ss}$, $V_{max} = V_{(ss)max}$.

Equation 1 were fitted to each set of data, and, in this way, values for v_i , v_{ss} and k_{obs} were obtained for each dGTP concentration. As shown in Figure 2A, initial velocity values determined from the computer-fitted curves varied hyperbolically with dGTP concentration. (Determination of initial velocity by assaying reactions at 1-min time points, before the slow transition in reaction velocity is detectable, yielded very similar values, attesting to the validity of the computer-derived parameters; Figure 2A.) The parameter $[\tau]$, which is the reciprocal of k_{obs} , was also a hyperbolic function of dGTP concentration (Figure 2B); similar data have been reported for the dog kidney Na^+/K^+ -ATPase, another hysteretic enzyme (23). In contrast, steady-state velocity, v_{ss} , showed a sigmoidal dependence on dGTP concentration (Figure 3A). A Hill plot (24) of the data was linear with an n_{app} of 1.5, indicating an element of cooperativity in the relation between dGTP concentration and v_{ss} (Figure 3B). Our data are, therefore, consistent with the prediction first made over a decade ago (19), that enzyme hysteresis may produce kinetic cooperativity of steady-state, but not initial, reaction velocity.

Exclusion of Artifacts

As previous authors have noted (25), the attainment of a distinct non-zero v_{ss} value, as seen in all of our time course studies (for example, Figures 1 and 9A), excludes substrate depletion, abortive complex formation due to product accumulation, and enzyme inactivation as potential causes of the observed time-dependent decrease in reaction velocity. For the work described here, the same conclusion is reached if one directly addresses each of these potential artifacts. The assay conditions used for our time course studies were designed

Figure 4. Time course after preincubation. Time course of the β -polymerase reaction was monitored after addition of $13 \mu\text{M}$ ^{32}P -dGTP to a reaction which had already been incubated for 60 min at 37°C in the presence of $2 \mu\text{M}$ unlabeled dGTP, $v_i = 0.87 \text{ pmol/min}$, $v_{ss} = 0.27 \text{ pmol/min}$, $k_{obs} = 0.13 \text{ min}^{-1}$.

to minimize substrate depletion; our calculations indicate that no more than 2% of the dGTP in the reaction mix was used in any of the assays, and that in no case was there a reduction in the concentration of useable 3'-OH primer-termini during the course of the reaction. These changes in substrate concentration are well within the 10% deemed acceptable in such studies (18). Data in Figure 3 effectively exclude abortive complex formation due to product accumulation. If abortive complex formation were occurring, inhibition of v_{ss} would be expected to increase with dGTP concentration, as product concentration would increase (18). The opposite relation between v_{ss} and dGTP concentration is observed, however (Figure 3).

To exclude directly enzyme inactivation as the cause of the decrease in polymerase activity seen in our time course studies, a reaction containing $2 \mu\text{M}$ unlabeled dGTP was initiated, and allowed to proceed for 60 min at 37°C . At this time essentially all of the enzyme would be predicted to be in the 'inactive' form since $1/k_{obs}$ at this dGTP concentration is less than 5 min (Figure 2B). After this 60-min incubation, ^{32}P -dGTP was added to the reaction to a final concentration of $15 \mu\text{M}$, and product appearance was monitored over time (Figure 4). A burst of product formation was observed with a v_i which was 13% greater than that predicted from the initial velocity data for reactions which did not undergo a 60-min preincubation (Figure 2A). This result is inconsistent with enzyme inactivation

Figure 5. Time course of β -polymerase reaction assayed under conditions modified from reference 27. Poly(dC)·oligo(dG) and β -polymerase concentration were as described in Materials and Methods. ^{32}P -dGTP concentration was $2 \mu\text{M}$. Reaction mixture included: 28 mM Tris·HCl (pH=7.6), 18% glycerol, 3 mM DTT, 633 μM MnCl_2 , 500 $\mu\text{g/ml}$ NFBSA, 133 μM EDTA and 20 mM NaCl. $v_i = 0.11 \text{ pmoles/min}$. For comparison, a β -polymerase reaction performed with $2 \mu\text{M}$ dGTP under our standard assay conditions had $v_i = 0.19 \text{ pmoles/min}$ and $\tau = 4 \text{ min}$.

