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Biochemistry

Catalytic editing properties of DNA polymerases

(nucleotide analogues/termination/active site/mechanism)

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ABSTRACT **Enzymatic incorporation of 2′,3′-dideoxynucleotides into DNA results in chain termination. We report that 3′-esterified 2′-deoxynucleoside 5′-triphosphates (dNTPs) are false chain-terminator substrates since DNA polymerases, including human immunodeficiency virus reverse transcriptase, can incorporate them into DNA and, subsequently, use this new 3′ end to insert the next correctly paired dNTP. Likewise, a DNA substrate with a primer chemically esterified at the 3′ position can be extended efficiently upon incubation with dNTPs and T7 DNA polymerase lacking 3′-to-5′ exonuclease activity. This enzyme is also able to use dTTP-bearing reporter groups in the 3′ position conjugated through amide or thiourea bonds and cleave them to restore a DNA chain terminated by an amino group at the 3′ end. Hence, a number of DNA polymerases exhibit wide catalytic versatility at the 3′ end of the nascent DNA strand. As part of the polymerization mechanism, these capabilities extend the number of enzymatic activities associated with these enzymes and also the study of interactions between DNA polymerases and nucleotide analogues.**

DNA polymerases are multifunctional enzymes involved in the replication and repair of DNA (1). Understanding the polymerase reaction mechanism might lead to major developments in modern molecular biology, medicine, and drug design. DNA polymerases, such as *Escherichia coli* DNA polymerase I, carry on the same polypeptide chain various enzymatic activities separated into different domains (2). Standard protein chemistry aimed at elucidating the enzymatic mechanism has not been as rewarding for the DNA polymerases as for other enzyme families such as the protease (3). Photolabeling of *E. coli* DNA polymerase I has been reported with an azido-DNA (4), dTTP (5), and dNTP analogues (6, 7) and has identified several amino acid residues in the dNTP binding site. Crystallographic studies and site-directed mutagenesis have provided insight into the structure–function relationship of polymerases, such as the Klenow fragment of DNA polymerase I (8), human immunodeficiency virus (HIV) reverse transcriptase (RT) (9), T7 RNA polymerase (10), and rat DNA polymerase β (11, 12). Crystal structure analysis of the 3′-to-5′ exonuclease domain of the Klenow fragment has also suggested a related polymerization mechanism involving two metal ions (13).

Thus, with sequence alignments (14), two crucial Asp residues have been pinpointed in the polymerase active site, but, to our knowledge, no direct evidence of their mode of action has been obtained to date. From the rat DNA polymerase β structure, Davies *et al.* (11) suggested that Asp-190 could activate the 3′-hydroxyl end of DNA to produce a nucleophilic attack on the α-phosphate of the bound nucleotide, while Pelletier *et al.* (12) suggested Asp-256 for this role and proposed a detailed polymerization mechanism. Thus, despite refined structural knowledge of these enzymes, precise assignments of catalytic roles of amino acid side chains remain to be made. With these results in mind, we felt that a modification of the 3′-hydroxyl group that would retain an oxygen atom might drastically alter the catalytic behavior of the enzyme. However, the effect of a substitution at this 3′ oxygen on the polymerization of nucleotides is not well documented, probably because it was anticipated that incorporation of these analogues would not occur or would lead to mere chain termination and would, thus, be of limited value for the understanding of the mechanism. During the course of synthesizing bonified 3′-modified dNTPs (15–17), we found various analogues unable to inhibit DNA synthesis. In the present report, we present evidence that DNA polymerases, including HIV-RT, exhibit catalytic versatility at the nascent 3′ end of DNA by removing 3′-oxygen and 3′-nitrogen substituents. We propose that this capability might be part of the polymerization mechanism.

MATERIAL AND METHODS

Reagents. DNA oligonucleotides were synthesized at the Institut Pasteur (Paris) or Eurogentec (Brussels) and purified by two successive polyacrylamide gel electrophoreses. Modified T7 DNA polymerase (Sequenase 2.0) was from United States Biochemical; *Taq* DNA polymerase was from Eurobio (Paris) or Boehringer Mannheim; HIV-RT was from Boehringer Mannheim. No significant 3′-to-5′ exonuclease activity was detected after prolonged incubation with a 5′-^{32}P-labeled primer/template.

