The Role of Base Flipping in Damage Recognition and Catalysis by T4 Endonuclease V*

Amanda K. McCullough, M. L. Dodson, Orlando D. Schärer‡§, and R. Stephen Lloyd¶

From the Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-1071 and the ¶Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

The process of moving a DNA base extrahelical (base flipping) has been shown in the co-crystal structure of a UV-induced pyrimidine dimer-specific glycosylase, T4 endonuclease V, with its substrate DNA. Compared with other enzymes known to use base flipping, endonuclease V is unique in that it moves the base opposite the target site extrahelical, rather than moving the target base itself. Utilizing substrate analogs and catalytically inactive mutants of T4 endonuclease V, this study investigates the discrete steps involved in damage recognition by this DNA repair enzyme. Specifically, fluorescence spectroscopy analysis shows that fluorescence changes attributable to base flipping are specific for only the base directly opposite either abasic site analogs or the 5'-thymine of a pyrimidine dimer, and no changes are detected if the 2-aminopurine is moved opposite the 3'-thymine of the pyrimidine dimer. Interestingly, base flipping is not detectable with every specific binding event suggesting that damage recognition can be achieved without base flipping. Thus, base flipping does not add to the stability of the specific enzyme-DNA complex but rather induces a conformational change to facilitate catalysis at the appropriate target site. When used in conjunction with structural information, these types of analyses can yield detailed mechanistic models and critical amino acid residues for extrahelical base movement as a mode of damage recognition.

The initiating events in base excision repair are performed by a class of enzymes, DNA glycosylases. These damage-specific enzymes are responsible for recognizing and binding to damaged bases and catalyzing the cleavage of the N-C1'-glycosydic bond linking the damaged base to the sugar phosphate backbone. Glycosylases thus provide the specificity to base excision repair. The precise mechanism by which these enzymes discriminate between nontarget and target DNA bases is beginning to be elucidated for a few glycosylases due to insights from high resolution x-ray crystallographic structures (reviewed in Ref. 1). In particular, uracil DNA glycosylases and a catalytically inactive mutant of T4 endonuclease V have been co-crystallized with their product or substrate DNAs, respectively, and shown to move a base extrahelical (nucleotide), placing the base in a pocket within the enzyme.

The mechanism of base flipping is not limited to DNA repair enzymes. In fact, it appears to be a generalized mechanism for catalytic DNA binding proteins (reviewed in Refs. 2 and 3). The first evidence that an enzyme flips a base extrahelical was revealed in the crystal structure of a DNA cytosine 5-methyltransferase, HhaI, complexed with its substrate DNA, in which the target cytosine was flipped out of the DNA helix and into the active site pocket of the enzyme where the methylation reaction can occur (4). A similar methyltransferase, HaeIII, has also been shown to move a base extrahelical (5), suggesting that this may be a conserved mechanism for this class of enzymes. As mentioned above, the two co-crystal structures for the glycosylases demonstrate a base (nucleotide) is flipped extrahelically; however, the mode of base flipping between the two enzymes is quite different. Uracil DNA glycosylases, like the methyltransferases, flip out the target base uracil into a uracil-specific pocket, thus providing the basis for specificity (6–8). T4 endonuclease V is unique in that it flips out the base in the opposite strand from the target site, suggesting a related but distinct mode of recognition, relying on the contacts with the nondamaged strand and the conformation of the phosphate backbone (9).

The catalytic mechanism of T4 endonuclease V has been extensively studied (reviewed in Refs. 10 and 11). It is a highly specific glycosylase that cleaves the glycosydic bond of the 5'-pyrimidine of a cis-syn cyclobutane pyrimidine dimer, a major photoproduct induced in DNA by exposure to ultraviolet light. This glycosylase also possesses a concomitant AP lyase activity, resulting in the production of a 3'-α,β-unsaturated aldehyde and a 5'-phosphate via a β-elimination reaction at the glycosylase generated abasic (AP) site. Biochemical and structural analyses have demonstrated the involvement of two key residues in the catalytic activity of the enzyme, Glu-23 and the N-terminal Thr-2 (12–18).

