1308–1313 *Nucleic Acids Research, 1996, Vol. 24, No. 7*

Differential discrimination of DNA polymerases for variants of the non-standard nucleobase pair between xanthosine and 2,4-diaminopyrimidine, two components of an expanded genetic alphabet

Michael J. Lutz, Heike A. Held, Michael Hottiger[1], Ulrich Hübscher[1] and Steven A. Benner*

Department of Chemistry, Swiss Federal Institute of Technology, Universitätsstraße 16 and CH-8092 Zürich, Switzerland and [1]Department of Veterinary Biochemistry, University of Zürich, CH-8057 Zürich, Switzerland

Received December 4, 1995; Revised and Accepted February 13, 1996

ABSTRACT

Mammalian DNA polymerases α and ε, the Klenow fragment of *Escherichia coli* DNA polymerase I and HIV-1 reverse transcriptase (RT) were examined for their ability to incorporate components of an expanded genetic alphabet in different forms. Experiments were performed with templates containing 2′-deoxyxanthosine (dX) or 2′-deoxy-7-deazaxanthosine (c^7dX), both able to adopt a hydrogen bonding acceptor–donor–acceptor pattern on a purine nucleus (puADA). Thus these heterocycles are able to form a non-standard nucleobase pair with 2,4-diaminopyrimidine (pyDAD) that fits the Watson–Crick geometry, but is joined by a non-standard hydrogen bonding pattern. HIV-1 RT incorporated d(pyDAD)TP opposite dX with a high efficiency that was largely independent of pH. Specific incorporation opposite c^7dX was significantly lower and also independent of pH. Mammalian DNA polymerases α and ε from calf thymus and the Klenow fragment from *E.coli* DNA polymerase I failed to incorporate d(pyDAD)TP opposite c^7dX.

INTRODUCTION

Nucleobases in oligonucleotide strands form Watson–Crick base pairs following two rules of complementarity: (i) a large purine from one strand pairs with a small pyrimidine from the other; (ii) hydrogen bond donors (NH groups) from one base are matched with hydrogen bond acceptors (lone pairs of electrons on oxygen or nitrogen) from the other. In DNA, for example, cytosine, implementing a hydrogen bond donor–acceptor–acceptor pattern (pyDAA), pairs as the small component with guanine, a large component implementing the hydrogen bond acceptor–donor–donor pattern (puADD).

Some time ago we pointed out that standard nucleobases exploit only part of the potential of the Watson–Crick formalism (1). When fully exploited the Watson–Crick formalism permits 12 independently replicatable nucleobases joined in six base pairs by mutually independent hydrogen bonding patterns (Fig. 1). Previous work in these and other laboratories has yielded implementations of all six hydrogen bonding patterns (2–6). Further, individual RNA and DNA polymerases have been found that catalyze template-directed incorporation of several non-standard base pairs into duplex DNA (7–9). However, DNA polymerases involved in DNA transactions in mammals have so far rejected non-standard base pairs.

As non-standard nucleobases are accepted by at least some polymerases, these bases must be intrinsically able to form Watson–Crick base pairs during a polymerization reaction, just as they contribute to the overall duplex stability in complementary oligonucleotide strands (4,10). The polymerases that do not accept non-standard nucleobases must, therefore, recognize some structural feature of the non-standard nucleobases incidental to their ability to form a competent Watson–Crick structure.

Recent studies in these laboratories have focused on the non-standard base pair between xanthosine (trivially designated X), which presents a hydrogen bond ‘acceptor–donor–acceptor’ (puADA) pattern to the complementary non-standard base 2,4-diaminopyrimidine (presenting a ‘donor–acceptor–donor’ hydrogen bonding pattern), designated here pyDAD (9). We have shown that the Klenow fragment of DNA polymerase I accepts dX as a nucleoside triphosphate opposite d(pyDAD) in the template, while human immunodeficiency virus type I (HIV-1) reverse transcriptase (RT) accepts dX and d(pyDAD) both in the template and as a triphosphate. The efficiency of incorporation of this non-standard base pair was generally lower, however, compared with the incorporation of standard nucleobases. Further, with a pK_a of 5.7 when free in solution (11), xanthosine is far more acidic as a heterocycle than standard nucleobases. While its pK_a should be higher when incorporated into an oligonucleotide, this acidity might also prove to be a problematical aspect of the non-standard nucleobase.