Chemical Synthesis of Nucleotide and Oligonucleotide Analogues. The 2′-deoxy-3′-anthranyloyl-dNTPs (3′-ant-dNTPs) were synthesized as described (15). The 3′-amino-3′-deoxy-dTTP (3′-NH_2-dTTP) and 3′-{N^3-[3-carboxylato-4-(3-oxido-δ-oxo-6*H*-xanthen-9-yl)phenyl]thioureido}-3′-deoxythymidine 5′-triphosphate (3′-fluothioureido-dTTP) were synthesized as described (18). Details of the syntheses of 3′-deoxy-3′-(*N*-methylanthranyloylamino)thymidine 5′-triphosphate (3′-amd-dTTP) from 3′-azido-3′-deoxythymidine (AZT), 3′-*O*-[N^6(*N*-methylanthranyl)amidohexanoyl]-dGTP (3′-chain-dGTP), and 3′-*O*-[N^6(anthranyl)amidohexanoyl]-dATP (3′-chain-dATP) will be described elsewhere. The synthesis and use of 3′-*O*-[N^6(*N*-methylanthranyl)amidohexanoyl]-dCTP (3′-chain-dCTP) are described (17). In all cases, successive HPLC purification procedures as described (17) gave homogenous products (>99.99% pure). Chemical synthesis of 3′-ant-21-mer will be described elsewhere. Briefly, 5′-DMT-ATACTTTAAGGATATGTATCC-3′ (where DMT is dimeth-

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Abbreviations: HIV, human immunodeficiency virus; RT, reverse transcriptase; FITC, fluorescein isothiocyanate; AZT, 3′-azido-3′-deoxythymidine; 3′-ant-, 3′-anthranyloyl-; 3′-fluothioureido-, 3′-{N^3-[3-carboxylato-4-(3-oxido-δ-oxo-6*H*-xanthen-9-yl)phenyl]thioureido}-; 3′-amd-, 3′-(*N*-methylanthranyloylamino)-; 3′-chain-dGTP and -dCTP, 3′-*O*-[N^6-(*N*-methylanthranyl)amidohexanoyl]-dGTP and -dCTP, respectively; 3′-chain-dATP, 3′-*O*-[N^6-(anthranyl)aminohexanoyl]-dATP; dd-, 2′,3′-dideoxy-.

oxytrityl) was treated with *N*-methylisatoic anhydride under slightly alkaline conditions and the mixture was desalted. UV (254 nm)-absorbing fractions were treated with glacial acetic acid, lyophilized, and purified by reverse-phase HPLC. Unreacted 21-mer, 3′-ant-21-mer, and 5′-DMT-21-mer were eluted at 9.9, 11.72, and 14.97 min, respectively. The homogenous peak corresponding to 3′-ant-21-mer was recovered by lyophilization.

Deprotection Assay of the 3′-Ant-21-mer. The 3′-ant-21-mer was annealed to its complementary template bearing $(dG)_5$ or $(dT)_5$ protruding from the 5′ termini under mild conditions (10 min at 37°C and pH 7.3). Subsequent incubation (1 min at 37°C) was at equimolar concentrations of primer/template and enzyme (from 50 to 500 nM) and [α-^{32}P]dCTP or [α-^{33}P]dATP at various concentrations. The reaction was stopped with 100 mM EDTA, aliquots were spotted onto DE81 filter papers and washed three times in 2× SSC, and radioactivity was measured by liquid scintillation counting. Unprotected 21-mer was treated similarly and taken as 100% incorporation control. Aliquots were also run through a 15% denaturing polyacrylamide gel, which was autoradiographed.

