Interaction of Deoxyinosine 3'-Endonuclease from Escherichia coli with DNA Containing Deoxyinosine*

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(Received for publication, April 24, 1995, and in revised form, August 16, 1995)

By using a band mobility shift assay, deoxyinosine 3'-endonuclease, an Escherichia coli enzyme which recognizes deoxyinosine, AP site, urea residue, and base mismatches in DNA, was shown to bind tightly to deoxyinosine-containing oligonucleotide duplexes. Two distinct protein-DNA complexes were observed, the faster migrating complex (complex I, \(K_d = 4 \times 10^{-9} M\)) contained one molecule of deoxyinosine 3'-endonuclease, while the slower migrating complex (complex II, \(K_d = 4 \times 10^{-7} M\)) contained two molecules of the protein bound to every molecule of duplex DNA. The endonucleolytic activity of deoxyinosine 3'-endonuclease paralleled the formation of the complex I. Interestingly, deoxyinosine 3'-endonuclease exhibited similar affinities for both the substrate and the nicked duplex product and thus remained bound to the DNA after the cleavage reaction. The formation of a stable complex required the presence of a duplex structure 5' to the deoxyinosine residue. DNase I footprinting revealed that deoxyinosine 3'-endonuclease protected 4–5 nucleotides 5' to the deoxyinosine, and when complex II was formed, at least 13 nucleotides 3' to deoxyinosine were protected. Based on these results, a model is proposed for the interaction of deoxyinosine 3'-endonuclease with DNA containing deoxyinosine.

Deoxyinosine in DNA can arise from deamination of deoxycytidine, which can be spontaneous or promoted by exposure of DNA to ionizing radiation, UV light, or nitrous acid (1–3). Deoxyinosine in DNA is premutagenic and can lead to A/T to C/G transition mutations (4). Deoxyinosine in DNA was thought to be removed by hypoxanthine DNA glycosylase, which has been partially purified from E. coli as well as other eu-karyotic sources (5–8). However, it was reported recently (9) that a well characterized repair enzyme, 3-methyladenine DNA glycosylase II, encoded by alkA gene in E. coli possesses hypoxanthine DNA glycosylase activity and releases hypoxanthine from deoxyinosine-containing DNA, suggesting that both 3-methyladenine DNA glycosylase and hypoxanthine DNA glycosylase activities reside in the same protein. It has also been shown that 3-methyladenine DNA glycosylase from human (ANPC protein), rat (APDG protein), and yeast (MAG protein) are able to excise hypoxanthine from DNA (9).

Recently, we identified and purified a novel deoxyinosine specific endonuclease from E. coli, deoxyinosine 3'-endonuclease (10). The enzyme hydrolyzes the second phosphodiester bond 3' to the deoxyinosine residue. In addition to deoxyinosine, deoxyinosine 3'-endonuclease also recognizes AP site, urea residue, and base mismatches in DNA and exhibits strand-specific cleavage of base mismatches in DNA (11). In contrast to hypoxanthine DNA glycosylase, deoxyinosine 3'-endonuclease does not remove deoxyinosine or other lesions from the DNA (10). Therefore, it is likely that other proteins are required to complete the repair process initiated by deoxyinosine 3'-endonuclease.

The initial binding of a protein to DNA is critical for multi-protein interaction with DNA (12, 13). The protein-DNA complexes formed promote the entry of other proteins by protein-protein interaction or by induced conformational changes of the DNA. In DNA repair systems, both nucleotide excision repair and methyl-directed mismatch repair require multiple protein-DNA and protein-protein interactions to remove the DNA damages (for reviews, see Refs. 14 and 15). Since deoxyinosine 3'-endonuclease might also initiate a repair process that requires more than one protein, it is of interest to determine whether deoxyinosine 3'-endonuclease can interact with DNA containing deoxyinosine to form a stable complex. In this paper, we show that deoxyinosine 3'-endonuclease forms two stable complexes with duplex oligodeoxynucleotides containing deoxyinosine, and we propose a model to explain its interaction with the DNA containing deoxyinosine.

