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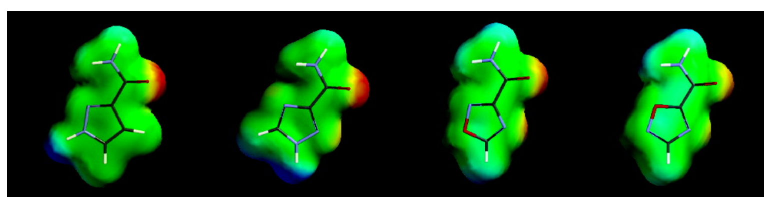

Isosteric Carboxamide Nucleobases

A) Pyrazole-3-, **B)** 1,2,4-Triazole-3-, **C)** 1,2,4-Oxadiazole-3-, **D)** 1,2,4-Oxadiazole-5-

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Efficient Primer Strand Extension beyond Oxadiazole Carboxamide Nucleobases

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Discrimination between matched or mismatched nucleotides by the DNA polymerase domains takes place during both the incorporation opposite a template nucleobase and the strand extension steps. It appears that for most DNA polymerases, especially those lacking proofreading activity, the structural requirements are more stringent during extension of a newly synthesized unnatural base pair.[1,2] In this paper, we describe the ability of *Taq* DNA polymerases to incorporate and efficiently continue DNA synthesis beyond the oxadiazole carboxamide nucleobases, **1** and **2**.

Our understanding of DNA polymerase recognition and fidelity has been substantially augmented by a number of studies utilizing nucleoside mimics as probes of specificity.[3−6] Exploration of DNA polymerase recognition of unnatural nucleosides has been critical for identification of orthogonal nucleobase pairs for expansion of the genetic code.[7,8] Degenerate recognition of certain nucleobase analogues by DNA polymerases has also served as the basis for development of random mutagenesis methodologies.[9,10] However, successful uses of modified nucleoside triphosphates as an orthogonal or mutagenic substrate have been greatly hindered due to the inability of many families of DNA polymerases to extend beyond most nonstandard nucleobases in a DNA strand.[4,11] Currently, there is essentially no clear structural and mechanistic information that can be used to predict the relative efficiencies of DNA polymerase mediated extension of artificial nucleobases.

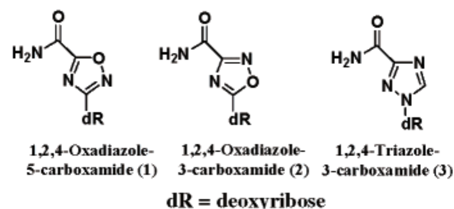
Oxadiazole carboxamide nucleoside analogues are C-linked deoxyribonucleoside base analogues designed such that they present heteroatoms (nitrogen or oxygen) on both minor and major groove sides of the DNA (Scheme 1, panel 1). As highlighted here, the comparison of the *Taq* DNA polymerase extension of DNA strands containing analogues **1** and **2** with azole nucleobases represented by **3** suggests that unique geometry and electronic properties of the oxadiazole nucleobases provide favorable interactions for successful recognition and extension.

The ability of *Taq* DNA polymerase to recognize an unnatural nucleobase was studied by a single nucleotide, steady-state incorporation assay. Nucleotide triphosphates were synthesized as described in Wu et al.[12,13] to enable the study of the substrate properties of these azole analogues. Primers modified with fluorescent dye (Cy5) nucleobase at the 5′-terminal end and the 28-nt templates used in these studies are shown in Scheme 1 (panel II). Briefly, the rate of single nucleotide incorporation was measured at different substrate concentrations, and the quantitative analysis of extension reactions was accomplished through capillary electrophoresis using a LIF detector (Supporting Information).[14] The steady-state kinetic constants, V_{max}/K_m, were obtained by fitting the data to the Michaelis−Menten equation as described earlier.[6]

To investigate the extension properties beyond these analogues in the forward strand, we synthesized primers containing 3′-terminal azole bases using *Taq* DNA polymerase followed by PAGE purification of the extended oligonucleotide. The 24-nt Cy5-labeled

Scheme 1. Primers and Template for Incorporation and Extension Studies

I



II.

