Adenine Release Is Fast in MutY-catalyzed Hydrolysis of G:A and 8-Oxo-G:A DNA Mismatches*

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MutY, a DNA repair enzyme, is unusual in that it binds exceedingly tightly to its products after the chemical steps of catalysis. Until now it was not known whether the product being released in the rate-limiting step was DNA, adenine, or both. MutY hydrolyzes adenine from 8-oxo-G:A (OG:A) base pair mismatches as the first step in the base excision repair pathway, as well as from G:A mismatches. The products are adenine and DNA containing an apurinic (AP) site. Tight product binding may have a physiological role in preventing further damage at the OG:AP site. We developed a rate assay using [8-¹⁴C]adenine in OG:A or G:A mismatches that distinguishes between adenine hydrolysis and adenine release. [8-¹⁴C]Adenine was released quickly from the MutY-AP-DNA-[8-¹⁴C]adenine complex, with a rate constant greater than 5 min⁻¹. This was much faster than the rate-limiting step, at 0.006–0.015 min⁻¹. Gel retardation experiments showed that AP-DNA release was very slow, consistent with it being the rate-limiting step. Thus, the kinetic mechanism involves fast adenine release after hydrolysis followed by rate-limiting AP-DNA release. Adenine appears to be buried deep in the protein-DNA interface, but there is enough flexibility or open space for it to dissociate from the MutY-AP-DNA adenine complex. These results have implications for the catalytic mechanism of MutY.

Unlike “normal” enzymes, MutY releases its products exceedingly slowly after the chemical steps of catalysis are complete. MutY is a DNA repair enzyme that recognizes 8-oxo-G:A (OG:A) base pair mismatches in the first step of the base excision repair pathway (1, 2) (Fig. 1). OG residues are one of the most common forms of oxidative DNA damage (3). If OG-containing DNA is replicated, A is incorporated with a 200-fold preference over C. If this OG:A mismatch is not repaired, a transition state or intermediates (1) with a positive charge on the sugar ring (15, 16), and they are often susceptible to inhibition by imino sugars such as 2. However, (OG:2)-DNA binding to MutY is no better than (OG:AP)-DNA, whereas an adenine ring mimic in (OG:3)-DNA improved binding by more than 65-fold (17, 18). This raises the possibility of a catalytic mechanism similar to purine nucleoside phosphorylase (PNP), which is believed to bind tightly with the leaving group and nucleophile but has little interaction with the ribose ring (19). Similarly, MutY could form tight interactions with adenine during catalysis and/or in the product complex. An activity assay was developed to detect free [8-¹⁴C]adenine, allowing us to distinguish between the kinetics of hydrolysis and adenine release.

Experimental Procedures
MutY—MutY was prepared (7) and the active site concentration was determined (6) using an overexpressing Escherichia coli strain supplied by Prof. Sheila David (University of Utah). Protein concentration was determined (6) using an overexpressing E. coli strain supplied by Prof. Sheila David (University of Utah). Protein concentration was determined by using A₂₈₀ using ε₂₈₀ = 77,400 M⁻¹ cm⁻¹ as determined by the method of Edelhoch (20).
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Unlabeled DNA Oligonucleotides—Oligonucleotides were synthesized at the Institute for Molecular Biology and Biotechnology (McMaster University). 5'-Oxy-5'-dethiophenyl-N2-isobutyl-2'-deoxyguanosine, 5'-[2-(cyanoethyl)-N,N-diisopropyl]-phosphoramidite (Glen Research) was used for the incorporation of OG. Oligonucleotides °(14A) residue was synthesized enzymatically using Moloney murine DNA duplexes were annealed as described above, except in 20 mM HCl, pH 7.6, 12.5 mM KCl, 10 mM EDTA and 1 M M MLV reverse transcriptase for 13 h at 37 °C and then concentrated under vacuum to 60 μl, and DNA was precipitated with an equal volume of isopropanol. The nucleotide composition of the 14A-DNA strand was determined (22), and it matched the expected values (data not shown). Overall radioactivity yields were 35–50%.