MATERIALS AND METHODS

Enzymes—Deoxyinosine 3'-endonuclease was purified from E. coli strain BW434 (nth–xth–, provided by Dr. B. Weiss, University of Michigan) as described previously (10, 11), except that Fraction III from the Mono S column was loaded onto a 50-m l Sepharose Fast Flow (Pharmacia Biotech Inc) column instead of Mono Q column to yield Fraction VI. Fraction IV was then further subjected to phenyl-Superose chromatography (Pharmacia) to yield Fraction V as described previously (11). Enzyme preparation from Fraction V was used in this study.

T4 polynucleotide kinase and deoxynucleotidyl terminal transferase were from U. S. Biochemical Corp. DNase I was from LifeTechnologies, Inc.

DNA Substrates—Single-stranded oligodeoxynucleotides were synthesized on an Applied Biosystem DNA synthesizer housed at the Department of Microbiology and Molecular Genetics, University of Vermont. The phosphoramidite derivatives used for dNMP incorporation were obtained from Glen Research (Sterling, VA). Oligodeoxynucleotides were purified by electrophoresis on a 20% polyacrylamide gel and were eluted from the gels by an IBI electroduster or purified by high performance liquid chromatography using a Mono Q 5/5 column (Pharmacia Biotech Inc) as described before (10). Oligodeoxynucleotides were 5'-end-labeled with \(\gamma\)-32P-ATP (Amersham Corp.) using T4 polynucleotide kinase or 3'-end-labeled with [\(\alpha\]-32P]cytidine 5'-triphosphate (DuPont NEN) using deoxynucleotidyl terminal transferase following instructions from the enzyme supplier (U. S. Biochemical Corp.). The labeled oligodeoxynucleotides were annealed to the appropriate complementary strands at 1:1 ratio in 10 mM Tris-HCl, pH 7.5, 0.1 M NaCl, and 2 mM 2-mercaptoethanol by heating the mixture to 90°C and cooling down gradually to room temperature. The following oligodeoxynucleotide duplexes were used in this study.

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To examine the specificity of the complexes formed, duplex 27-mers containing either an A/T or a C/G pair in place of an I/T or an I/C pair were used as controls (duplex A/T and duplex C/G). No complexes were found with these control duplexes containing deoxyinosine 3'-endonucleotides even at the highest protein concentration used (1.28 μM). The specificity of the complex formation was further demonstrated by a competition assay in which increasing amounts of unlabeled duplex containing deoxyinosine or control duplex were added to the reaction mixture prior to the addition of the enzyme (5 nM). Fig. 2 shows the results of the competition reactions. As it was shown above, there was no detectable complex II formed at this enzyme concentration. The addition of an equal amount of unlabeled specific oligodeoxynucleotide duplex containing deoxyinosine to the reaction mixture (lane 5) reduced by approximately 50% the amount of labeled complex I when no competitor was present (lane 2); the addition of large excess (32-fold) of unlabeled specific duplex abolished the binding of the deoxyinosine 3'-endonuclease to the labeled oligodeoxynucleotide. However, it required more than 40–80-fold excess of unlabeled nonspecific control oligodeoxynucleotide duplex to obtain a 50% reduction in the formation of labeled complex I. These data suggest that complex I is formed by specific binding of deoxyinosine 3'-endonuclease to DNA containing deoxyinosine. In order to examine the specificity of complex II, an enzyme concentration of 0.32 μM was used. Under this condition, the reaction mixture yielded approxi-
Deoxyinosine 3'-endonuclease

Fig. 1. Binding of deoxyinosine 3'-endonuclease to DNA containing deoxyinosine. The binding reactions contained 1 nM of 5'-labeled deoxyinosine-containing oligodeoxynucleotide (duplex I/T or duplex I/C) and the indicated amounts of deoxyinosine 3'-endonuclease under standard conditions. The reaction mixtures were analyzed by a band mobility shift assay as described under "Materials and Methods." Panel A, binding of deoxyinosine 3'-endonuclease to duplex I/T and duplex I/C at the enzyme concentration ranged from 0.001 to 0.16 μM. Panel B, binding reactions with higher enzyme concentrations.