To explore this idea 2′-deoxy-*N*1-methyloxoformycin B, trivially designated dπ, was examined. The nucleoside bearing this heterocycle also presents a pu(ADA) hydrogen bonding

* To whom correspondence should be addressed

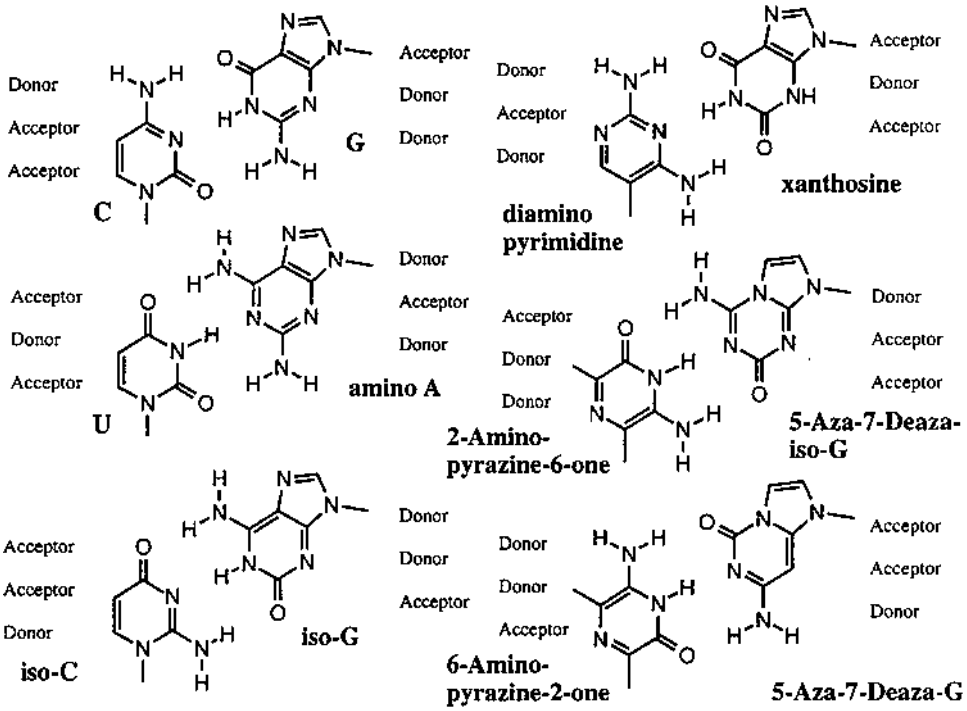

Figure 1. Six base pairs that meet the constraints imposed by the Watson–Crick base pairing geometry.

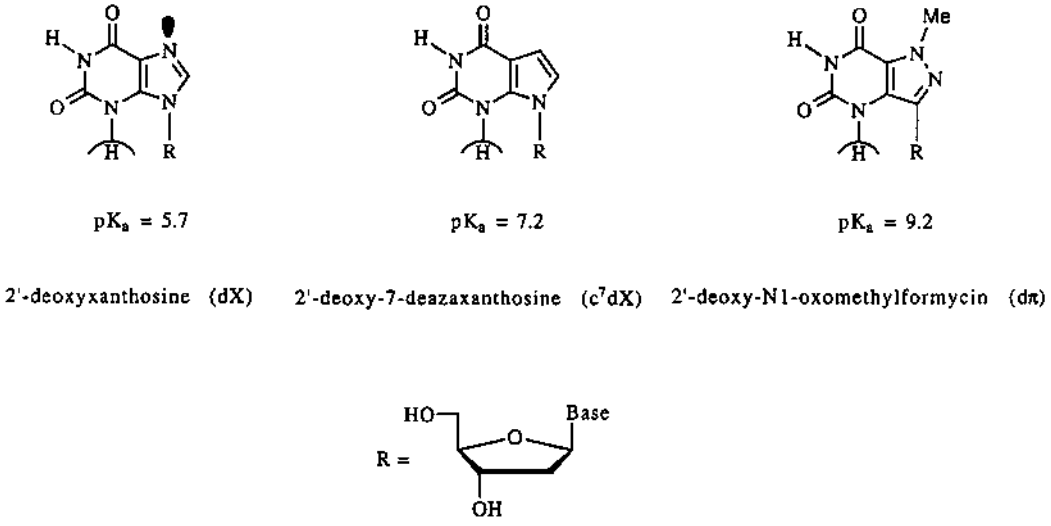

Figure 2. Three implementations of the 'acceptor–donor–acceptor' hydrogen bond pattern on variants of xanthosine.

pattern (Fig. 2), but has a pK_a of 9.2 (12). No polymerase was found to be able to synthesize a base pair between dπ and d(pyDAD), either when dπ was in the template or when it was presented as the triphosphate (9). However, dπ has other differences that distinguish it from dX. First, it is a C-glycoside, the heterocyclic base being joined to the 2′-deoxysugar by a carbon–carbon bond. This was known to exert a small but significant (~3-fold) effect on incorporation with some polymerases (13). Further, dπ is modified at the N-7 position, replacing the lone pair of electrons in xanthosine at this position by a methyl group. Each of these differences could also account for the ability of HIV-1 RT to accept dX but not dπ.