The co-crystal structure of a catalytically inactive mutant of T4 endonuclease V (E23Q) bound to thymine dimer-containing DNA has been solved (9). Interestingly, the structure revealed not only that the adenine base opposite the 5'-thymine of the pyrimidine dimer was flipped out of the DNA duplex and into a pocket on the protein surface but that the DNA substrate was sharply bent (60°) at the thymine dimer (9). Fig. 1 shows the overall structure of the co-crystal complex with the key active site residues and the extrahelical adenine indicated. Interestingly, the adenine in the pocket does not form hydrogen bonds with any protein residues but rather is held in place by weak van der Waals interactions (9). The thymine opposite the adenine remains within the helix although the base stacking interactions are disrupted. Thus, the enzyme appears to induce a conformational change in the DNA to allow the active site

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† Present address: Dept. of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands.

‡ To whom correspondence should be addressed: University of Texas Medical Branch, 5138 Medical Bldg., Bldg. 1071, Galveston, TX 77555-1071, Tel.: 409-772-2179; Fax: 409-772-1790.

§ The abbreviations used are: 2-Ap, 2 aminopurine; AP, abasic site; rAP, reduced abasic site; BSA, bovine serum albumin; bp, base pairs.

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residues access to the glycosidic bond to catalyze the reaction. Interesting questions arise from the structural data; at what step in the enzyme reaction does the extrahelical capture of the base occur? Is it simultaneous and/or necessary for specific binding? These questions can be addressed using the well-characterized T4 endonuclease V system that provides an opportunity for studying the pre-catalytic events involving specific damage recognition and the role of base flipping in establishing specificity.

In this study we utilize the fluorescent properties of the base analog, 2-aminopurine (2-Ap), to monitor the binding of the enzyme and, more importantly, the relative helical position of the 2-Ap when it is in complex with the T4 endonuclease V. The use of 2-Ap in examining local changes in DNA structure has been well documented for several enzyme systems (19–25). It is an effective fluorophore for probing enzyme-induced conformational changes in the DNA helix as it forms a Watson-Crick base pair with thymine (26), is sensitive to the surrounding environment (27), and is substantially quenched when present in duplex DNA as compared with single-stranded DNA (21), thus providing an ideal system to analyze local DNA structure.

By using modified oligonucleotides previously shown to form stable-specific complexes with T4 endonuclease V (28), and by replacing the adenine with 2-Ap placed directly opposite the 5'-thymine of a pyrimidine dimer. Furthermore, the process of base flipping, specific binding, and catalysis can be dissected using this technique in combination with various substrate analogs and catalytic mutants of the enzyme.

**EXPERIMENTAL PROCEDURES**

**T4 Endonuclease V—**T4 endonuclease V (wild type) was purified from *Escherichia coli* AB2480 (recA, uvrA) cells transformed with a den expression vector as described previously (29). E23Q endonuclease V mutants were created by site-directed mutagenesis and purified from *E.* coli (28), and its expression vector as described previously (29).

**Oligonucleotide Substrates—**The duplex DNAs that were used for this study are shown in Table I. DNA oligonucleotides containing a site-specific pyrrolidine residue or a reduced abasic site residue (rAP) were synthesized as described previously (30). Complementary sequences were synthesized using standard procedures, and the deprotected oligonucleotides were purified electrophoretically on 20% denaturing polyacrylamide gels. The 2-Ap phosphoramidite was purchased from Glen Research (Sterling, VA) and incorporated into the designated oligonucleotide sequences (Table I) following standard synthesis procedures. Complementary oligonucleotides were annealed by mixing the DNAs, heating to 75 °C, and slow cooling to room temperature. The non-2-Ap strand was present in 2-fold excess to ensure that the majority of the fluorophore was present in duplex DNA. The presence of duplex DNA was confirmed by electrophoresis through a native polyacrylamide gel.