14A-DNA—A DNA strand with a centrally located 14A (14A) residue was synthesized enzymatically using Moloney murine leukemia virus (MLV) reverse transcriptase (Invitrogen) (Fig. 2). Primers (20 μM) and 20 μM [14C]dATP (500,000 cpm) were incubated in 230 μl of 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl2, 10 μM dithiothreitol, 200 μM TTP, 200 μM GTP, 200 μM dCTP, and 1150 U M-MLV reverse transcriptase for 13 h at 37 °C and then concentrated under vacuum to 60 μl, and DNA was precipitated with an equal volume of isopropanol. The nucleotide composition of the 14A-DNA strand was determined (22), and it matched the expected values (data not shown). Overall radioactivity yields were 35–50%.

14A-DNA Oligonucleotide—A DNA strand was 5'-32P-phosphorylated using γ-[32P]ATP (Amersham Biosciences) and T4 polynucleotide kinase (Invitrogen) (6).

Duplex Substrate DNAs—14A-DNA or 33A-DNA (4 μl) was annealed with equal amounts of OG-DNA or G-DNA in 150 μl of 20 mM Tris-HCl, pH 7.6, 150 mM KCl, and 10 mM EDTA at 90 °C for 5 min and then cooled to 25 °C over 12 h.

2P-Labeled and Unlabeled (OGAP)-DNA—(OG-33A)-DNA or (OG)-DNA duplexes were annealed as described above, except in 20 mM Tris-HCl, pH 7.6, 12.5 mM KCl, 10 mM EDTA, and 1 μl oligonucleotide for 32P incorporation. After annealing, 0.1 mg/ml bovine serum albumin was added followed by a 2-fold excess of MutY, and the reaction was incubated at 37 °C for 10 min before being extracted three times with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) to remove MutY. All reactions (2.5 ml) were collected, mixed with 18 ml of scintillation fluid (Liquiscint, National Diagnostics), and 14C was counted. Free [8-14C]adenine concentration was determined from 14C in the low and high molecular weight peaks (Equation 1),

\[
\text{[8-14C]adenine}_{low} = \left(\text{[14A]} - 30\right) \times \left(\frac{14\text{C}_{\text{low, M}}}{{14}\text{C}_{\text{low, M}} + {14}\text{C}_{\text{high, M}}}\right)
\]

where [8-14C]adenine}_{low} is the concentration of free [8-14C]adenine at time = 0, [14A] is the initial substrate DNA concentration, and 14C_{low, M} and 14C_{high, M} are the amounts of 14C (in cpm) eluted in the low molecular weight and high molecular weight peaks, respectively.

No MutY and excess MutY (1.76 μl) reactions were used to determine [8-14C]adenine present in substrate DNA, and any unreactive substrate DNA and [8-14C]adenine_{low} were corrected.

Rate constants and apparent [MutY] were derived by fitting the experimental data to Equation 2.

\[
[\text{PI}] = \frac{[\text{MutY}] (1 - e^{k_{s2} \cdot t})}{k_{s2}}
\]

where [PI] is product concentration at time = t, k_{s2} is the burst phase rate constant, and k_{s2} is the steady state rate constant, equal to k_{s2}[MutY], where k_{s2} is the rate of product release (Fig. 3) (6).

Chromatographic Resolution—The adenine release rate calculation was based in part on the time needed for high molecular weight species (DNA and protein) to be separated from adenine on the gel filtration column. Chromatographic resolution, R_s, is given by Equation 3,

\[
R_s = \frac{t_{1/2}}{t_{max}}
\]

where t_{1/2} is the difference in retention time between two peaks, and t_{max} is the average width of the two peaks (25). Two peaks are fully resolved when R_s > 1.5, with <0.1% overlap.