Figure 6. BuPdGTP inhibition of the initial velocity of the β -polymerase reaction on two different primer-templates. dGTP-deficient truncated assays (■) were performed with 50 μ M activated DNA as template-primer, 32 P-TTP at 10 μ M, dCTP and dATP at 100 μ M each, 40 ng of β -polymerase, and BuPdGTP at the indicated concentrations. The uninhibited reaction velocity was 2.95 pmol/min. In addition, standard assays (●) were performed with 30 μ M poly(dC)·oligo(dG) as substrate, 32 PdGTP at 16 μ M, 40 ng of β -polymerase, and BuPdGTP as indicated. The uninhibited velocity was 15.44 pmol/min.

as a cause of the observed time-dependent decay in reaction velocity. We conclude that the time-dependent change in the velocity of the poly(dC)·oligo(dG)-directed β -polymerase reaction seen in Figures 1 and 2B is due to enzyme hysteresis.

Expression of Hysteresis is Affected by Reaction Conditions

Above we suggest that non-linearity in the time course of the β -polymerase could account for the several examples of unexplained non-hyperbolic kinetics that are documented for this enzyme (20–22). However, other groups studying the β -polymerase (e.g. 13,26) have failed to detect secondary effects for substrates in replots of kinetic data. A third group, moreover, has published a time course for the poly(dA)·oligo(dT)-directed β -polymerase reaction which is linear for approximately 1 hr (27). To test whether differences in reaction conditions may affect the time course of the β -polymerase, we have assayed the recombinant β -polymerase with 30 μ M poly(dC)·oligo(dG) and 2 μ M 32 P-dGTP under reaction conditions modified from reference 27 (see Figure Legend to Figure 5). Note that when 2 μ M dGTP is used under our standard assay conditions, a τ of 4 min is obtained. By contrast, under the assay conditions of (27), dGMP incorporation was linear with time for the entire length of the assay, which was 6 τ (Figure 5). We conclude that differences in reaction conditions affect expression of the slow transition in polymerase activity, and must be considered when comparing data from different laboratories.

BuPdGTP is an Inhibitor of the β -Polymerase

Using the dGTP-deficient truncated assay (16) on activated DNA, we have found that BuPdGTP inhibits the initial velocity of the β -polymerase reaction by 50% at 100 μ M (Figure 6). This value lies within the range of BuPdGTP concentrations required for significant inhibition of polymerase δ on this template (2,3). By comparison with initial inhibition of the poly(dC)·oligo(dG)-directed β -polymerase reaction (Figure 6), inhibition on activated DNA was 100-fold weaker. Because divalent cations have been shown to affect β -polymerase activity in, as yet, ill-defined ways (28), we considered the possibility that the observed difference in sensitivity to the inhibitor on the two template-primer systems was due to the difference in divalent cations used (Mg^{2+} in activated DNA and Mn^{2+} in homopolymer reactions). However, BuPdGTP displayed comparably-weak inhibition against β -polymerase on activated DNA when assayed with Mn^{2+} as the divalent cation under otherwise identical conditions (not shown).

Figure 7. Analysis of BuPdGTP inhibition of the initial velocity of the poly(dC)·oligo(dG)-directed β -polymerase reaction. Initial velocities of reactions containing the indicated concentrations of dGTP and BuPdGTP were determined by assaying nucleotide incorporation after 1 min. Panel A: Lineweaver-Burk plot with ^{32}P -dGTP as varied substrate. (○) no BuPdGTP; (■) 2 μM BuPdGTP; (▲) 4 μM BuPdGTP; (●) 8 μM BuPdGTP. V_{max} by linear regression analysis of data for the uninhibited reaction was 2.28 pmol/min. Panel B: Slope (●) and intercept (■) replots of primary data presented in Panel A.

Using poly(dC)·oligo(dG) as template·primer, we have studied the mechanism of inhibition of the initial velocity of polymerase β by BuPdGTP. The data indicate that BuPdGTP is competitive with dGTP (Figure 7A), with a K_i (determined from a Dixon plot, not shown) of 1.3 μM . The linearity of the slope replot (Figure 7B) confirms that our 1-min assay excluded any significant contribution of the slow transition to the observed inhibition. The K_i extrapolated from the slope replot is 1.2 μM , in agreement with that derived from the Dixon plot.

Kinetic analysis of inhibition of the β -polymerase by BuPdGTP on activated DNA revealed a mechanism distinct from that on poly(dC)·oligo(dG), with a K_i of 74 μM , 60-fold higher than that on poly(dC)·oligo(dG) (DiGiuseppe, J.A. and Dresler, S.L., unpublished data). This novel mechanism of inhibition is the subject of ongoing study.