The 49-mer containing a *cis*-syn cyclobutane thymine dimer (CS-49) was used as substrate for activity assays was prepared as follows: a 12-mer containing a thymine dimer (TT), GCCGGAATTAAG (a gift from J-S. Taylor, Washington University), was 5'-end-labeled (1:10 γ-32P-ATP: unlabeled ATP) and annealed to a complementary 37-mer. The 12-mer was extended to a 36-mer in a reaction containing 0.3 mM dNTPs, Sequenase (13 units, U. S. Biochemical Corp.), 40 mM Tris-HCl, pH 7.5, 20 mM MgCl2, and 50 mM NaCl incubated at 25 °C for 2 h, followed by the addition of another 13 units of Sequenase and 0.2 mM dNTPs and 2 mM 7-Methylguanine DNA methyltransferase.
incubation at 25 °C for another 24 h upon which gel analysis indicated the extension of the 12-mer to a 36-mer was essentially complete. A 13-mer was then ligated to this 36-mer annealed to the complementary 49-mer forming fully duplex 49-bp DNA. The resulting 49-mer was then purified by gel electrophoresis through a 15% denaturing polyacrylamide (8 m urea), and the oligonucleotides recovered were allowed to re-anneal. All oligonucleotide strands containing the base analogs, thymine dimer, and a control sequence were γ-32P-labeled on the 5'-end with T4 polynucleotide kinase (New England Biolabs) following standard procedures and annealed to their complementary strands as shown in Table I. The DNA concentrations were determined by measuring the absorbance at 260 nm.

**Enzyme Activity Assays**—For the 25-bp duplexes, 1.25 nM duplex, with the indicated strand labeled, was incubated with enzyme (6.25 nM) in a standard reaction buffer (25 mM sodium phosphate, pH 6.8, 100 μg/ml BSA, 100 mM KCl) in a total volume of 20 μl for 1 h at 25 °C. For the 12-bp DNAs, 6.24 nM duplex and 25 nM enzyme were incubated as described above. An equal volume of loading buffer (95% (v/v) formamide, 20 mM EDTA, 0.02% (w/v) bromophenol blue, 0.02% (w/v) xylene cyanol) was added, and the samples were heated to 90 °C for 5 min prior to loading on a 15% denaturing polyacrylamide gel (8 m urea) in 1 × TBE buffer (90 mM Tris borate, 2 mM EDTA, pH 8.0). The DNAs were separated by electrophoresis for 3 h at 800 V. Bands were visualized by autoradiography of the wet gels using Hyperfilm-MP x-ray film (Amersham Corp.).

**Fluorescence Assay**—Fluorescence changes induced by wild type endonuclease V were assayed using gel mobility shift analysis. The reactions contained 25 mM sodium phosphate, pH 6.8, 100 mM KCl, 5% glycerol, 100 μg/ml BSA, 2 mM (25 bp) or 0.5 mM (12 bp) DNA duplex, and 200 nM T4 endonuclease V in a total volume of 20 μl. Following a 30-min incubation at 25 °C, the free DNA and enzyme-bound DNA were separated by electrophoresis through a 7.5% (10% for 12 bp) native polyacrylamide gel in 0.5 × TBE buffer (45 mM Tris borate, 1 mM EDTA) for 2 h at 120 V. Bands were visualized by autoradiography of the wet gels using Hyperfilm-MP x-ray film (Amersham Corp.).

**Acrylamide Quenching Assays**—Quenching of the enhanced fluorescence signal produced upon endonuclease V binding to the pyrrolidine/9-Ap duplex was investigated using acrylamide as a quencher. The fluorescence emission scan for the DNA alone (200 nM in 25 mM sodium phosphate, pH 6.8, 100 mM KCl, in a total volume of 200 μl), enzyme was added at the indicated concentrations, mixed, allowed to bind for 5 min at 25 °C, and the emission scan observed from 340 to 420 nm. The reactions were held at 25 °C during the scan time (approximately 3 min).