Fig. 2. Competition of unlabeled specific and nonspecific oligonucleotide with the binding of deoxyinosine 3'-endonuclease to duplex I/T. 5'-End-labeled duplex I/T (1 nM) was premixed with unlabeled duplex I/T (specific) or duplex A/T (nonspecific) at the indicated ratio (labeled:unlabeled) in the binding reaction buffer. Deoxyinosine 3'-endonuclease was added to the mixture at a final concentration of 5 nM. The reaction was incubated at 20 °C for 10 min and analyzed by nondenaturing polyacrylamide gel electrophoresis as described.

The Endonucleolytic Activity of Deoxyinosine 3'-Endonuclease

Approximately 75% complex II, 20% complex I, and 5% free labeled duplex DNA. The preformed complex II was then chased by addition of unlabeled specific or control oligodeoxynucleotide duplex. Fig. 3 shows that the addition of an excess amount of specific duplex led to a reduction of complex II and a concomitant increase in the amount of complex I. As will be shown later, this is due to the formation of a tight complex, complex I, between deoxyinosine 3'-endonuclease and deoxyinosine-containing DNA. The addition of unlabeled specific DNA trapped any enzyme that dissociated from the labeled complex II, thus regenerating labeled complex I. No such trapping was observed, even when a large excess (100-fold) of unlabeled control DNA was added, suggesting that complex II is also a specific complex with deoxyinosine-containing DNA. Similar to the competition reaction shown above for complex I, unlabeled specific DNA premixed with labeled specific DNA also led to drastic inhibition of the formation of complex II (data not shown).

When using single-stranded oligodeoxynucleotide containing deoxyinosine as a substrate for a band mobility shift assay, two electrophoretic bands were observed. One migrated as the free oligodeoxynucleotide, and the other faster migrating species corresponded to the cleaved single-stranded product (10). No stable protein-DNA complex was observed with single-stranded DNA containing deoxyinosine. Even in the absence of Mg²⁺, a reaction condition in which the enzyme does not nick DNA, stable complexes were not formed with single-stranded DNA. Thus, it appears that the formation of a stable DNA-protein complex requires a duplex DNA substrate.

The Endonucleolytic Activity of Deoxyinosine 3'-Endonuclease

Fig. 3. Chase of complex II by unlabeled specific and nonspecific oligonucleotide. Deoxyinosine 3'-endonuclease (0.32 μM) was incubated with 5'-end-labeled duplex I/T or duplex I/C (1 nM) at 20 °C for 10 min. Ten-fold, 50-fold, or 100-fold excess of unlabeled specific (duplex I/T or duplex I/C) or nonspecific (duplex A/T or duplex C/G) oligodeoxynucleotide duplex was added to the reaction mixture. The reactions were incubated at 20 °C for another 10 min and analyzed by nondenaturing polyacrylamide gel electrophoresis as described.
nicking activity. When the enzyme concentration was higher than 8 nM, the amount of complex I started to decrease, which was accompanied by a concomitant increase in the amount of complex II formed. Thus, the formation of complex I was sufficient to induce the cleavage of DNA containing deoxyinosine, and deoxyinosine 3'-endonuclease was enzymatically active as a monomer. However, these data does not exclude the possibility that complex II formed could also initiate the cleavage reaction. Similar results were obtained with both I/T and I/C pair-containing oligodeoxynucleotides.

Factors That Affect Complex Formation—Deoxyinosine 3'-endonuclease has an obligatory requirement for Mg2+ for its nicking activity. No activity was found in 50 mM Tris-HCl buffer alone or in Tris-HCl buffer supplemented with different amounts of NaCl and/or EDTA. Addition of ATP, GTP, or NAD+ does not affect its nicking activity. Similar to its nicking activity, the binding activity of deoxyinosine 3'-endonuclease was not affected by the addition of ATP, GTP, or NAD. However, in contrast to the nicking activity, the formation of the complexes was independent of Mg2+. Deoxyinosine 3'-endonuclease binds to duplex I/T and duplex I/C with the same pattern and similar binding activity with or without Mg2+ (data not shown). The apparent Kd determined in the presence or absence of Mg2+ was similar, i.e. 4 and 400 nM for complexes I and II, respectively.