The intention was to separate adenine from DNA based on size, but it adsorbed on the gel resin, leading to an elution volume of 19 ml for a 9.9 ml bed volume (Fig. 4) and better than expected resolution. A 50–μl test injection containing 2.5 μg of denatured herring sperm DNA and 3.5 μg of adenine was monitored by A_260 and gave R_s = 3.8 (Fig. 4c). The UV chromatogram matched the peak position and shape of 14C elution from an acid-quenched reaction with 15-s fractions. The rate of peak travel and widening was simulated numerically based on chromatogram. These values were used to calculate peak travel, peak widths, and R_s within the gel filtration column at increasing times throughout the separation. R_s was also measured for test runs with injection volumes from 50 to 2000 μl. The numerical simulations agreed well with experimental values of R_s, lending support for their accuracy.

DNA Product Release Assay—Pulse-chase experiments were used to detect transient dissociation of MutY-AP-DNA complexes during gel filtration. 32P-DNAs were reacted with MutY and then chased with unlabeled AP-DNAs followed by gel retardation PAGE (24). Reactions were as described above, with 200 nM (OG-33A)-DNA incubated with 250 nM MutY for 30 s. A 20–50 μl aliquot was then added to 400 μl of (OG:AP)-DNA. The mixture was chromatographed as described above but on a 5-ml HiTrap Desalting column (G-25 Sephadex Superfine, Amersham Biosciences) to reduce sample dilution. Loading buffer (20 μl, 200 mM Tris, pH 7.6, 200 mM KCl, 100 mM EDTA, 0.25% bromphenol blue) was added to the fraction with the highest [DNA] (the 10th 200–500 μl). The numerical simulations agreed well with experimental values of R_s, lending support for their accuracy.

RESULTS

Adenine Hydrolysis/Release Assays—Previous MutY assays took advantage of facile β-elimination at the AP site to cleave the DNA backbone at 90 °C in 0.2 M NaOH. This allowed separation and quantitation of the resulting fragments by PAGE. An assay was developed in this study to report directly on the adenine product by using gel filtration chromatography to separate [8-14C]adenine from 14A-DNA substrate (Fig. 4). Unquenched reactions were analyzed under non-denaturing conditions, with samples cooled rapidly in an ice-cold syringe and injected immediately onto the gel filtration column. If the MutY-AP-DNA-[8-14C]adenine complex were present, it would co-elute with 14A-DNA in the high molecular weight peak. In acid-quenched reactions, MutY was denatured, releasing all of the [8-14C]adenine into solution. Reactions contained 110 nM MutY and 200 nM 14A-DNA. At the end of the pre-steady state burst, roughly half of the substrate had been hydrolyzed. If adenine release were slow, most or all of the adenine would have been trapped in the MutY-AP-DNA-[8-14C]adenine com-
plex in the unquenched reactions, and free [8-14C]adenine would have been lower than in the acid-quenched reactions. There was no detectable difference between the acid-quenched and unquenched reactions (Fig. 5, Table I). That is, the free [8-14C]adenine concentration in solution was equal to total [8-14C]adenine, indicating that adenine was released quickly from the MutY active site after the chemical steps of catalysis.

The only source of 14C in these experiments was [8-14C]A residues in the substrate, which were converted to [8-14C]adenine upon hydrolysis. No detectable 33P co-eluted with adenine (Fig. 4a), demonstrating that the 14C detected with 14A-DNA substrates was truly from free [8-14C]adenine and not a chromatographic artifact. The small 33P peak at t = 3.5 min corresponded to free [33P]phosphate, which co-eluted with other nonadsorbed low molecular weight species.

For the (OG:14A)-DNA reactions, the burst phase was essentially complete in the first time points, and thus the fitted values of k_{+3} were not considered reliable even though they were in good agreement with literature values. The magnitude of the (OG:14A)-DNA burst phase, 10^9 M, agreed with the value expected from active site titration, 110 nM. [MutY] values with (G:14A)-DNA were slightly higher but still within 15% of the active site titration.