Incorporation of Nucleotide Analogues into DNA by Using an End-Labeled Primer-Extension and Gel Assay. The standing start reaction was performed as described (15, 19). A synthetic 5′-^{32}P-labeled DNA primer/template (4 μM) made of 5′-ATACTTTAAGGATATGTATCC-3′ annealed to 5′-TTTTTTTTTCGGATACATATCCTTAAAGTAT-3′ (template C) was incubated with 3′-esterified dNTPs and 5 units of Sequenase or 0.5 unit of HIV-RT in 20 μl. The same primer/template was also used with 5 units of *Taq* DNA polymerase and 3′-esterified dNTPs at 45°C in Mn^{2+}/citrate buffer (20). The nature of the base adjacent to the primer 3′ end was varied depending on the assay (template G, A, T, or C) and is underlined above. Samples were electrophoresed through a denaturing 15% polyacrylamide gel, which was autoradiographed.

3′-Ester Displacement Monitored with an End-Labeled Primer-Extension and Gel Assay. The same primer/template C was incubated with 3′-ant-dGTP (2 mM) and 5 units of Sequenase for 3 min, 5 units of *Taq* DNA polymerase for 15 min in Mn^{2+}/citrate buffer, or 3′-chain-dGTP (1 mM) and 0.5 unit of HIV-RT, as indicated. When needed, the reaction mixture was heat-treated for 15 min at 68°C, and the oligonucleotides were reannealed for 1 h in a waterbath from 68°C to room temperature. Chase experiments used dATP (200 μM), 3′-ant-dATP (2 mM), 3′-chain-dATP (1 mM), or 2′,3′-dideoxyadenosine 5′-triphosphate (ddATP) (200 μM) and were incubated for 15 min at 37°C for Sequenase or HIV-RT or for 15 min at 45°C for *Taq* polymerase.

Incorporation of 3′-amd-dTTP, 3′-NH_2-dTTP, and 3′-fluothioureido-dTTP. The same 5′-P^{32}-labeled primer was annealed to template A and incubated with 1 mM 3′-amd-dTTP, 1 mM 3′-fluothioureido-dTTP, or 100 μM 3′-NH_2-dTTP and 5 units of Sequenase at 37°C or 5 units of *Taq* DNA polymerase at 45°C. When needed, the incorporation mixture was heat-treated for 15 min at 68°C, the oligonucleotides were reannealed as above, and 5 units of fresh *Taq* DNA polymerase was added with 200 μM dATP. When indicated, *Taq* DNA polymerase was added in the presence of 200 μM dATP.

Labeling of the Incorporation Products with Fluorescein Isothiocyanate (FITC). The incorporation products were ethanol-precipitated, dried under vacuum, and treated with FITC in $NaHCO_3$ buffer exactly as described (18).

RESULTS

Incorporation of 3′-Modified Nucleotides into DNA. The sugar ring of nucleotide analogues can reportedly tolerate considerable structural modifications and still be a substrate for DNA polymerases. However, when present with classical dNTPs, nucleotide analogues can be discriminated several hundredfold when carrying modest chemical modifications such as a 3′-deoxy group (20). We have reported (15) that high concentrations of one 3′-modified analogue (3′-ant-dNTP) were required to get good incorporation yields. This was expected to preclude their use in conjunction with classical dNTPs because of the large discrimination ratio against them. Moreover, an unnoticed contamination of the 3′-ester analogue with its corresponding dNTP might lead to the favored incorporation of the latter if the former is not a substrate for the DNA polymerase. Consequently, we positioned a 6-carbon tether between the sugar ring and the fluorescent reporter group (Fig. 1*a*) (17). This allowed us to greatly decrease the analogue concentration to get the same incorporation yield with Sequenase. As reported (17), DNA chains terminated with a 3′-chain-dCMP showed two products on gel autoradiograms. The upper product was more fluorescent than the lower after gel purification, but cross-contamination of the latter by the former was hard to assess (data not shown). Because the lower band comigrated with authentic 3′-dCMP-terminated DNA chain, it prompted us to test the possibility that free 3′-OH groups had been generated during the incorporation reaction. This was done with a standard Sanger sequencing reaction in which dCTP had been replaced with 3′-chain-dCTP. We found that this analogue was unable to stop DNA synthesis at several dCMP insertion sites but produced a specific DNA chain-termination at others. This incorporation pattern was rather unexpected since dCTP and 3′-chain-dCTP are widely separated on HPLC chromatograms that yield analogue with no detectable accompanying dCTP (<0.01%). Moreover, putative contaminating dCTP would likely be used first by the polymerase, producing gradually more stops at dCMP insertion sites because of gradual dCTP pool exhaustion. This was clearly not the case (data not shown). Thus, it raised the possibility that, at least at specific sites in a cDNA sequence, esters at the nascent 3′ end of the DNA were cleaved off and did not prevent chain extension.