23-nt Primer 5'-[Cy5]-TAATACGACTCACTATAGGGAGA

24-nt Primer 5'-[Cy5]-TAATACGACTCACTATAGGGAGAAz

28-nt Template 3'- ATTATGCTGAGTGATATCCCTCTNGTCA

Az = 1, 2 or 3; N = dA, T, dG, or dC

Table 1. Single Base Incorporation Kinetics: Extension beyond the Unnatural Base at the Primer Terminus

substrate	base pair	V_{max} (pmol/U·min)	K_m (μM)	V_{max}/K_m ($\times10^{-3}$)
dCTP	A:A	0.026 ± 0.004	131 ± 56	0.20
dCTP	G:T	0.554 ± 0.084	242 ± 90	2.29
dCTP	1:A	1.96 ± 0.08	1.43 ± 0.34	1370
dCTP	1:T	9.18 ± 0.83	5.44 ± 1.35	1687
dCTP	1:G	10.02 ± 1.01	7.65 ± 1.94	1309
dCTP	1:C	1.77 ± 0.08	1.33 ± 0.36	1330
dCTP	2:A	1.01 ± 0.04	1.39 ± 0.29	726
dCTP	2:T	7.16 ± 0.66	4.73 ± 1.24	1513
dCTP	2:G	6.61 ± 0.57	3.60 ± 0.96	1836
dCTP	2:C	0.92 ± 0.05	1.12 ± 0.33	823
dCTP	3:A	too low		
dCTP	3:T	0.920 ± 0.005	164 ± 22	5.61
dCTP	3:G	0.126 ± 0.004	369 ± 28	0.34
dCTP	3:C	0.052 ± 0.005	621 ± 127	0.08

primers and the 28-nt templates shown in Scheme 1 were used to determine the kinetic constants for incorporation of the next correct nucleotide beyond the artificial base. The data in Table 1 demonstrate the striking ability of *Taq* DNA polymerase toward primer extension beyond the artificial nucleobases **1** and **2** with the V_{max}/K_m similar to the values for canonical Watson−Crick base pair (Supporting Information, Table S2). In comparison, the observed value for extension beyond G:T mismatch is about 600-fold lower than that of the natural match incorporation (Table 1 and Supporting Information). Similarly, the V_{max}/K_m value was more than 7000-fold lower for an A:A mismatch. Most importantly, there is less than 2-fold discrimination in the case of compound **1** and less than 3-fold in case of compound **2** for extension beyond all four nonstandard opposite natural nucleobase pairs (Table 1). Also, when the reactions were carried out in the presence of all four canonical triphosphates, the incorporation was not limited to single nucleotide incorporation but yielded full-length product (29-nt) (Supporting Information Figure S2).

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These results indicate that **1** and **2** are recognized without significant discrimination during incorporation after the nonstandard base pairs, and that the presence of oxadiazole base does not perturb the interactions at the enzyme active site as it translocates beyond the artificial base. Extension with all four canonical bases in the gel-based assay shows the insignificant level of stalling at the sites distal to the unnatural base (Supporting Information). This is in contrast to the natural mismatch extensions with Pol I family DNA polymerases, where the rates are affected for up to four bases beyond the lesion.[15] Structural studies on polymerase complex during mismatch extension indicate participation of electrostatic and steric forces that lead to stalling at and beyond the mismatch.[16,17] Oxadiazole nucleobase seems to satisfy these electrostatic contacts with the polymerase residues during translocation. At the same time, the relatively small size of these analogues facilitates unhindered extension. Interestingly, compound **3**, which is structurally similar to **1** and **2**, shows extension kinetics similar to that of the natural mismatches (Table 1).

The V_{max}/K_m values for incorporation of azole nucleobases are shown in Table S1 (Supporting Information) along with the data for natural base incorporations. The relative V_{max}/K_m values for the incorporation of all three analogues, **1**−**3**, are significantly lower as compared to the natural match incorporation. For example, the V_{max}/K_m for incorporation of **1**TP opposite T is more than 4000-fold lower than the value for dATP opposite T incorporation. These data also indicate that all three compounds favor incorporation opposite dG and T in the template strand as compared to dA and dC. Similar preferences are observed during the extension beyond compounds **1**−**3** (Table 1). The ability of *Taq* polymerase to selectively incorporate and extend beyond dG as compared to dA in the template strand clearly indicates the nonconformity of these data to the steric-fit model of base selection by DNA polymerases.[5]

Despite the common structural framework of oxadiazoles, **1** and **2**, and the triazole carboxamide nucleobase, **3**, the observed distinctions in the context of *Taq* DNA polymerase catalyzed incorporation and extension steps offer a new perspective on the mechanism of recognition. The distinct effects of heteroatom substitution in azole carboxamide nucleoside analogues (e.g., compound **3**) that leads to reduced extension rates may be due to the relatively larger size of these bases causing unfavorable steric interactions, destabilizing the duplex at the primer terminus and distortion of the geometry at the site of catalysis (Figure S3 and Table S2). The unique electronic properties of oxadiazoles lead to relatively smaller size and possible deviations from the classical base pair geometry, and/or protrusion into the major and minor grooves are minimal and well tolerated by the DNA polymerase (Table S2 and Figure S4). In accordance with our previous studies with a series of azole bases, it seems that the presence of heteroatoms at positions 2, 3, and 5 are probed by *Taq* DNA polymerase when the azole base is in the template strand.[6,18] These interactions may contribute to the overall geometry of the terminal base pair and a proper alignment of the terminal 3′ OH group for the chemistry to occur. A number of recent studies[19−21] also illustrated that, by mimicking the electrostatic and geometric features of the classical Watson−Crick base pair and inducing local conformation change in a phosphodiester backbone, a potentially mutagenic 8-oxoG·A base pair can be readily extended by some DNA polymerases. Similar conformational change could be brought by interactions between the α heteroatom (N or O) of the oxadiazole moiety with the phosphodiester linkage and/or the O4′ atom of the deoxyribose ring. Another feature of analogues **1** and **2** is the C-glycosidic linkage in contrast to the classical N-linkage in **3**; the consequences on DNA polymerase extension are not clear at this time.