MutY/AP-DNA Release—With adenine release being fast, an important question became whether it is released directly from the MutY/AP-DNA adenine complex or whether transient release and rebinding of AP-DNA allows adenine release. Pulse-chase experiments and gel retardation PAGE (24) were used to examine the high molecular weight peak from gel filtration. Gel retardation exploits the change in DNA mobility on nondenaturing PAGE when complexed with protein and has been used with MutY to probe DNA binding (9, 11–13).

(OG:14A)- or (G:14A)-DNA was reacted with MutY, and then excess unlabeled (OG:AP)-DNA was added immediately before gel filtration chromatography. If 33AP-DNA dissociated from MutY during chromatography, unlabeled (OG:AP)-DNA would out-compete it to rebind MutY, resulting in displacement of 33AP-DNA. The small proportion of free 33AP-DNA (Fig. 6a, Table II) demonstrated that most of the product DNA did not dissociate even once from MutY during the 9 min between the start of the reaction and separation on gel retardation PAGE. The fraction of free 33AP-DNA was in excellent agreement with that calculated from k_{-3} and was far from the equilibrium value expected if there was dissociation/rebinding of AP-DNA.
The complementary experiment with unlabeled substrate DNA and an (OG:33AP)-DNA chase gave the same result, with only a small fraction of labeled 33P appearing in the MutY/H18528 AP-DNA complex (Fig. 6b, Table II).

Adenine Release Rate—The adenine release rate was estimated from the chromatographic data. The acid- and unquenched reactions were essentially identical for both (OG:A)- and (G:A)-DNA, arguing that adenine release was complete on the time scale of the assay. For short reaction times, the time on the gel filtration column was longer than the reaction time, and adenine release could have occurred during chromatography. If this were the case, the high molecular weight peak would be normal, but the adenine peak would have a long leading edge due to adenine being transported partway down the column in the MutY/H18528 AP-DNA/adenine complex before release. Also, the peak would be smaller for the unquenched reaction. There was no detectable 14C eluted between the high molecular weight peak and adenine (Fig. 4b), and [8-14C]-adenine free was the same for acid-quenched and unquenched reactions (Fig. 5). Thus, most or all adenine release occurred before chromatographic separation.

The shortest elapsed time between the start of reaction and separation on the gel filtration column was 23 s, comprising 7 s reaction time, 5–7 s to withdraw an aliquot into an ice-cold syringe and inject it, and 7.8 s for the sample to reach the top of the resin, based on the internal volume of the tubing and fittings. Once on the resin, numerical simulations showed that 1.0 s was required to separate [8-14C]adenine from 14A-DNA and MutY-AP-DNA/8-14C]adenine. Assuming that 23 s represented at least 3 half-lives of adenine release (87.5% released), then $t_{1/2} = 8$ s and $k_{32} = 0.09$ s$^{-1}$ or 5 min$^{-1}$ (Fig. 3).

**DISCUSSION**

**Reaction Rates and Adenine Release**—The rate constants $k_{12}$ and $k_{32}$ determined in this study were very similar to those

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**Table I**

| Substrate | Treatment | [MutY] | $k_{12}$ | $k_{32}$ | $k_{33}$ |
|-----------|-----------|--------|---------|---------|---------|
| OG:14A    | Acid-quenched | 109 ± 3 | 0.7 ± 0.2 | 0.007 ± 0.002 |
|           | Unquenched  | 109 ± 2 | 0.6 ± 0.2 | 0.006 ± 0.002 |
| G:14A     | Acid-quenched | 126 ± 8 | 1.4 ± 0.2 | 0.012 ± 0.004 |
|           | Unquenched  | 118 ± 6 | 2.2 ± 0.2 | 0.015 ± 0.003 |

**Fig. 5.** Adenine hydrolysis/release with (G:14A)-DNA (a) and (OG:14A)-DNA (b) substrates. [8-14C]Adenine$\text{free}$ versus time is shown for reaction times of up to 2 or 60 min (insets). Curves were fitted to Equation 2. G, dashed line, acid-quenched reaction; C, solid line, unquenched reaction; dotted line, expected curve if adenine release were rate-limiting. [MutY] = 110 nM; [DNA] = 200 nM.