The Catalytic Editing of 3′-Esters by DNA Polymerases. When a mixture of all four 3′-ant-dNTPs was used, one product corresponding to multiple additions was observed with several DNA polymerases, such as Sequenase (Fig. 2) or HIV-RT. However, the very same analogue mixture gave rise to only one addition product of the expected size with *Taq* DNA polymerase. This clearly demonstrates that read-through was not due to a minute concentration of 3′-unprotected deoxynucleotides because one would expect a distribution of products larger than the primer rather than a single adduct. Mn^{2+}-containing buffers (20) gave the high incorporation levels and thus were used for subsequent editing experiments

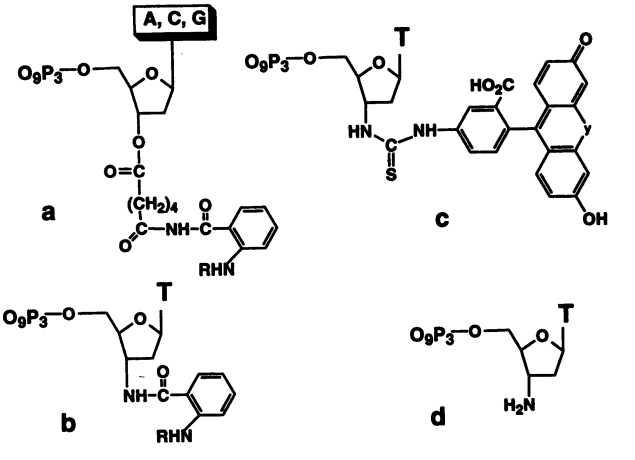

FIG. 1. Structure of 3′-modified dNTP substrates. (*a*) 3′-chain-dATP for R = H or 3′-chain-dGTP and 3′-chain-dCTP for R = CH_3. (*b*) 3′-amd-dTTP (R = CH_3). (*c*) 3′-fluothioureido-dTTP. (*d*) 3′-NH_2-dTTP.

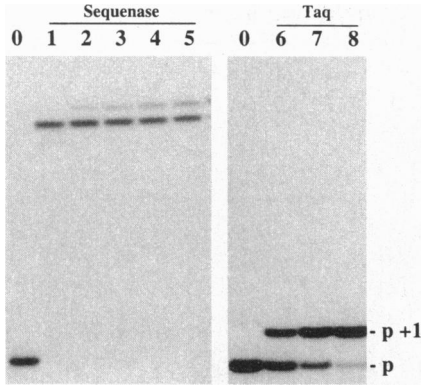

FIG. 2. End-labeled primer extension and gel assay using 3′-ant-dNTPs and DNA polymerases. 3′-ant-dNTPs were as described (15). Lanes: 1–5, 3′-ant-dNTPs (400 μM) incubated with primer/template C and Sequenase for 1, 2, 3, 4, and 5 min, respectively; 6–8, the same 3′-ant-nucleotide mixture (all four nucleotides, each at 2 mM) and *Taq* DNA polymerase for 5, 10, 15 min, respectively; 0, no enzyme. p, Primer (21-mer).

(see below), but same results were obtained with 3′-chain-dNTPs at lower concentrations (2 μM, around the sensitivity limit of this assay, see below). When 3′-esterified nucleotides were preincubated with Sequenase in the absence of DNA, heated to inactivate the Sequenase, and then mixed with a DNA template/primer and *Taq* DNA polymerase and incubated, a single addition product was observed as before, indicating that the 3′ esters were not hydrolyzed prior to incorporation and that the ternary enzyme–DNA–analogue complex was a prerequisite for the editing reaction.