**RESULTS**

**Binding and Activity of Endonuclease V on 2-Aminopurine-containing Duplexes**—DNAs were constructed for binding analysis of endonuclease V in which the adenine opposite the modification or target site was replaced with the 2-Ap fluorophore. To demonstrate the utility of the 2-Ap-containing duplexes for use in the fluorescence assay, we first examined if the enzyme binds specifically to these DNAs. Previously, it has been shown that endonuclease V binds with relatively high affinity to duplexes containing a site-specific pyrrolidine or rAP residue with an unmodified base in the opposite strand (28). Gel mobility shift analyses were used to demonstrate that duplexes containing a 2-Ap residue placed opposite these modified abasic sites are specifically bound by endonuclease V.

As shown in Fig. 2A, both wild type and mutant E23Q enzyme bind to the 2-Ap 25-bp duplexes (lanes 1–6) in a manner similar to the non-2-Ap duplex (lanes 7–9, and see Ref. 28). Fig. 2A also shows that E23Q binds to the 12-bp duplexes containing a thymine dimer with a 2-Ap opposite either the 5’- or 3’-thymine (lanes 10–13) but not to a control duplex containing a normal thymine opposite the 2-amino-purine base (lanes 14–15). The higher molecular weight bands seen in lanes 3, 6, and 9 upon E23Q binding have been observed previously under conditions of excess enzyme and determined to be due to more than one enzyme molecule bound to a DNA molecule (18). To ensure that the 2-Ap does not disrupt the catalytic activity of the enzyme on thymine dimer (TT)-containing DNA, wild type endonuclease V was incubated with TT/2-Ap-containing 12-bp duplex (Table I, part II), and the products were analyzed by denaturing gel electrophoresis. As shown in Fig. 2B, placing the 2-Ap residue opposite either the 5’- or 3’-thymine (lanes 1–3) or the 3’-thymine (lanes 4–6) of the TT did not affect catalysis by endonuclease V as evidenced by the complete conversion of the labeled 12-mer to the expected product at approximately the 7-mer position (lanes 3 and 6). No cleavage products were observed with the catalytically inactive E23Q enzyme and the same duplexes as expected (lanes 2 and 5). DNA not containing a dimer opposite 2-Ap was not processed by endonuclease V (lanes 7–9). These data establish the ability to use 2-Ap opposite a dimer as an appropriate substrate for endonuclease V.

The duplexes containing abasic site analogs and 2-Ap were incubated with wild type and mutant endonuclease V to ensure that these oligonucleotides were not cleaved by the enzymes which could generate misleading results in the fluorescence studies. As shown in Fig. 2, C and D, none of the 2-Ap/abasic site analog paired duplexes are substrates for endonuclease V. (Note: the light bands present in Fig. 2D, lanes 4–6, are background degradation of the DNA as evidenced by the appearance in lane 4, the no enzyme control; thus, this band is not a product band.) Thus, any enhancement in fluorescence will not be attributable to release of a base from the duplex or cleavage of the DNA backbone.

**Acrylamide Quenching Assays**—Quenching of the enhanced fluorescence signal produced upon endonuclease V binding to the pyrrolidine/2-Ap duplex was investigated using acrylamide as a quencher. The fluorescence emission scan for the DNA alone (200 nM in 25 mM sodium phosphate, pH 6.8, 100 mM KCl, in a total volume of 200 μl), with endonuclease V (200 nM), and both with the addition of increasing amounts of acrylamide (10, 20, 50, 100, 200, 250, 300 mM) were examined using the standard procedure described above. 200 mM single-stranded 2-Ap DNA was also incubated with and without acrylamide.