Most importantly, a number of recent studies have described base analogues that are readily incorporated by DNA polymerases as substrate triphosphates but lead to chain termination.[22] Most of these analogues are hydrophobic bases that show enhanced stacking ability. These results in the context of the data presented here highlight that significantly different selection rules are utilized by DNA polymerases during the incorporation and extension steps to avoid mismatches, that is, stacking and/or hydrophobic interactions likely play a major role during the incorporation step in contrast to the steric and electrostatic interactions that contribute significantly to the selection during extension past the base pair.

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Supporting Information Available: Experimental data including kinetics of incorporation, electrophoresis gels, and molecular calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Henry, A. A.; Olsen, A. G.; Matsuda, S.; Yu, C.; Geierstanger, B. H.; Romesberg, F. E. *J. Am. Chem. Soc.* **2004**, *126*, 6923−6931.
(2) Morales, J.; Kool, E. *J. Am. Chem. Soc.* **1999**, *121*, 2323−2324.
(3) Matsuda, S.; Henry, A. A.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2003**, *125*, 6134−6139.
(4) Henry, A. A.; Yu, C.; Romesberg, F. E. *J. Am. Chem. Soc.* **2003**, *125*, 9638−9646.
(5) Kool, E. T. *Annu. Rev. Biochem.* **2002**, *71*, 191−219.
(6) Paul, N.; Nashine, V. C.; Hoops, G.; Zhang, P.; Zhou, J.; Bergstrom, D. E.; Davisson, V. J. *Chem. Biol.* **2003**, *10*, 815−825.
(7) Henry, A. A.; Romesberg, F. E. *Curr. Opin. Chem. Biol.* **2003**, *7*, 727−733.
(8) McMinn, D.; Ogawa, A.; Wu, Y.; Liu, J.; Schultz, P.; Romesburg, F. *J. Am. Chem. Soc.* **1999**, *121*, 11585−11586.
(9) Zaccolo, M.; Williams, D. M.; Brown, D. M.; Gherardi, E. *J. Mol. Biol.* **1996**, *255*, 589−603.
(10) Strobel, H.; Dugue, L.; Marliere, P.; Pochet, S. *Nucleic Acids Res.* **2002**, *30*, 1869−1878.
(11) Tae, E. L.; Wu, Y.; Xia, G.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2001**, *123*, 7439−7440.
(12) Wu, W.; Bergstrom, D. E.; Davisson, V. J. *J. Org. Chem.* **2003**, *68*, 3860−3865.
(13) Adelfinskaya, O.; Davisson, V. J.; Bergstrom, D. E. *Nucleosides Nucleotides*, in press.
(14) Hanes, J. W.; Johnson, K. A. *Anal. Biochem.* **2005**, *340*, 35−40.
(15) Miller, H.; Grollman, A. P. *Biochemistry* **1997**, *36*, 15336−15342.
(16) Johnson, S. J.; Beese, L. S. *Cell* **2004**, *116*, 803−816.
(17) Johnson, S. J.; Taylor, J. S.; Beese, L. S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3895−3900.
(18) Hoops, G. C.; Zhang, P.; Johnson, W. T.; Paul, N.; Bergstrom, D. E.; Davisson, V. J. *Nucleic Acids Res.* **1997**, *25*, 4866−4871.
(19) Hsu, G. W.; Ober, M.; Carell, T.; Beese, L. S. *Nature* **2004**, *431*, 217−221.
(20) Krahn, J. M.; Beard, W. A.; Miller, H.; Grollman, A. P.; Wilson, H. R. *Structure* **2003**, *11*, 121−127.
(21) Brieba, L. G.; Eichman, B. F.; Kokoska, R. J.; Doublie, S.; Kunkel, T. A.; Ellenberger, T. *EMBO J.* **2004**, *23*, 3452−3461.
(22) Reineks, E. Z.; Berdis, A. J. *Biochemistry* **2004**, *43*, 393−404.

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