The 2′-deoxy-7-deazaxanthosine (c^{7}dX) (Fig. 2) nucleoside also implements the pu(ADA) hydrogen bonding pattern found in dX and dπ. Like dπ, c^{7}dX is missing the lone pair of electrons at N-7 through replacement of N-7 with a CH group, but does not have a bulky methyl group at this position. Further, the nucleobase has a pK_a value of 7.2 (14), presumably corresponding to deprotonation at N-3. We report here the enzymology of the py(DAD)·c^{7}dX base pair.

MATERIALS AND METHODS

Synthesis of non-standard nucleobases

2,4-Diamino-5-(β-D-ribofuranosyl)pyrimidine (pyDAD) was synthesized using the route of Chu *et al.* (3). This compound was converted to the 2′-deoxygenated nucleoside analog as described by Piccirilli *et al.* (4). The triphosphate d(pyDAD)TP was synthesized by a published procedure (15). 5′-Dimethoxytrityl-2′-deoxyxanthosine with both heterocyclic ring oxygens protected as *p*-nitrophenylethyl ethers was prepared by the procedure of Van Aerschot *et al.* (16) and converted to the phosphoramidite following a standard method (17). 2′-Deoxy-7-deazaxanthosine (c^7dX) was synthesized as 7-deaza-2′-deoxy(4,4′-dimethoxytrityl)xanthosine-3′-H-phosphonate as recently described (18). Standard dNTPs were from Pharmacia.

Oligonucleotides

The oligonucleotide bearing 2′-deoxyxanthosine was prepared by solid phase synthesis (Applied Biosystems) from the β-cyanoethyl-protected phosphoramidite, purified by the trityl-on procedure, deprotected and purified again by HPLC (19). The oligonucleotide bearing c^7dX was synthesized by Dr L.Arnold (Czech Academy of Chemistry and Biochemistry, Prague) using H-phosphonate technology.

The primer (5′-GCATGGATCCCACTGCACTCCAGGG-3′) was synthesized by Microsynth (Windisch, Switzerland) and purified by PAGE. Labelling of the primer at the 5′-end with Redivue [γ-^{32}P]ATP (Amersham) was performed using T4 polynucleotide kinase (Life Technologies).

Nucleic acid substrates

The primer was annealed with a template (5′-ACCCCqCCCCCC-CTGGAGTGCAGTGGGATCCATGC-3′), where q is either dX or c^7dX, in 500 µl total buffer containing 50 pmol template and 15 pmol labelled primer in 1.8 mM Tris–HCl, pH 7.0, 0.5 mM $MgCl_2$, 23 mM NaCl by heating the mixture at 85°C for 15 min followed by subsequent slow cooling to room temperature over a period of 1 h.

DNA polymerases

HIV-1 RT, overexpressed using the plasmid pJS3.7 in *Escherichia coli*, was purified by a published procedure (20). Calf thymus DNA polymerases α and ε were purified according to the methods of Podust *et al.* (21) and Weiser *et al.* (22) respectively. Enzymatic activity was determined as described in these references. The Klenow fragment of DNA polymerase I was from Boehringer Mannheim.