Activity assays of wild type endonuclease V in the presence of acrylamide on the CS-49 substrate were performed by incubating the DNA (1 nM) and endonuclease V (20 nM) in the standard reaction buffer for 30 min at 25 °C. Where indicated, acrylamide at the following concentrations, 10, 50, 100, 200, 300 mM was added to DNA prior to the addition of T4 endonuclease V. Preincubation of 400 mM acrylamide with the enzyme (20 μM) for 10 min at 25 °C prior to the addition of the DNA was also performed. The reaction products were separated through a 15% denaturing polyacrylamide (8 m urea) gel. Bands were visualized by autoradiography of the wet gels using Hyperfilm-MP x-ray film (Amersham Corp.). The appearance of the product band was quantitated using a Molecular Dynamics PhosphorImager and ImageQuant software (Sunnyvale, CA).
maxima are given in Table I and correspond well with previously published excitation maxima for 2-Ap in various duplex sequence and size contexts (21, 23, 24, 31). As several of the duplexes lacked a base opposite the fluorophore (pyrrolidine and reduced AP), the relative intensity of the fluorescence of these DNAs was higher than those with a base opposite the fluorophore, indicative of the quenching effects of a base paired to 2-Ap. The fluorescence signal of 2-Ap was quenched by duplex formation for the pyrrolidine/2-Ap duplex (Fig. 3A). Thus, even though there is no base pairing, the location of the 2-Ap in a double helical environment does quench the signal as compared with the single-stranded 2-Ap oligonucleotide.

Once the intrinsic fluorescence of the pyrrolidine/2-Ap DNA was determined (Fig. 3A), wild type endonuclease V was added to the reaction, and an emission scan was taken (Fig. 3, A and B). As shown in Fig. 3B, the increase in fluorescence intensity
occurred within seconds. The reaction was followed for 15 (Fig. 3B) and 30 min (data not shown), and after the initial enhancement, no significant increase in fluorescence was observed. Thus, all subsequent enzyme binding reactions were incubated for 5 min for consistency. This enhanced fluorescence was dependent on duplex DNA, since no increase was observed when endonuclease V was incubated with single-stranded 2-Ap DNA (Fig. 3A). This was expected as it has been shown previously that endonuclease V does not bind significantly to single-stranded DNA (32). The enhanced fluorescence was also dependent on DNA as no interaction was observed between free 2-Ap and endonuclease V (data not shown).

The intensity of the signal was dependent on the enzyme concentration, as increasing amounts of endonuclease V resulted in increased fluorescence of the pyrrolidine/2-Ap duplex (Fig. 4). Enhanced fluorescence was also observed when wild type endonuclease V was incubated with the reduced abasic site/2-Ap duplex (Fig. 4B). However, no increase in fluorescence was observed when a non-modified base (cytosine) was placed directly opposite the 2-Ap base (Fig. 4B). The enhanced fluorescence is thus directly associated with a specific binding event. The enhanced fluorescence observed for endonuclease V binding was typically 2–5-fold for the pyrrolidine/2-Ap duplex and 2–7-fold for the reduced AP/2Ap duplex. These fluctuations appear to be dependent on the preparation of the substrate and the relative enzyme activity.

As evidenced by the emission scans (Fig. 4A), there does not appear to be any spectral shift associated with endonuclease V binding to these duplexes. The increased fluorescence observed upon enzyme binding is more substantial than the fluorescence.
of the single-stranded 2-AP oligonucleotide (Fig. 3A), suggesting it is not just enzyme induced local unwinding of the helix resulting in a region of single-stranded DNA surrounding the 2-Ap (see below for discussion).