Effect of BuPdGTP on the Time Course of the β -Polymerase

We next pursued the possibility that BuPdGTP, in addition to its inhibition of initial velocity, is also capable of modulating the full time course of the β -polymerase. As shown in Figure 8, with poly(dC)·oligo(dG) as template·primer, τ is reduced in the presence of BuPdGTP, yielding a premature bend in the time course of dGMP incorporation. Also, inhibition of v_{ss} by BuPdGTP was apparent, and about as potent as that of v_i (Figure 8). Finally, using poly(dA)·oligo(dT), we found that the β -polymerase reaction in the absence of inhibitor resembles that on poly(dC)·oligo(dG); a burst is observed (Figure 9A). When relatively high concentrations of BuPdGTP are present, however, a lag in activity is seen (Figure

Figure 8. Time course of the β -polymerase reaction on poly(dC)·oligo(dG) in the presence and absence of BuPdGTP. $^{32}\text{PdGTP}$ concentration was $16\ \mu\text{M}$. For the reactions containing BuPdGTP, the curves shown were obtained by fitting Eq. 1 to the data; in this way, we also obtained values of v_i , v_{ss} , and k_{obs} for each of the reactions. (●) no BuPdGTP, $v = 0.70\ \text{pmol/min}$; (▲) $1.5\ \mu\text{M}$ BuPdGTP, $v_i = 0.47\ \text{pmol/min}$, $v_{ss} = 0.16\ \text{pmol/min}$, $k_{obs} = 0.47\ \text{min}^{-1}$; (■) $3\ \mu\text{M}$ BuPdGTP, $v_i = 0.35\ \text{pmol/min}$, $v_{ss} = 0.092\ \text{pmol/min}$, $k_{obs} = 0.80\ \text{min}^{-1}$. For comparison, $v_{ss} = 0.25\ \text{pmol/min}$ with no inhibitor at $16\ \mu\text{M}$ dGTP (Figure 3).

9B); the velocity of incorporation slowly increases from an apparent value of zero to a steady-state value.

DISCUSSION

During our study of the sensitivity of rat recombinant DNA polymerase β to BuPdGTP, it became apparent that there are substantial kinetic complexities associated with the β -polymerase reaction. When polymerizing on synthetic polynucleotides, the polymerase undergoes a slow transition in activity of the sort which has previously been described as enzyme hysteresis (17, 18). Through appropriate experimental design and specific control experiments, we have excluded the potential artifacts which may produce similar kinetic patterns and have established that a slow transition from initial to steady-state velocity is an intrinsic feature of the β -polymerase reaction.

The concept of enzyme hysteresis was first developed (29) to explain slow ligand-induced changes in enzyme activity of the sort described above. It has been extended (19), however, to explain the occurrence of positive or negative cooperativity in the dependence of steady-state reaction velocity on substrate concentration in enzymes which lack multiple substrate binding sites. Examples of cooperative behavior in β -polymerase reactions have been reported (20,22). In one study (20), which used both poly(rA)·oligo(dT) and poly(dA)·oligo(dT) as template·primers, a sigmoidal dependence of nucleotide incorporation on TTP concentration was observed. Hill coefficients of 1.8 and 1.4 were calculated for the reactions on poly(rA)·oligo(dT) and poly(dA)·oligo(dT), respectively, but a mechanism for the apparent cooperativity was not elucidated (20). At least two other groups, while studying purified polymerase β , have noted a sigmoidal dependence on TTP of homopolymer-directed TMP incorporation (21, 22). Our conclusion that the homopolymer-directed β -polymerase reaction displays enzyme hysteresis provides a potential explanation for these hitherto unexplained examples of cooperativity.