Catalytic Editing Is Dependent on the Next Correctly Paired Nucleotide. Thus, we synthesized chemically a 3′-esterified primer that mimicked an incorporation product as well as a putative substrate for 3′ editing. This 3′-blocked primer (Fig. 3*a*) was hybridized to its cognate template bearing a $(dG)_5$ or $(dT)_5$ protruding from the 5′ terminus and incubated with Sequenase and increasing [α-^{32}P]dCTP or [α-^{33}P]dATP, respectively. Fig. 3*b* shows that this template/primer was able to be extended and that [α-^{32}P]dCTP titrated the 3′-ant-primer/template, as expected for the reaction depicted in Fig. 3*a*. Incorporation of radioactive label onto the 3′-blocked primer relative to an unblocked primer was assessed by using a filter-binding assay and showed that the amount of label in Fig. 3*b*, lane 9, accounted for at least 20% of the primer under these conditions. Comparatively, terminal deoxynucleotidyltransferase did not incorporate any label into 3′-ant-21-mer.

We then evaluated the ability of several 2′-deoxynucleotides and analogues to enhance the 3′-esterase-like activity intrinsic to the DNA polymerase once the DNA primer had been terminated by a 3′-esterified nucleotide. By using Sequenase or HIV-RT, we found that dATP, ddATP, 3′-chain-dATP, or 3′-ant-dATP was able to displace the ester present at the 3′ end of the dGMP incorporated previously (Fig. 4), whereas only dATP was able to do so when *Taq* DNA polymerase was used. These results indicated that correct positioning of the next nucleotide in the nucleotide binding site is important for full expression of this 3′-esterase-like activity and that *Taq* polymerase did not accommodate ddNTPs well enough in its dNTP binding site to promote efficient removal of the ester. We conclude that Sequenase exhibited a strong 3′-esterase-like activity on the 3′ end of DNA that allowed the use of 3′-esterified DNA primer and the DNA polymerization to proceed in the presence of 3′-esterified dNTPs. Furthermore, the displacement of the 3′ ester during the addition of the next correct nucleotide suggests that the 3′ esterase and the polymerase active sites may be the same or at least be near one another and work closely together.

The 3′-Ester-Related Bonds Are Subjected to DNA Polymerase-Mediated Hydrolysis. The 3′ substituents with related 3′ bonds would be instrumental in gaining insight into this last point. Amides and thioureas are related to ester bonds but harder to hydrolyze. We reasoned that if the ester was subjected to a polymerization-mediated hydrolysis, positioning another carbonyl or thiocarbonyl group in a spatially similar position might lead to the same elimination should the analogue be incorporated. Therefore, we synthesized 3′-amd-dTTP (Fig. 1*b*) from AZT and used it with Sequenase and *Taq* DNA polymerases in a similar fashion (Fig. 5). This analogue alone was not a substrate for *Taq* DNA polymerase (Fig. 5, lane 1, and data not shown) unless the next correct nucleotide (dATP) was present in the reaction mixture (Fig. 5, lane 2). With Sequenase, a unique addition product with a slightly smaller R_f was observed (Fig. 5, lanes 3, 5, and 9), indicating that the chemical nature of the addition products was probably