Correlation of Enhanced Fluorescence with the Extrahelical Base of the Co-crystal Structure of E23Q Bound to Dimer-containing DNA—To support further the idea that the enhanced fluorescence observed with the wild type enzyme on the non-cleavable substrates was indeed due to the 2-aminopurine being moved extrahelically by the enzyme, fluorescence studies were conducted using the E23Q enzyme and TT/2AP duplexes. The duplexes containing a site-specific thymine dimer with a 2-Ap base placed opposite either the 5’-thymine (5’ T/2-Ap; Table I, part IIa) or the 3’-thymine (3’ T/2-Ap; Table I, part IIb) showed similar excitation and emission scans. Likewise, the fluorescence of the 2-Ap base was quenched significantly upon duplex formation (Fig. 5A). Following an emission scan of the 5’ T/2-Ap at the optimum excitation wavelength, E23Q was titrated into the binding reaction at the indicated concentrations (Fig. 5B). As seen in the emission scans, a significant increase in the fluorescence was observed in a concentration-dependent manner upon E23Q binding to the duplex containing the 2-Ap directly opposite the 5’-thymine of the TT. This increase was typically 3–4-fold over the base intensity of the duplex DNA in the absence of enzyme. At high concentrations of E23Q, the enhanced fluorescence begins to be quenched (Fig. 5B, dotted lines) suggesting saturation of the specific binding sites. This is most likely due to the presence of multiple enzyme molecules bound to the DNA, which has been shown to occur on this substrate as evidenced by gel mobility shift analysis (Fig. 2A).

The most convincing evidence that the enhanced fluorescence upon endonuclease V binding is due to movement of a base extrahelically and not to general disturbances in the local helical structure is shown in Fig. 5C. The placement of the 2-Ap base opposite the 3’-thymine of the dimer (i.e. one base 5’ to the previous duplex, see Table I, part IIb) resulted in no apparent increase in fluorescence intensity of the duplex upon E23Q binding (Fig. 5C). As shown in Fig. 2A, this duplex is bound by the enzyme; thus, the enhanced fluorescence due to E23Q binding to TT/2AP DNA is highly specific for only the base that is predicted to be moved extrahelically based on the co-crystal structure. Binding of E23Q to a control duplex containing a normal thymine opposite the 2-Ap produced no increase in fluorescence intensity, indicating the enhanced fluorescence is due to a specific binding event and does not occur on nontarget DNA as detected by this method.

Interestingly, when the effect of E23Q binding to the pyrrolidine/2-Ap and the reduced AP/2-Ap duplexes was investigated, no increase in fluorescence was observed (Fig. 5C). This was not expected as E23Q binds to these substrates with relatively high affinity similar to wild type endonuclease V (Fig. 2A and Ref. 28), and the wild type enzyme does increase the fluorescence upon binding to these two duplexes (Fig. 4). Thus, despite the specific binding to the pyrrolidine and reduced AP site-containing duplexes, the E23Q does not appear to move the 2-Ap extrahelically in these duplexes. Thus, specific binding can be achieved without a stable base flipping event.

Quenching of the Enzyme-enhanced 2-Aminopurine Fluorescence—To assess the environment of the extrahelical 2-Ap, quenching studies were performed using acrylamide as the probe. The fluorescence emission scans were obtained for the pyrrolidine/2-Ap duplex, the single-stranded 2-Ap oligonucleotide, and the pyrrolidine/2-Ap with endonuclease V bound. Acrylamide was added at increasing concentrations as indicated, and emission scans were observed. The data were analyzed.
Base Flipping by T4 Endonuclease V

The movement of bases extrahelically as a means of promoting specificity for DNA repair enzymes is a process that is difficult to ascertain in the absence of x-ray crystallographic structures. This study has demonstrated that the movement of the base opposite the target site by a repair enzyme may be measured by fluorescence techniques. More significantly, the discrete steps along the pathway leading to catalysis, such as specific binding and base flipping, can be investigated by using a combination of modified oligonucleotides that form stable, pre-catalytic complexes representing intermediates along the reaction pathway. The evidence that the wild type enzyme exhibits base flipping on the pyrrolidine and reduced AP DNAs, both abasic site analogs, suggests that base flipping does occur for endonuclease V on both the abasic site target as well as the thymine dimer target. However, these data await confirmation by x-ray crystallographic studies on the pyrrolidine DNA-endonuclease V complex. These studies will provide evidence as to whether this base flipping event does occur when endonuclease V encounters abasic sites in the DNA (not associated with glycosylase action of the enzyme). It is interesting that the catalytic mutant E23Q, which does move the adenine opposite the thymine dimer extrahelically, is not capable of stably “trapping” the same base extrahelically, when it is placed opposite an abasic site analog. Interestingly, another catalytic mutant E23D also exhibits no detectable base flipping on the pyrrolidine/2Ap duplex (data not shown). However, due to the E23D mutant having general lower binding affinity to these substrates, it is difficult to determine if the lack of flipping is due to steric interference in the case of E23D, if the charge difference on E23Q has any effect on base flipping opposite an abasic site, or if subtle structural differences at the location of the mutant residues destabilize the extrahelical base conformation. A more detailed analysis is necessary to understand the differences between these catalytically inactive mutants which appear to exhibit some differences in base flipping as compared with the wild type enzyme.