Assays to detect incorporation of the bases

Incorporation of a non-standard base opposite the complementary non-standard base was performed in a total volume of 25 µl using 0.15 pmol labelled and annealed primer and all required dNTPs, at a final concentration of 5 µM each. Reaction buffers contain the following: for HIV-1 RT, 50 mM Tris–HCl, pH 7.2 (unless otherwise stated), 5 mM $MgCl_2$, 100 mM KCl, 1 mM DTT, 0.5 mM EDTA; for DNA polymerases α and ε from calf thymus, 50 mM Tris–HCl, pH 6.5, 1 mM DTT, 0.25 mg/ml BSA; for Klenow fragment of DNA polymerase I, 50 mM Tris–HCl, pH 7.5, 1 mM DTT, 0.1 mg/ml BSA. The amount of enzyme used was 0.1 U Klenow fragment and HIV-1 RT, 0.11 U DNA polymerase α and 0.04 U DNA polymerase ε. The reactions were started by adding the enzyme and incubated for 15 min at 37°C and finally quenched by adding 5 µl of a mixture of stop/loading dye (New England Biolabs), which contains 0.3% xylene cyanol, 0.3% bromphenol blue and 0.37% Na EDTA, pH 8.0. The samples were heated (20 min, 95°C) and aliquots (5 µl) were loaded onto a 17% polyacrylamide gel containing 7 M urea. Following electrophoresis (constant power 25 W) the gels were fixed (12% MeOH, 10% HOAc, diluted with water), dried and autoradiographed. Radioactivity was quantified using a PhosphorImager (Molecular Dynamics), with 3 h exposures and the ImageQuant program from Molecular Dynamics. To determine the amount of specific formation of the non-standard base pairs the amount of full-length product was quantified, divided by the total amount of radioactivity in the lane and expressed as a percentage. To correct for non-specific misincorporation of standard nucleobases opposite the non-standard nucleotides the amount of misincorporation of natural dNTPs, determined in a control experiment, was subtracted.

RESULTS

No evidence could be obtained for incorporation of d(pyDAD)TP opposite c^7dX in a template when Klenow fragment of DNA polymerase I from *E.coli* was incubated at pH 7.5. Oligonucleotide products indicating extension of the primer past the non-standard base were found both in the presence and absence of d(pyDAD)TP. It is possible that the Klenow fragment misincorporates dGTP opposite c^7dX (Fig. 3, lanes 5–7). However, the principal product is shorter than the full-length product by one base. Why this $n-1$ product is formed is not known. It may arise from the DNA polymerase skipping over the non-standard nucleobase or may be a response of the DNA polymerase to a mismatch in the template–primer complex. Similar production of $n-1$ product has been observed with other unsuccessful fill-in experiments using Klenow fragment (4, 9). In any case, a quantitative analysis using a PhosphorImager shows that at most 1% of the longest product is derived from specific incorporation of d(pyDAD)TP opposite c^7dX in the template under these conditions.

Similarly, neither calf thymus DNA polymerases α nor ε incorporated d(pyDAD)TP opposite c^7dX in a template at pH 6.5. Less misincorporation was observed with these DNA polymerases (Fig. 3, lanes 8–13), consistent with the overall higher fidelity of these polymerases in general (9). The quantitative analysis yields ~1.5% specific formation of the non-standard base pair for DNA polymerase α and ~1.9% for DNA polymerase ε, within the experimental error. These mammalian DNA polymerases also yielded full-length product missing the final base.

When HIV-1 RT was incubated with (pyDAD)TP and a template containing c^7dX and d(pyDAD)TP at pH 7.2 (Fig. 3, lanes 2–4) full-length product was observed in excess of that formed when d(pyDAD)TP was omitted. This suggested that (pyDAD)TP was successfully incorporated opposite c^7dX. However, the efficiency of incorporation of d(pyDAD)TP was much lower than that observed with an analogous template containing dX instead of c^7dX (Fig. 4a). Furthermore, evidence for misincorporation of dGTP opposite c^7dX could be seen. Quantitative analysis shows that only ~8% of the amount of