**Fig. 6.** AP-DNA release. a, 200 nM (G:32A)- or (OG:32A)-DNAs were incubated with 250 nM MutY, chased with 400 nM (OG:AP)-DNA, and chromatographed before electrophoresis. b, same as a but with 200 nM (G:A)- or (OG:A)-DNA, 150 nM MutY, and 50 nM (OG:33AP)-DNA chase. Lanes 1, 3, 5, and 7, (OG:32AP)-DNA.
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TABLE II

| Substrate | Chase molecule | Elapsed timea | Fraction of free [33P]DNAb | Expected fraction of free [33P]DNAc | Fraction of free [33P]DNA at equilibriumd |
|-----------|----------------|--------------|-----------------------------|---------------------------------|----------------------------------------|
| G:32A    | OG:AP          | 9            | 0.16                        | 0.12                            | 0.58                                    |
| G:A      | OG:32AP        | 11           | 0.83                        | 0.86                            | 0.40                                    |
| OG:32A   | OG:AP          | 9            | 0.07                        | 0.05                            | 0.58                                    |
| G:A      | OG:32AP        | 10           | 0.91                        | 0.94                            | 0.40                                    |

* From the start of the reaction through gel filtration until entire sample had entered the polyacrylamide gel.

+ From Fig. 6, lanes 2, 4, 6, 8.

+ Calculated from $k_{-2}$ for (G:AP)-DNA and (OG:AP)-DNA (Table I), assuming release was irreversible.

+ Fraction of free [33P]DNA if unlabeled and labeled AP-DNA were fully equilibrated.

+ No loading buffer (see text).

Reported previously, where $k_{+2} = 14–15 \text{ min}^{-1}$ (OG:A) and 1.3–2.5 min$^{-1}$ (G:A), and $k_{-3} = 0.002–0.005 \text{ min}^{-1}$ (OG:A) and 0.02–0.03 min$^{-1}$ (G:A) (6–10). There was no sequence similarity between the DNAs used here and in previous studies, showing that MutY is generally insensitive to the sequence context of the mismatch.

Acid-quenched and unquenched reactions were identical within experimental error for both (OG:14A)- and (G:14A)-DNA, indicating that adenine release was complete on the time scale of these experiments. If adenine release could only occur after rate-limiting AP-DNA release, there would have been a lag in its appearance followed by release at the steady state rate (Fig. 5, dotted line, insets). The estimated lower limit for the adenine release rate constant, $k_{-3} \leq 5 \text{ min}^{-1}$, was faster than the chemical step, $k_{-3} \approx 2 \text{ min}^{-1}$, for (G:14A)-DNA. For (OG:A)-DNA $k_{-3} \approx 15 \text{ min}^{-1}$, and so it was not possible to determine which was faster in this case, the chemical step or adenine release. Adenine release was 1000- and 300-fold faster than $k_{-3}$ for (OG:AP)- or (G:AP)-DNA, respectively.

Consequences of Fast Adenine Release—Fast adenine release has several important consequences for understanding MutY. First, in the kinetic mechanism (Fig. 3), the lower pathway predominates with $k_{-2} \approx 5 \text{ min}^{-1}$, and therefore, presumably, $k_{-3} \approx 0.015 \text{ min}^{-1}$ for G:A and 0.006 min$^{-1}$ for OG:A.

Second, contrary to appearances in the MutY-adenine co-crystal structure (Fig. 7), adenine is not trapped in a deep pocket by bound AP-DNA. This implies either structural flexibility in the complex and/or that a solvent-accessible route remains open in the MutY-AP-DNA-adenine complex.