Deoxyinosine 3'-Endonuclease Has the Same Affinity for Both the Substrate and the Product—The correlation between the binding and nicking activities suggested that deoxyinosine 3'-endonuclease remained bound to the DNA even after the DNA was cleaved, and thus the enzyme might be capable of binding to nicked DNA. The complexes were stable under non-denaturing conditions as for the gel retardation assay, but were disrupted in the gels containing 8 M urea used for the activity assay. Furthermore, since deoxyinosine 3'-endonuclease cleaves the DNA at the second phosphodiester bond 3' to deoxyinosine in the presence of Mg2+, the similarity in the Kd values of the enzyme determined with and without Mg2+ implied that deoxyinosine 3'-endonuclease binds to both the substrate and the product with the same affinity. To test this, the following nicked DNA duplexes were prepared.

**Fig. 4. Comparison of binding and nicking activity of deoxyinosine 3'-endonuclease on deoxyinosine-containing oligodeoxynucleotide.** Standard binding reactions were performed with 1 nM 3'-end-labeled duplex I/T and the indicated amounts of deoxyinosine 3'-endonuclease. An aliquot (2 μl) of reaction mixture was withdrawn and added to an equal volume of loading buffer. The mixture was electrophoresed in 20% polyacrylamide gel containing 8 M urea as described under “Material and Methods.” The remaining binding reaction mixture was analyzed by gel retardation. The gels were dried under vacuum and quantitated with a Bio-Rad PhosphorImager. The percent cleavage or percent complex formation was plotted against the enzyme concentration for comparison of these two activities. ○, percent total complex formation; □, percent complex I; X, percent complex II.

**Duplex A:** 5′-GGTCGACTI *p*GGAGGATCCCCGGGTAC-3′
3′-ACGTCCAGCTGAT-CTCTCTAGGGGCC-5′
**Duplex B:** 5′-GGTCGACTI *p*GGAGGATCCCCGGGTAC-3′
3′-ACGTCCAGCTGACT-CTCTCTAGGGGCC-5′

These oligodeoxynucleotide duplexes contain a nick 1 base 3′ to deoxyinosine. The nick contains a 3′-hydroxyl- and 5′-phosphoryl terminus, which is identical to the products that would be generated by deoxyinosine 3'-endonuclease on duplex I/T or duplex I/C. Duplexes A and B were 32P-labeled at the 5′ termini of the top oligonucleotide GGTCGACTA. Band mobility shift assay (Fig. 5) showed that deoxyinosine 3'-endonuclease formed stable complexes with these duplexes. No complex was formed with nicked DNA duplexes containing either A/T or C/G pair replacing the I/T or I/C pair (data not shown), suggesting that the binding was deoxyinosine-specific but not nick-specific. Binding of deoxyinosine 3'-endonuclease to nicked deoxyinosine-containing DNA was also Mg2+-independent.

**Contribution of the Neighboring DNA Segment to the Formation of Complexes—**Since deoxyinosine 3'-endonuclease formed stable complexes with nicked duplexes, we next tested the relative contribution of the DNA segment flanking deoxyinosine and the nick to the recognition and binding of the enzyme. To do this, partial duplexes containing 5′-IAGGAGGATCCCCGGGTAC-3′, 5′-TIAGGAGGATCCCCGGGTAC-3′, 5′-GGTCGACTI-3′, or 5′-TGCCAGGTCGACTI-3′ were an-
ing reactions were performed with 1 nM of DNA duplex and indicated amount of deoxyinosine 3'-endonuclease. The reaction mixture was analyzed by band mobility shift assay.

nealed to the complementary strand separately to form following duplexes.