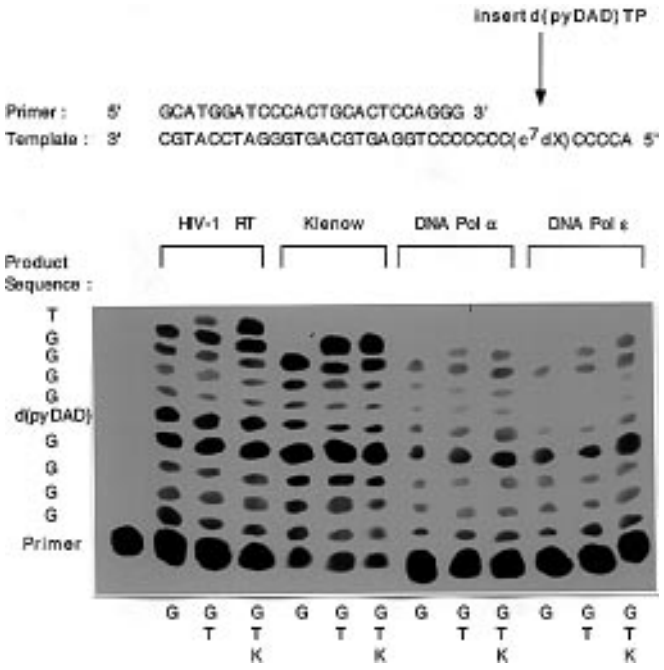

Figure 3. Primer extension by mutants of HIV-1 RT, Klenow fragment and mammalian DNA polymerases α and ε. Deoxynucleoside triphosphates present are indicated below, where K stands for d(pyDAD)TP. Deoxynucleoside triphosphates (5 µM) were incubated at 37°C for 15 min with 0.15 pmol primer–template complex containing the c^7dX nucleobase in the template and 0.1 U HIV-1 RT and Klenow fragment, 0.11 U DNA polymerase α and 0.04 U DNA polymerase ε in a final volume of 25 µl.

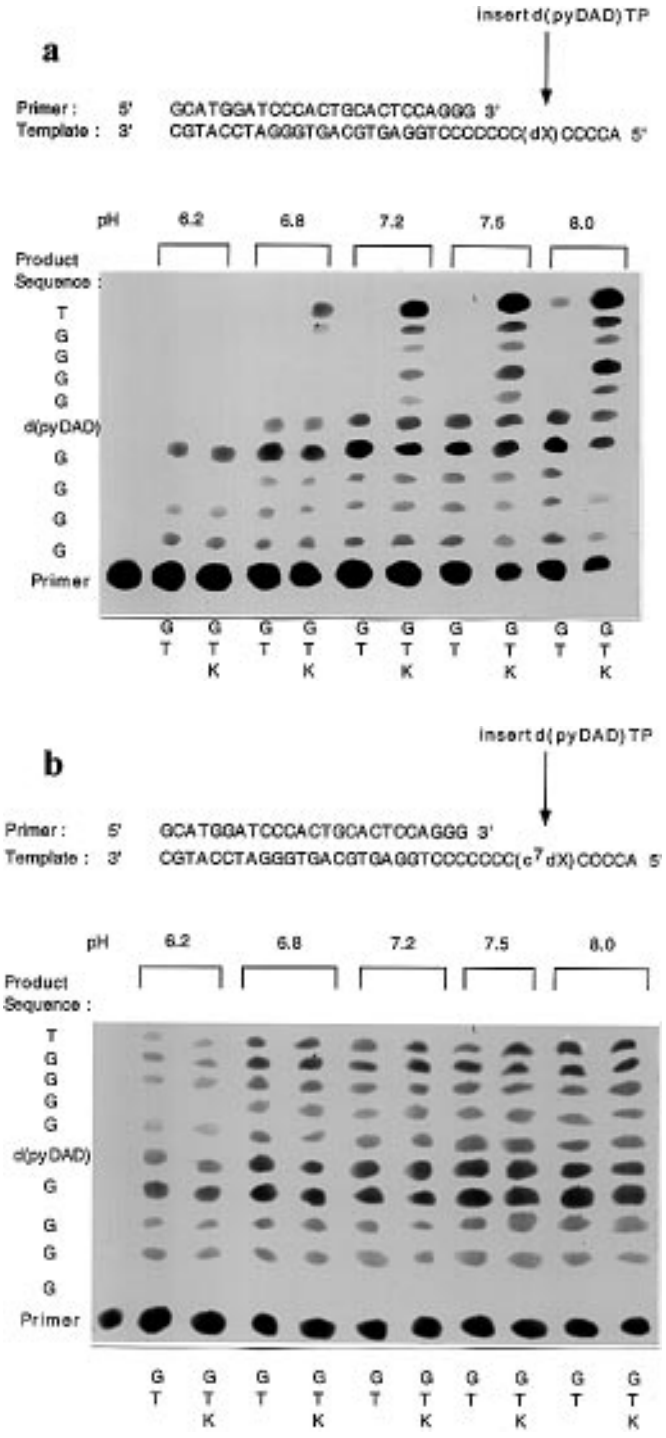

Figure 4. pH-dependent primer extension by HIV-1 RT. Deoxynucleoside triphosphates present are indicated below, where K stands for d(pyDAD)TP. Deoxynucleoside triphosphates (5 µM) were incubated at 37°C for 15 min with 0.15 pmol primer–template complex containing the (**a**) dX and (**b**) c^7dX nucleobase in the template and 0.1 U HIV-1 RT in a final volume of 25 µl.

full-length product derives from the incorporation of d(pyDAD)TP opposite c^7dX in the template.