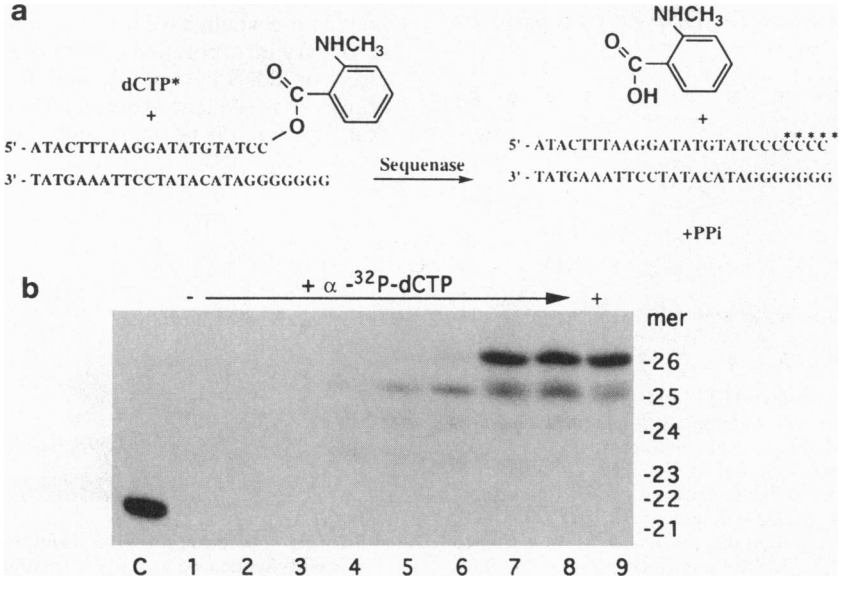

FIG. 3. (*a*) Structure and deprotection reaction of 3′-ant-21-mer with Sequenase and [α-^{32}P]dCTP. (*b*) 3′-ant-21-mer extension with Sequenase and [α-^{32}P]dCTP at various concentrations, as in *a*. Primer/$(dG)_5$ template concentration was 50 nM. Lanes: C, kinase-treated control; 1–9, dCTP at 2.5, 5, 10, 25, 50, 100, 250, 500, and 1000 nM, respectively.

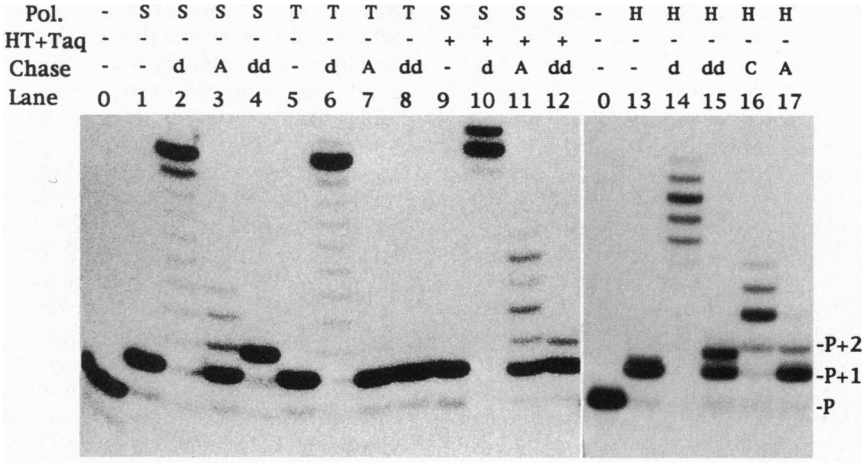

FIG. 4. Displacement of 3′ ester monitored with an end-labeled primer-extension and gel assay. Primer/template was incubated with 3′-ant-dGTP and Sequenase for 3 min (lanes 1–4 and 9–12), *Taq* DNA polymerase for 15 min in Mn^{2+}/citrate buffer (lanes 5–8), or HIV-RT and 3′-chain-dGTP for 15 min (lanes 13–17). For lanes 9–12, the reaction mixture was heat-treated and the oligonucleotides were reannealed. Reactions were chased with dATP (lanes 2, 6, 10, and 14), 3′-ant-dATP (lanes 3, 7, 11, and 17), 3′-chain-dATP (lane 16), or ddATP (lanes 4, 8, 12, and 15). After heat treatment (HT), a further 5 units of fresh *Taq* polymerase was added for lanes 10–12. P, primer (21-mer); A, 3′-ant-dATP; C, 3′-chain-dATP; d, dATP; dd, ddATP; Pol., polymerase; S, Sequenase; T, *Taq* polymerase; H, HIV RT.

different (Fig. 5, lane 4). Consequently, we treated part of the incorporation mixture with FITC, a fluorescent labeling reagent for primary amines that does not react with DNA (21). This resulted in a large shift in mobility for the Sequenase product only (Fig. 5, lanes 6 and 11). Furthermore, the 3′-amd-dTTP Sequenase addition product comigrated with the 3′-NH_2-dTTP (Fig. 1*d*) addition product (Fig. 5, lanes 9 and 10), and both were converted to the same major product upon FITC treatment (Fig. 5, lanes 11 and 12). Fluorescence spectroscopy analysis also indicated that an anthranylic derivative and a fluorescein derivative were present in the *Taq* and in the *FITC*-treated Sequenase addition products, respectively. With the same objective in mind, we tested 3′-fluothioureido-dTTP (18), an analogue on which a fluorescein molecule is attached to the 3′ position through a thiourea bond (Fig. 1*c*). Complete incorporation and removal of the 3′ reporter group were observed with Sequenase (Fig. 6) as well as with HIV-RT (data not shown).