The finding that polymerase β shows hysteretic behavior is not without precedent. Evidence for hysteresis in *E. coli* DNA polymerase I has also been presented. The 3' → 5' exonuclease activity of polymerase I has recently been shown to increase with increasing nucleotide incorporation by the polymerase (30,31). In addition, earlier studies of the time course of nucleotide incorporation by polymerase I into the cohesive ends of λ DNA (32,33)

Figure 9. Panel A: Time course of a representative uninhibited poly(dA)·oligo(dT)-directed β -polymerase reaction. Poly(dA)·oligo(dT) was 30 μ M, 32 P-TTP was 1.5 μ M. The curve shown resulted from fitting Eq. 1 to the data; $v_i = 2.16$ pmol/min, $v_{ss} = 0.78$ pmol/min, $k_{obs} = 0.13$ min $^{-1}$. Panel B: BuPdGTP induces a lag in the poly(dA)·oligo(dT)-directed β -polymerase reaction. Time courses were obtained with poly(dA)·oligo(dT) at 5 μ M, 32 P-TTP at 10 μ M, and BuPdGTP as indicated. For the reactions with BuPdGTP, the curves shown resulted from fitting Eq. 1 to the data, assuming a v_i of 0. (▲) no BuPdGTP, $v = 7.2$ pmol/min; (■) 40 μ M BuPdGTP, $v_{ss} = 2.21$ pmol/min, $k_{obs} = 2.5$ min $^{-1}$; (●) 100 μ M BuPdGTP, $v_{ss} = 0.90$ pmol/min, $k_{obs} = 1.2$ min $^{-1}$.

led to the conclusion that the enzyme exists in active and inactive forms, based on burst data similar to those in Figures 1 and 9A.

An ordered bireactant kinetic scheme has been proposed for the β -polymerase, with binding of DNA followed by binding of dNTP (13). Since that study was performed exclusively under initial-velocity conditions, its conclusions are in no way inconsistent with ours. In fact, rate equations have been derived (17) which demonstrate the potential for kinetic cooperativity in the steady-state velocity of a monomeric enzyme whose mechanism is ordered-bireactant. Thus, the cooperativity with respect to dGTP demonstrated in Figure 3 does not require the presence of multiple binding sites; rather, it is due to the presence of 2/1 functions in the steady-state rate equation describing the reaction (17).

Our finding that BuPdGTP substantially inhibits polymerase β on activated DNA at concentrations similar to those which inhibit polymerase δ has considerable practical significance. It is clearly necessary to determine the aphidicolin-sensitivity of any cellular DNA polymerase activity which is inhibited only by relatively high concentrations of BuPdGTP, so that one can distinguish β from δ activity. As mentioned above, we have encountered such a situation in our permeable human cell system. The apparent discrepancy between our data and the previously-reported resistance of the β -polymerase reaction on activated DNA to 400 μ M BuPdGTP (8, 9) deserves comment. The previous experiments were apparently performed in the presence of 100 μ M dGTP (9), which may have obscured any response of polymerase β to the inhibitor. At the time of those reports, the finding

of major interest was that polymerase α was 100,000-fold more sensitive to BuPdGTP than any of the other mammalian DNA polymerases. Thus the possibility that β -polymerase might show some degree of inhibition at high BuPdGTP concentrations was not explored.

It is surprising that BuPdGTP inhibits the activity of polymerase β on poly(dA)·oligo(dT) only about 20-fold less strongly than it inhibits the enzyme on poly(dC)·oligo(dG) (compare Figure 9B with Figure 8). Polymerase β is a small enzyme and probably has a single dNTP binding site (34). One would thus expect deoxyribonucleotide analogues to be potent inhibitors of β -polymerase activity only on templates complementary to the inhibitory nucleotide. Such is the case for 2',3'-dideoxythymidine-5'-triphosphate, which inhibits polymerase β synthesis on a non-complementary template, poly(dC)·oligo(dG), 1000-fold less strongly than it inhibits the activity of the enzyme on a complementary template, poly(dA)·oligo(dT) (our unpublished data). The relatively strong inhibition by BuPdGTP of β -polymerase activity on a non-complementary template, poly(dA)·oligo(dT), suggests that BuPdGTP may inhibit the polymerase by a different mechanism at high inhibitor concentrations. [Preliminary studies indicate that this hypothesis also applies to inhibition on activated DNA, which likewise, requires relatively high concentrations of BuPdGTP]. We also note that, in the presence of BuPdGTP, β polymerase reactions on poly(dA)·oligo(dT) and poly(dC)·oligo(dG) show dramatically different kinetic characteristics (compare Figure 9B with Figure 8). On poly(dC)·oligo(dG) burst kinetics are seen, in contrast to the lag seen on poly(dA)·oligo(dT). This difference may indicate that polymerase β is sensitive to the structural differences which have been demonstrated crystallographically between these two template·primer systems (e.g. 35,36).

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