Templates containing dX successfully direct incorporation of d(pyDAD)TP at pH 7.2 when HIV-1 RT is the catalyst. Remarkably, very little (if any) misincorporation is observed opposite dX when HIV-1 RT is used (Fig. 4a). The pH dependence of this incorporation was then studied (Fig. 4a) with a template containing dX and d(pyDAD)TP to be incorporated. A quantitative analysis shows that the amount of full-length product increases by ~3-fold with increasing pH over the range 6.8–8.0 (Fig. 4a). The maximum amount of full-length product formed under these conditions was ~30% at pH 7.5 and then drops to ~26% at pH 8.0. However, virtually all of the increase in the synthesis of full-length product is due to increased activity of the enzyme (~3-fold) at higher pH. Slight misincorporation of standard nucleobases opposite dX was observed, but only at pH 8.0.

Incorporation of d(pyDAD)TP opposite c^7dX in the template showed only slight pH dependency. With c^7dX the increase in enzymatic activity over the pH range 6.2–8.0 is only about a factor of two. Quantitative analysis using a PhosphorImager shows for this pH-dependent study that the amount of full-length product formed by specific incorporation of d(pyDAD)TP opposite c^7dX in a template reaches a maximum at pH 7.2 of ~5.5% under these conditions and then drops to a value of ~1.5% at pH 8.0.

Further pH dependence studies were performed at pH values of 8.0–9.5. Experiments with dX in the template show that the amount of full-length product due to specific incorporation of d(pyDAD)TP decreases with increasing pH. However, the amount of full-length product due to misincorporation of standard nucleobases increases with increasing pH. At pH 9.5 full-length product derives only from misincorporation (data not shown). Similiar results were seen when c^7dX was in the template. The amount of full-length product decreases with increasing pH and

no specific incorporation of d(pyDAD)TP opposite c^7dX was observed over this pH range (data not shown). Needless to say, HIV-1 RT has low catalytic activity under these high pH conditions.

DISCUSSION

The standard model of nucleic acid structure, proposed in its original form over four decades ago by Watson and Crick (23), invokes the stacking of hydrophobic nucleobases as a central determinant of the stability of the double helix. In its simplest form this model suggests that the less hydrophobic a nucleobase, the less likely it is to be accepted into a duplex structure by a DNA polymerase. Naively, this implies that given the choice between a more acidic nucleobase (in this example dX) and a less acidic nucleobase (c^7dX), both meeting the minimum hydrogen bonding requirements, the latter would be more easily accepted than the former.

This is not the case. A variety of polymerases accept c^7dX as a complement for (pyDAD)TP more poorly than dX; several do not accept it at all. Further, incorporation of (pyDAD)TP opposite dX in a template is largely independent of pH over the range 6.2–9.5. This pH range is expected to span the pK_a of dX in a template, as the pK_a of dX free in solution (5.7) is expected to be increased by 2 to 3 pK_a units when incorporated into a polyanionic oligonucleotide, according to the observed shift with 7-methyl-2′-deoxyguanine and guanylic acid when embedded in a DNA oligonucleotide (24,25). As the pK_a of the nucleobase can be further perturbed in the active site of a DNA polymerase, the ionization state of dX in a template at the instant when the molecular recognition event occurs is not easily known. However, it is clear that the intrinsic acidicity of dX does not present an obvious impediment to its serving as a partner in a Watson–Crick base pair.

Why is c^7dX accepted less efficiently (or not at all) than its analog dX? Three explanations might be considered.

(i) Substitution of N-7 in dX by a CH group in c^7dX might create structural perturbations that might be invoked to explain this discrimination against c^7dX. For example, the conformation of the base or the sugar might be influenced by this substitution.

(ii) Alternatively, the DNA polymerase might actually recognize the deprotonated form of dX, a form that cannot be attained by c^7dX due to its higher pK_a.

(iii) The DNA polymerase might itself interact with N-7 in a way that causes it to reject c^7dX as foreign. This proposal suggests that the DNA polymerase is ‘scanning’ the major groove of duplex DNA.

Each of these possibilities raises interesting questions concerning the event by which DNA polymerases recognize base pairs. Explanation (i) is problematical, because structural differences induced by the N-7 substitution are expected to be subtle. Further, HIV-1 RT seems to be largely indifferent to subtle structural features of the nucleobase. For example, it accepts both DNA and RNA as template, which have quite different conformations.

Explanation (ii) is problematical considering the fact that incorporation of dX is essentially pH independent. If the DNA polymerase indeed prefers a deprotonated form of the nucleobase over the protonated form, one might expect the efficiency of incorporation of dX to increase with increasing pH. This is not the case. Further, if the relative pK_a values of dX and c^7dX in the template are the same as the relative pK_a values of dX and c^7dX free in solution and if the only impact of the substitution at position 7 is the shift in pK_a then c^7dX at pH 8.5 should behave the same as dX at pH 7.0, but it does not.