Thus, Sequenase is able to incorporate 3′-amido and 3′-thioureido analogues and hydrolyze them leaving a 3′-amino-terminated DNA chain, whereas *Taq* DNA polymerase in the presence of the next correct nucleotide utilizes 3′-amd-dTTP without hydrolyzing the 3′-amido group once incorporated. We conclude that Sequenase is also able to cleave amido and thioureido bonds at the nascent 3′ end of DNA.

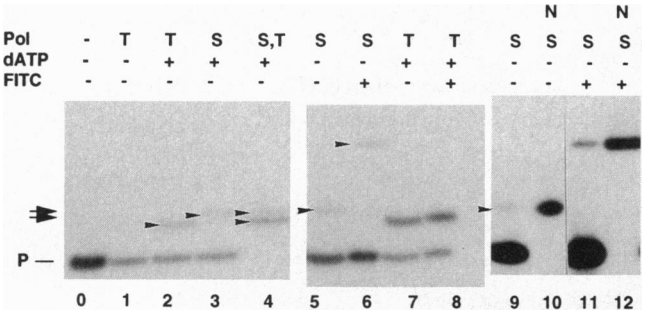

FIG. 5. Incorporation of 3′-amd-dTTP and 3′-NH_2-dTTP. The 5′-^{32}P-labeled primer was annealed to template A and incubated with 3′-amd-dTTP (lanes 0–9 and 11) and Sequenase (lanes 3–6, 9–12) or *Taq* DNA polymerase (lanes 1, 2, 7, and 8). For lane 4, the incorporation mixture was heat-treated, the oligonucleotides were reannealed, and 5 units of *Taq* DNA polymerase was added with 200 μM dATP. For lanes 2, 3, 7, and 8, incorporation was performed in the presence of 200 μM dATP. For lanes 6, 8, 11, and 12, the incorporation products were treated with FITC. Arrows and arrowheads indicate the position of migration of the two incorporation products. P, primer (21-mer); S, Sequenase; T, *Taq* DNA polymerase; N, when 3′-amd-dTTP was replaced by 3′-NH_2-dTTP in the incorporation reaction.

DISCUSSION

We have shown that nucleotide analogues with a bulky 3′-O-substituent are incorporated into DNA and block further incorporation of nucleotide analogues by *Taq* DNA polymerase, and the fluorescent reporter group can be observed at the nascent 3′ end of DNA. This is consistent with the fact that the 3′ block can be released upon alkali treatment (15). The incorporation products observed here cannot be due to contaminating deprotected dNTPs that would have been incorporated in place of the analogue (*i*) because a single incorporation product is obtained with *Taq* polymerase and a high concentration (2 mM) of the 3′-ant-dNTP mixture, whereas a distribution of products larger than P+1 (22-mer) would be expected with contaminating dNTPs (Fig. 2), (*ii*) because *Taq* polymerase would have used this new 3′-OH primer end upon secondary incorporation of putative contaminating dNTP, 3′ ester, or ddNTP (Fig. 4), and (*iii*) because the "editing" efficiency is sequence-specific. More interestingly, we report that 3′ esters do not absolutely block the ability of several

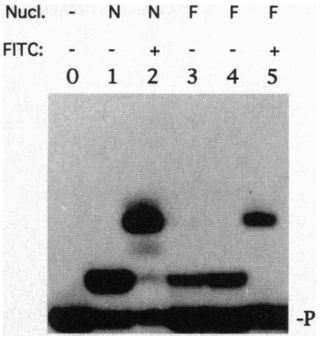

FIG. 6. Incorporation of 3′-fluothioureido-dTTP and 3′-NH_2-dTTP with Sequenase and FITC treatment. Lanes: 0, no enzyme; 1 and 2, 3′-NH_2-dTTP for 5 min; 3–5, 3′-fluothioureido-dTTP for 1, 5, and 5 min, respectively. Postincorporative derivatization with FITC is in lanes 2 and 5. P, primer (21-mer); N, 3′-NH_2-dTTP; F, 3′-fluothioureido-dTTP.