Third, adenine does not contribute to the observed affinity of MutY for AP-DNA. The previously determined values of $K_d$ for (OG:AP)-DNA (50 to 120 pm) and (G:AP)-DNA (2 to 21 nm) were measured under conditions in which adenine would have been released and therefore reflect the equilibrium dissociation constants for AP-DNA only (11–13).

The fact that $K_d$ reflects AP-DNA binding means that the presence of an oxocarbenium ion mimic in the MutY inhibitor (OG:2)-DNA, $K_d = 65 \text{ pm}$, does not contribute to binding. Iminosugars such as 2 and deoxyoxorimycin are oxocarbenium ion (1) mimics that are glycosylase inhibitors (25, 26). The lack of effect with MutY could reflect a catalytic mechanism similar to PNP. PNP catalysis is believed to involve tight binding of the leaving group and phosphate, the nucleophile, but relatively little interaction with the ribose ring (19). PNP can transiently bind the hypoxanthine product very tightly, $K_d = 1 \text{ pm}$ (27).

Inamoribitol (4), an analog of 2, is a poor inhibitor of M. tuberculosis PNP, $K_d = 2.9 \text{ mm}$, whereas immucillin-H (5), containing an analog of the leaving group, is potent, with $K_d = 28 \text{ pm}$ (28, 29). The fact that (OG:3)-DNA binds to MutY > 65-fold more tightly than (OG:2)-DNA indicates a role for adenine ring binding. Two other nucleic acid N-glycosylases, ricin (30) and uracil DNA glycosylase (31), show improved binding of oxocarbenium ion-mimic inhibitors in the presence of their base products, adenine or uracil.

Rapid adenine release from MutY-AP-DNA does not, of course, preclude tight binding in the transition state. Tight transition state binding could be catalytically important, with those interactions lost in the product complex. Also, rapid adenine release does not rule out significant affinity of MutY for adenine. With a rate constant of $\approx 5 \text{ min}^{-1}$ for adenine release, a $K_d$ as low as 1 nM is possible, assuming diffusion rate-limited association ($10^6 \text{ m}^{-1} \text{ s}^{-1}$). Transition state analysis of MutY will elucidate adenine binding at the transition state.

Conclusions—A new activity assay for MutY-based separation of free [8-14C]adenine from 14C-labeled DNA and MutY-AP-DNA-[8-14C]adenine was used to examine adenine release. Adenine release was fast for both (OG:AP)- and (G:AP)-DNA substrates and was faster than hydrolysis in the case of G:A. Gel retardation PAGE demonstrated that AP-DNA release was much slower, consistent with its being the rate-limiting step. The high affinity of MutY for (OG:AP)- and (G:AP)-DNA was not due to tight binding of the product complex, but rather to slow product release, consistent with the catalytically significant role of adenine release.
AP-DNA is due to DNA binding alone, and contrary to the appearances of the MutY/H18528 adenine crystal structure, there is enough flexibility or open space for adenine to dissociate from the MutY/AP-DNA/adenine complex.

Acknowledgments—We thank Prof. Sheila David (University of Utah) for enlightening discussions and for generously providing purified MutY, Prof. Joanne Stubbe (MIT) for the strain that overexpresses ribonucleotide reductase, as well as Karim Soliman, Alex Avila, and Bryan Davies for assistance in the early parts of this study.