The wild type endonuclease V binds tightly to both the pyrrolidine/2Ap and the reduced AP/2Ap and moves the opposing base extrahelically, and yet these are not catalytic substrates for the enzyme. Therefore, using these substrate analogs, it appears that base flipping may occur in the absence of catalysis. Base flipping by T4 endonuclease V is not sufficient for catalysis to occur. Likewise, using the E23Q catalytic mutant, we can observe wild type levels of binding to the pyrrolidine or reduced AP, yet no movement of the opposing base is detected. Thus, we can separate tight, specific binding from base flipping (i.e. base flipping does not result from all specific binding events), as well as base flipping from catalysis. This implies that base flipping does not add to the stability of the specific enzyme-DNA complex but rather induces a conformational change to facilitate catalysis at the appropriate target site. The fact that this assay cannot detect base flipping associated with nonspecific binding events is evidence that a stable extrahelical conformation is not maintained; however, this does not address the possibility that endonuclease V may use base flipping as a means of scanning nontarget DNA (34, 35), transiently moving bases extrahelically to probe for the target base. These transient species would not be detected using this method. Therefore, it is still plausible that although base flipping does not occur with every specific binding event on these substrate analogs, endonuclease V may still utilize this process as a means to discriminate target from nontarget DNA.

The quenching study reveals that the protein residues in close proximity to the flipped 2-Ap are not interfering with the ability of the relatively large acrylamide molecule to quench the enzyme-induced fluorescent enhancement. This approach may prove useful in revealing the microenvironment of the extrahelical base. These data taken together with the enhanced fluorescence and the x-ray crystallographic structures for T4 endonuclease V indicate that the fluorescence approach is indeed measuring a base flipping mechanism. We should point out, however, that caution should be taken when interpreting these types of fluorescence analyses in the absence of independent corroborating data. There is much evidence in the literature for enhanced 2-Ap fluorescence upon binding of enzymes...
which is not attributable to bases being moved out of the DNA helix. For example, changes in 2-AP fluorescence have been used to demonstrate helicase activity (21) and nucleotide incorporation and exonucleolytic removal of bases by polymerases (19). These studies give similar enhanced fluorescence signals as seen for T4 endonuclease V, yet when taken together with other biochemical and structural data, the mechanisms of the enhanced fluorescence are quite distinct.

In this regard, while these studies were in progress, Allan and Reich (25) presented data using a similar assay to study the EcoRI DNA methyltransferase binding to its target adenine. In their system, the enhanced fluorescence by the methyltransferase was much greater and accompanied by a blue spectral shift. This is interesting because it implies an altered yltransferase was much greater and accompanied by a blue shift. In their system, the enhanced fluorescence are quite distinct.

other biochemical and structural data, the mechanisms of the incorporation and exonucleolytic removal of bases by polymerases used to demonstrate helicase activity (21) and nucleotide incorporation, another repair enzyme which recognizes cyclobutane dimers, presents a unique opportunity for examining whether the DNA conformational change and the base movement occur for both DNA modifications to a similar extent and at a similar rate. Using these types of analyses, combined with structural information, detailed mechanistic models for extrahelical base movement as a mode of damage recognition and/or active site positioning used by DNA repair enzymes can be established.

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