Duplex C: 5'-IAGAGGATCCCGGTTAC-3' 3'-AGGCCAGCTGTTCCTCTAGGGGCC

Duplex D: 5'-IAGAGGATCCCGGTTAC-3' 3'-AGGCCAGCTGTTCCTCTAGGGGCC

Duplex E: 5'-GTTGGAGCTA 3'-AGGCCAGCTGTTCCTCTAGGGGCC

Duplex F: 5'-GTTGGAGCTA 3'-AGGCCAGCTGTTCCTCTAGGGGCC

Band mobility shift assays were performed with these duplexes under the same conditions as described above. Deoxyinosine 3'-endonuclease bound to duplex C and duplex D poorly; less than 25% of the DNA was bound as complex I even when the enzyme concentration was 128 nM. Furthermore, no complex II was observed with these duplexes, even at very high enzyme concentration (1.28 μM). However, as shown in Fig. 6, the ability of deoxyinosine 3'-endonuclease to form stable complexes with duplex E was comparable with that with duplex I/T. Similar results were obtained with duplex F. Thus, the data obtained with partial duplexes suggest that the presence of duplex structure 5' to the deoxyinosine is essential for the formation of stable complexes.

Stoichiometry of Protein-DNA Complex Formation—The stoichiometry of the protein-DNA complex formation was determined following the method of Orchard and May (16). The molecular weights of the first and second complex were estimated to be around 42,400 and 65,000, respectively (Fig. 7). An estimate of the molecular weight of the protein component in the complexes was then obtained by subtracting the contribution from the DNA component (approximate 17,145 for a 27-mer oligodeoxynucleotide). Thus, a value of 25,255 and 47,855 for the protein component was obtained for the first and the second complex, respectively. Since the molecular weight of deoxyinosine 3'-endonuclease is around 25,000 (10), it can be concluded that complex I contains a single molecule of deoxyinosine 3'-endonuclease, while complex II contains two molecules of the protein/DNA molecule.

DNase I Footprinting Assay—Since deoxyinosine 3'-endonuclease cleaves DNA at the second phosphodiester bond 3' to the deoxyinosine in the presence of Mg²⁺, therefore, DNase I footprinting was performed with both 5'-end-labeled and 3'-end-labeled oligodeoxynucleotides. The 27-mer containing an I/T pair (duplex I/T) used in the gel retardation assay was also used in the footprinting experiments.

Fig. 8 shows the results of DNase I footprinting with 5'-end-labeled oligodeoxynucleotide (the strand containing deoxyinosine was labeled), demonstrating that deoxyinosine 3'-endonuclease protected five bases of the DNA 5' to the nick site (this appeared as a thick dark band). The protection of band I and band II was quantitated and plotted against the protein concentration (Fig. 9). From these data, an apparent Kₐ (calculated as the enzyme concentration at which 50% protection from DNase I digestion was attained) was found to be about 4 nM, a value in good agreement with the Kₐ determined for complex I using the band mobility shift assay. This suggests that the protection of DNA 5' to the nick is consistent with the formation of complex I. Due to the cleavage catalyzed by deoxyinosine 3'-endonuclease, the protection pattern 3' to deoxyinosine was not able to be obtained using the 5'-end-labeled oligodeoxynucleotide.

To obtain the protection pattern of the DNA 3' to the deoxyinosine by the enzyme, the oligodeoxynucleotide was 3'-end-labeled with [α-³²P]deoxyctydine triphosphate, and DNase I footprinting assay was performed as described with the 5'-end-labeled oligodeoxynucleotide. The results are shown in Fig. 9. In contrast to the 5'-labeled substrate, very little protection was observed when the protein concentration was below 16 nM; however, up to 20–40% protection was observed when the protein concentration was increased to 32 nM (panel A), a concentration shown previously to give rise to complex II. Thus, it appears that the protection of the DNA 3' to the deoxyinosine was not due to the formation of complex I but to complex II which contains two molecules of protein. Furthermore, at the higher protein concentration when complex II is formed, the segment of DNA protected by deoxyinosine 3'-endonuclease dimer was very broad (panel B). The exact border of protection was not clear with this oligodeoxynucleotide. Based on the data presented in Fig. 9, we concluded that at least 13 bases 3' to deoxyinosine were protected from DNase I digestion upon formation of complex II. However, the 2 to 3 bases immediate 3' to the nick appeared to be hypersensitive to DNase I digestion when complex II formed.