REFERENCES
1. Au, K. G., Clark, S., Miller, J. H., and Modrich, P. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8977–8981
2. David, S. S., and Williams, S. D. (1998) Chem. Rev. 98, 1221–1261
3. Grollman, A. P., and Moriya, M. (1993) Trends Genet. 9, 246–249
4. Shibutani, S., Takeshita, M., and Grollman, A. P. (1991) Nature 349, 431–434
5. Michaels, M. L., Tchou, J., Grollman, A. P., and Miller, J. H. (1992) Biochemistry 31, 10964–10968
6. Porello, S. L., Leyes, A. E., and David, S. S. (1998) Biochemistry 37, 14756–14764
7. Chmiel, N. H., Golinelli, M. P., Francis, A. W., and David, S. S. (2001) Nucleic Acids Res. 29, 553–564
8. Bernards, A. S., Miller, J. K., Bao, K. K., and Wong, I. (2002) J. Biol. Chem. 277, 29960–29964
9. Noll, D. M., Gogos, A., Granek, J. A., and Clarke, N. D. (1999) Biochemistry 38, 6374–6379
10. Chmiel, N. H., Livingston, A. L., and David, S. S. (2003) J. Mol. Biol. 327, 431–443
11. Wright, P. M., Yu, J., Cillo, J., and Lu, A. L. (1999) J. Biol. Chem. 274, 29011–29018
12. Chepanoske, C. L., Porello, S. L., Fujitara, T., Sugiyama, H., and David, S. S. (1999) Nucleic Acids Res. 27, 3197–3204
13. Lu, A. L., Tsai-Wu, J. J., and Cillo, J. (1995) J. Biol. Chem. 270, 23582–23588
14. Guan, Y., Manuel, R. C., Arvai, A. S., Parikh, S. S., Mol, C. D., Miller, J. H., Lloyd, S., and Tainer, J. A. (1998) Nat. Struct. Biol. 5, 1058–1064
15. Berti, P. J., and Tanaka, K. S. E. (2002) Adv. Phys. Org. Chem. 37, 223–234
16. Schramm, V. L. (1999) Methods Enzymol. 308, 301–355
17. Deng, L., Scharer, O. D., and Verdine, G. L. (1997) J. Am. Chem. Soc. 119, 7865–7866
18. Scharer, O. D., Nash, H. M., Jirinj, J., Laval, J., and Verdine, G. L. (1998) J. Biol. Chem. 273, 8592–8597
19. Fedorov, A., Shi, W., Kicska, G., Fedorov, E., Tyler, P. C., Furneaux, R. H., Hanson, J. C., Gainsford, G. J., Larese, J. Z., Schramm, V. L., and Almo, S. C. (2001) Biochemistry 40, 853–860
20. Pace, C. N., Vajdos, F., Fee, L., Grimsley, G., and Gray, T. (1995) Protein Sci. 4, 2411–2423
21. Chen, X.-Y., Berti, P. J., and Schramm, V. L. (2000) J. Am. Chem. Soc. 122, 6527–6534
22. Connolly, B. A. (1997) in Oligonucleotides and Analogues: A Practical Approach. (Eckstein, F., ed) pp. 179–181, IRL Press, Oxford
23. Harris, D. C. (1995) Quantitative Chemical Analysis, pp. 635–636, W. H. Freeman, New York
24. Carey, J. (1991) Methods Enzymol. 208, 103–117
25. Lilledund, V. H., Jensen, H. H., Liang, X., and Bls, M. (2002) Chem. Rev. 102, 515–533
26. Legler, G. (1990) Adv. Carbohydr. Chem. Biochem. 48, 319–384
27. Kline, P. C., and Schramm, V. L. (1992) Biochemistry 31, 5964–5973
28. Miles, R. W., Tyler, P. C., Furneaux, R. H., Bagdassarian, C. K., and Schramm, V. L. (1998) Biochemistry 37, 8615–8621
29. Basso, L. A., Santos, D. S., Shi, W., Furneaux, R. H., Tyler, P. C., Schramm, V. L., and Blanchard, J. S. (2001) Biochemistry 40, 8196–8203
30. Tanaka, K. S. E., Chen, X.-Y., Ichikawa, Y., Tyler, P. C., Furneaux, R. H., and Schramm, V. L. (2001) Biochemistry 40, 8645–8651
31. Jiang, Y. L., Ichikawa, Y., and Stivers, J. T. (2002) Biochemistry 41, 7116–7124