The remaining possibility is that the DNA polymerase is itself examining structural features of the nucleobases, presumably in the major and minor grooves, to discard ‘unnatural’ structures. At one level this proposal is reasonable. To enforce a Watson–Crick geometry the DNA polymerase must interact in some way with the nucleobases, in either the major or minor groove. This interaction presumes a direct contact between functionality on the bases and functionality in the protein. This proposal is problematical, however, as different nucleobases present different functionality in these grooves and DNA polymerase should have no intrinsic preference for one nucleobase over another, once the nucleobase has been accepted by the template.

Thus DNA polymerases, if they are to interact with the nucleobases to enforce a Watson–Crick geometry, must do so by identifying features in the grooves of duplex oligonucleotides that are constant for all four nucleobases. One such feature exists. In the minor groove the lone pair of electrons on N-3 of both purines approximately overlap in space the lone pair of electrons presented by the 2-position carbonyl oxygens of both thymine and cytosine. Thus it is conceivable that a DNA polymerase might present a hydrogen bond donor to this lone pair in all four bases, allowing it to control the geometry of the incoming nucleobase without having a preference for one over the other. Several years ago Steitz noted that such minor groove ‘scanning’ might be used by DNA polymerases to improve their fidelity (26). Furthermore, the recently published crystal structure of mammalian DNA polymerase β co-crystallized with template, primer and a triphosphate analog identified three amino acid residues that make contacts with these lone pairs (27).

The results reported here are inconsistent with the scanning proposal in its broadest form, as a lone pair of electrons at position O-2 in pyrimidines is not an absolute requirement for recognition by DNA polymerases. The pyDAD nucleobase lacks the exoxyclic oxygen and would not be accepted by any polymerase if the lone pair were an absolute specificity determinant. As we have shown here and elsewhere (9), pyDAD is accepted by many polymerases, either in the template or as a triphosphate. Further, in its protonated form dX also lacks the lone pair of electrons at N-3 and yet is also accepted by DNA polymerases, although the possibility remains that the polymerase is accepting the N-3 deprotonated form of the nucleobase, which carries the lone pair.

Explanation (iii) requires, however, a new type of scanning, in the major groove. This scanning is also problematical, as no functional group is consistently presented to the major groove by the standard nucleobases. For example, thymine presents a hydrophobic methyl group to one region of the major groove, cytosine presents a hydrogen atom and both purines present a hydrogen bond acceptor, a lone pair of electrons on N-7. These functionalities are different and it is difficult to imagine a DNA polymerase making a contact with this region of the major groove without causing it to favor one of the standard bases over any other in a way that would diminish faithful reproduction of information in the template.

The disfavoring of c^7dX is more perplexing in the light of former results showing that Klenow fragment and Taq DNA polymerase both accept 7-deaza-dGTP (28,29), as well as dGTP substituted at the N-7 position with either a methyl group or cyanoborane (24,30). Because Klenow fragment rejects

d(pyDAD) as the triphosphate, both opposite dX and c^7dX, its rejection of c^7dX is more difficult to interpret. Nevertheless, Klenow fragment does not seem to require a lone pair of electrons on N-7 in the major groove for all purines.

These data suggest a paradox in the 'model' for the selectivity of polymerases. The selectivity of individual polymerases (such as Klenow fragment) with respect to variants of non-standard nucleobases seems to be unrelated to their selectivity with respect to analogous variants of the standard nucleobases. There is no simple structural explanation for this fact. Further, even though crystal structures compellingly argue that all polymerases are related by common ancestry (31), it is clear that the details of the molecular recognition process diverge greatly with their sequences. There is not likely to be a general model describing DNA polymerase specificity generally; each polymerase will need to be described individually, with more work both in solution with non-standard nucleobases and other nucleotide analogs and in the crystal.

ACKNOWLEDGEMENTS

We thank Drs J.Opitz and A.Roughton for helpful discussions. We are indebted to Dr L.Arnold from the Czech Academy of Organic Chemistry and Biochemistry in Prague for oligonucleotide synthesis. MJL was supported by a scholarship from the German Academic Exchange Service (DAAD) in the program HSP II/AUFE and MH and UH were supported by the Swiss National Science Foundation (grants 3135-36713.92 and 31.37146.93).