When the complementary strand (the DNA strand that does not contain deoxyinosine) of duplex I/T was labeled and used in DNase I footprinting assay, no protection by deoxyinosine 3'-endonuclease was observed (data not shown). This suggests that the enzyme binds only to the DNA strand containing
deoxyinosine and makes no contact with the opposite strand. Furthermore, no footprinting was observed when the DNA digestion was performed with 1,10-phenanthroline-copper ion system (17, 18), suggesting that the protein interacts with the DNA along the major groove (data not shown).

As shown above, deoxyinosine 3'-endonuclease can also form stable complex I and complex II with deoxyinosine-containing DNA duplexes lacking the DNA segment 3' to the deoxyinosine residue (duplex F) and 3' to the nick (duplex E). This suggests that the DNA sequences 3' to the deoxyinosine residue are not essential for the binding of the enzyme. However, the results of the DNase I footprinting assay with duplex I/T revealed that the formation of complex II provided protection to the DNA segment 3' to the nick. Thus, it is possible that the second molecule of the enzyme binds to complex I by interacting with the enzyme already bound to the DNA. Consistent with the results of the DNase I footprinting assay with the labeled complementary strand of duplex I/T, no significant protection from DNase I digestion was observed on the complementary strand (the strand that does not contain deoxyinosine) of duplex E and duplex F, even at high concentration of deoxyinosine 3'-endonuclease (data not shown). These data suggest that the second molecule of deoxyinosine 3'-endonuclease binds to complex I through protein-protein interaction.

DISCUSSION

In this paper, we show that deoxyinosine 3'-endonuclease from E. coli interacts with deoxyinosine-containing DNA and forms two stable protein-DNA complexes, complex I and complex II. Complex I contains a single molecule of protein, and complex II has two molecules of protein bound to one molecule of DNA. An unusual feature of the interaction of deoxyinosine 3'-endonuclease with deoxyinosine-containing DNA is that the
enzyme can bind to the DNA under optimal cleavage conditions, exhibiting identical affinity for both the DNA substrate and the product. This is different from most DNA-binding proteins, which usually do not have specific enzymatic functions. Their role is to locate the specific sequence on the DNA and bring other proteins with specific enzymatic functions to the site. For example, in nucleotide excision repair (14), the function of the UvrA protein is to recognize and locate DNA damage, such as a UV photodimer, and help to deliver the UvrB protein to the damage site. After UvrB forms a stable complex with the lesion, UvrA then dissociates from the DNA (13, 24). Similarly, MutS protein in a methyl-directed mismatch repair system binds to base mismatches and helps to activate the endonucleolytic activity of MutH protein through interaction with the MutL protein (15, 25, 26). These proteins have high affinity to their recognition sequences and form very stable complexes with the DNA, since they have no need for rapid dissociation to continue a catalytic cycle. In contrast to these proteins, deoxyinosine 3'-endonuclease exhibits not only high affinity to DNA containing deoxyinosine, but also an endonucleolytic activity which cleaves the DNA in the protein-DNA complex.

Since deoxyinosine 3'-endonuclease can form stable complexes with partial duplexes E and F, but not partial duplexes C and D, DNA duplex structure 5' to the deoxyinosine residue must be essential for complex formation. Furthermore, the binding activities of the enzyme to partial duplexes E and F are comparable with those of duplex I/T or I/C. This demonstrates that duplex structure 5' to deoxyinosine residues is not only essential but also sufficient for the enzyme to bind to DNA. Combined with the results of the DNase I footprinting, we propose that a single molecule of deoxyinosine 3'-endonuclease binds to a deoxyinosine-containing DNA duplex by direct contact with the four bases 5' to the deoxyinosine residue, leading to the formation of complex I. DNase I footprinting also shows that the second molecule of deoxyinosine 3'-endonuclease protects the DNA 3' to the nick. However, the formation of complex II does not require the DNA segment 3' to the nick (as with duplex E and duplex F) nor does it provide protection to the complementary strand from DNase I digestion. Therefore, the second molecule of deoxyinosine 3'-endonuclease appears to bind to complex I by interacting with the first molecule of the enzyme.