polymerases to synthesize DNA. Indeed, once a primer has been terminated with a 3′ ester, addition of the next correctly paired dNTP leads to further extension by several DNA polymerases lacking 3′-to-5′ exonucleolytic activity. Also, a chemically attached 3′ ester can be removed upon incubation with dNTP, indicating that this "editing" activity is distinct from pyrophosphorolysis and inherent with the DNA polymerization mechanism. The incubation times (1 min at 37°C) used to deprotect the 3′-blocked primer make it unlikely that an unwanted exonuclease (not detected) would first act to remove the last 3′-esterified nucleotide before addition of labeled dCTP. Furthermore, identical results were obtained by using either labeled dATP or dCTP as chasing agents. Exonucleolytic cleavage of 3′-ant-dCMP, if any, would yield a 20-mer primer that would be extended by dCTP and not dATP. We conclude that only the 3′ ester has been removed to allow chain extension. The 3′-esterified 21-mer substrate is invaluable in establishing that this 3′ editing occurs at the DNA level but does not rule out that editing could also occur before or simultaneously with the phosphodiester bond formation, as shown with 3′-N-substituted analogues. Indeed, 3′-amido-dTTP and 3′-thioureido-dTTP are incorporated and edited quantitatively into 3′-amino-dTMP-terminated DNA chain by Sequenase in the absence of the next correct nucleotide (dATP). Interestingly, 3′-amido-dTTP is incorporated by *Taq* polymerase without being edited into 3′-amino only when the next correct nucleotide (dATP) is present, consistent with incorporation without edition of 3′ esters by this enzyme.

DNA polymerases are able to use modified nucleotides relative to their classical deoxynucleotide (dNTP) counterparts (1, 15, 16, 20, 22, 23). The 3′-deoxy analogues, such as AZT (22), have been investigated mainly as inhibitors but have been of moderate value as active-site mapping probes. Catalano and Benkovic (24) reexamined the work of Abboud *et al.* (25) by using adenosine 2′,3′-epoxide triphosphate and concluded that the strong inhibition observed was due to tight binding of the 2′,3′-epoxide-terminated DNA chain without detectable covalent modification of the polymerase. The results reported here may indicate that the polymerase-bound 3′ end of DNA lies in the vicinity of a powerful nucleophilic group in the active site involved either in polymerization or in 3′-substituent removal, a finding in accordance with crystallographic data for several DNA polymerases (8–12). "Read-through" (16), "quenching" (18), and results reported by others (23, 26, 27) may be explained by a quantitative removal of the fluorescent reporter upon enzymatic chain extension.

These catalytic properties (that we call catalytic editing) raise several important questions. Indeed, these activities may be a fortuitous side reaction or have a biological meaning, i.e., to cope with illegitimate 3′ modifications that may occur *in vivo*. The 3′-hydroxyl group of a 2′-deoxynucleotide is its most chemically reactive functional group. Thus, it makes sense that DNA polymerases are able to cope with potential illegitimate 3′-coupling that can stop DNA synthesis. Interestingly, 3′-phosphates are potent blocks to DNA polymerization (1). This may reflect the fact that a phosphate cannot receive a nucleophilic attack unless it is precisely ligated to a metal ion and that enzymes exist *in vivo* that can remove phosphates at 3′ termini (1). Several retroviral RTs, including that of HIV, also utilize tRNA primers (28) that may be acylated with amino acids whose structures resemble the anthranyloyl substituents used here. The HIV-RT capability that we demonstrated above may be relevant to the initiation of reverse transcription during early retroviral life cycle. Other substituents at the 3′ end of DNA at such a position and distance of the sugar ring may also receive a nucleophilic attack. Strikingly, the central nitrogen of the 3′-azido group of AZT bears a partial positive charge (29) in a position similar to the carbonyl group of 3′-esterified nucleotides, and the 3′-azido group of AZT is metabolized into a 3′-amino group *in vivo* (30). Finally, this also identifies a position to be modified for DNA polymerase substrate analogues that does not prevent incorporation into DNA.

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