REFERENCES

1 Benner,S.A., Allemann,R.K., Ellington,A.D., Ge,L., Glasfeld,A., Leanz,G.F., Krauch,T., MacPherson,L.J., Moroney,S.E., Piccirilli,J.A. and Weinhold,E.G. (1987) *Cold Spring Harbor Sym. Quant. Biol.*, **52**, 53–63.
2 Sepiol,Z., Kazimierczuk,D. and Shugar,Z. (1976) *Z. Naturforsch.*, **31c**, 361–370.
3 Chu,C.K., Reichman,U., Watanabe,K.A. and Fox,J.J. (1977) *J. Org. Chem.*, **42**, 711–714.
4 Piccirilli,J.A., Krauch,T., Moroney,S.E. and Benner,S.A. (1990) *Nature*, **343**, 33–37.
5 Voegel,J.J. and Benner,S.A. (1994) *J. Am. Chem. Soc.*, **116**, 6929–6930.
6 von Krosigk,U. and Benner,S.A. (1995) *J. Am. Chem. Soc.*, **117**, 5361–5362.
7 Switzer,C.Y., Moroney,S.E. and Benner,S.A. (1989) *J. Am. Chem. Soc.*, **111**, 8322–8323.
8 Switzer,C.Y., Moroney,S.E. and Benner,S.A. (1993) *Biochemistry*, **32**, 10489–10496.
9 Horlacher,J., Hottiger,M., Podust,V.N., Hübscher,U. and Benner,S.A. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 6329–6333.
10 Leach,A.R. and Kollman,P.A. (1992) *J. Am. Chem. Soc.*, **114**, 3675–3683
11 Roy,K.S and Miles,H.T. (1983) *Nucleosides Nucleotides*, **2**, 231–242.
12 Krauch,T.(1990) Dissertation, ETH Zürich no. 8940.
13 Piccirilli, J.A., Moroney,S.E. and Benner,S.A. (1991) *Biochemistry*, **30**, 10350–10356
14 Seela,F., Driller,H. and Liman,U. (1985) *Liebigs Ann. Chem.*, 321–320.
15 Ludwig,J. and Eckstein,F. (1989) *J. Org. Chem.*, **54**, 631–635.
16 Van Aerschot,A., Mag,M., Herdewijn,P. and Vanderhaeghe,H. (1989) *Nucleosides Nucleotides*, **8**, 159–178.
17 Sinha,N.D., Biernat,J. and Köster,H. (1983) *Tetrahedron Lett.*, **24**, 5843–5846.
18 Milligan,J.F., Krawczyk,S.H., Wadwani,S. and Matteuci,M.D. (1993) *Nucleic Acids Res.*, **21**, 327–333.
19 Horlacher,J. (1995) Dissertation, ETH Zürich no. 11084.
20 Hafkemeyer,P., Ferrari,E., Brecher,J. and Hübscher,U. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 5262–5266.
21 Podust,V.N., Mikhailov,V., Georgaki,A. and Hübscher,U. (1992) *Chromosoma*, **102**, 133–141.
22 Weiser,T., Gassmann,M., Thömmes,P., Ferrari,E., Hafkemeyer,P. and Hübscher,U. (1991) *J. Biol. Chem.*, **266**, 10420–10428.
23 Watson,J.D. and Crick,F.H.C. (1953) *Nature*, **171**, 964–967.
24 Hendler,S., Furer,E. and Srinivasan,P.R. (1970) *Biochemistry*, **9**, 4141–4135.
25 Pochon,F. and Michelson,A.M. (1965) *Proc. Natl. Acad. Sci. USA*, **53**, 1425–1430.
26 Steitz,T. (1987) In Burnett,R.M. and Vogel,H.J. (eds), *Biological Organization: Macromolecular Interactions at High Resolution*. Academic Press, New York, NY, pp.45–55.
27 Pelletier,H., Sawaya,M.R., Kumar,A., Wilson,S.H. and Kraut,J. (1994) *Science*, **264**, 1891–1903.
28 Mizusawa,S., Nishimura,S. and Seela,F. (1986) *Nucleic Acids. Res.*, **14**, 1319–1324.
29 McConlogue,L., Brow,M.A.D. and Innis,M.A. (1988), *Nucleic Acids. Res.*, **16**, 9869.
30 Porter,K.W., Tomasz,J., Huang,F., Sood,A. and Shaw,B.R. (1995) *Biochemistry*, **34**, 11963–11969.
31 Steitz,T.A., Smerdon,S.J., Jäger,J. and Joyce,C.M. (1994) *Science*, **266**, 2022–2025.