The biological role of the tight binding of deoxyinosine 3'-endonuclease to deoxyinosine-containing DNA is not clear. Even though the binding and cleavage reaction of deoxyinosine 3'-endonuclease to deoxyinosine-containing DNA do not lead to the removal of the lesion, the formation of stable complexes might be important for other biological processes. Hypoxanthine DNA glycosylase activity has been reported to be very low in E. coli (5, 8). Although the AlkA protein releases hypoxanthine from DNA containing deoxyinosine, the enzyme shows a very high Km (420 nm) (9) for deoxyinosine. In contrast, the affinity of deoxyinosine 3'-endonuclease to deoxyinosine-containing DNA is 100-fold lower (Kd about 4 nm). Furthermore, there are about 100 molecules of deoxyinosine 3'-endonuclease in a E. coli cell (10). It is likely that deoxyinosine in DNA is primarily trapped in a complex with deoxyinosine 3'-endonuclease. We have found that the binding of deoxyinosine 3'-endonuclease to the DNA blocks the recognition of hypoxanthine by the AlkA protein, leading to a strong inhibition of the removal of hypoxanthine by AlkA protein.1 Therefore, based on the current biochemical data, it is unlikely that AlkA protein will be able to remove deoxyinosine from DNA when deoxyinosine 3'-endonuclease is present. Binding to DNA, even after nicking, indicates that deoxyinosine 3'-endonuclease not only acts as an endonuclease but also as a DNA binding protein. It is possible that the formation of stable complexes might be important for targeting other repair enzyme(s) to the damage site, which then leads to efficient removal of the lesion. The binding activity of deoxyinosine 3'-endonuclease would be sim-

1 M. Yao and Y. W. Kow, unpublished observation.
ilar then to the function of the UvrA protein in nucleotide excision repair or the MutS protein in the methyl-directed mismatch repair in E. coli. The strong binding of deoxyinosine 3'-endonuclease to deoxyinosine-containing DNA prevents the dissociation of the enzyme from the DNA after cleavage. Thus, no turnover of the enzyme cleavage activity was observed when duplex I/T or duplex I/C was used as a substrate. However, deoxyinosine 3'-endonuclease retains nicking activity on duplex C and duplex D and exhibits a turning over of cleavage, since the enzyme cannot form stable complexes with these duplexes. One can speculate that other repair protein(s) brought to the damage site by interacting with deoxyinosine 3'-endonuclease in the protein-DNA complex would make an incision in the DNA 5'-to deoxyinosine. This would lead to the dissociation of deoxyinosine 3'-endonuclease from the DNA and generation of a small gap on DNA with the removal of the lesion. Alternatively, the binding of deoxyinosine 3'-endonuclease to the DNA might expose the nick, thus facilitating the entry of a 3'-to 5'-helicase. This is supported by the finding that complex II renders the 2 to 3 bases immediate 3' to the nick hypersensitive to DNase I digestion. Actually, although the binding of deoxyinosine 3'-endonuclease to the DNA blocks the hypoxanthine base, it does not block the nick, since DNA polymerase I can nick-translate the DNA in the complex (10). Recently, we have cloned the gene for deoxyinosine 3'-endonuclease from E. coli, thus allowing us to investigate the biological function of the enzyme in vivo.

Acknowledgment—We are grateful to Dr. Susan Wallace for critical reading of the manuscript. We also thank Drs. Robert Melamede, Zafer Hatahet, and Andrei Purmal for helpful discussions.

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