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Actions of Aprataxin in Multiple DNA Repair Pathways*

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Mutations in the *Aptx* gene lead to a neurological disorder known as ataxia oculomotor apraxia-1. The product of *Aptx* is Aprataxin (Aptx), a DNA-binding protein that resolves abortive DNA ligation intermediates. Aprataxin catalyzes the nucleophilic release of adenylate groups covalently linked to 5′ phosphate termini, resulting in termini that can again serve as substrates for DNA ligases. Here we show that Aprataxin acts preferentially on adenylated nicks and double-strand breaks rather than on single-stranded DNA. Moreover, we show that whereas the catalytic activity of Aptx resides within the HIT domain, the C-terminal zinc finger domain provides stabilizing contacts that lock the enzyme onto its high affinity AMP-DNA target site. Both domains are therefore required for efficient AMP-DNA hydrolase activity. Additionally, we find a role for Aprataxin in base excision repair, specifically in the removal of adenylates that arise from abortive ligation reactions that take place at incised abasic sites in DNA. We suggest that Aprataxin may have a general proofreading function in DNA repair, removing DNA adenylates as they arise during single-strand break repair, double-strand break repair, and in base excision repair.

Ataxia oculomotor apraxia-1 (AOA1)[4] is a human autosomal recessive syndrome whose clinical features include early onset cerebellar ataxia, oculomotor apraxia, and late peripheral neuropathy (1). The cause of AOA1 is linked to mutations in a gene that encodes a 342-amino acid protein called Aprataxin (2, 3). *Aptx*-defective cell lines are sensitive to DNA-damaging agents such as methyl methanesulfonate, hydrogen peroxide, and camptothecin (4–7), indicating a role for Aprataxin in DNA repair. Recently, it was shown that Aprataxin resolves abortive DNA ligation intermediates (8), such as those that arise at "dirty" single-strand breaks formed by oxygen radical attack. Abortive ligation leads to the covalent attachment of an AMP moiety to the 5′ terminus at the break site. Aprataxin repairs these lesions by catalyzing the nucleophilic release of 5′-adenylate groups from the single-strand breaks, resulting in the restoration of 5′ phosphate termini that can be efficiently rejoined.

Aprataxin protein has three distinct domains: an N-terminal FHA domain, a central histidine triad (HIT) domain, and a C-terminal domain with a zinc finger motif (3). The FHA domain mediates the association of Aprataxin with two distinct DNA repair complexes, as it specifically interacts with XRCC1 and XRCC4 (4–6, 9). The catalytic HIT domain (Fig. 1*A*, *upper sequence*) is responsible for adenylate hydrolase activity (8, 10), which is abolished by a mutation in the histidine triad motif (8). Finally, although the C-terminal domain of Aprataxin has been implicated in possessing general DNA binding capacity (11), the importance of the zinc finger motif (*lower sequence*) for DNA binding, DNA adenylate binding, and DNA de-adenylation activity has not been addressed. Here, we define the substrate specificity of Aptx and show that the zinc finger motif plays an important role in DNA adenylate recognition and hydrolysis. Furthermore, we show that Aptx fulfills a more general proofreading function in DNA repair, extending beyond the repair of dirty oxidative single-strand breaks.

EXPERIMENTAL PROCEDURES

Proteins—Human Aprataxin was PCR-amplified from a HeLa cDNA library (Invitrogen) and cloned into pET41 using the SpeI and BamHI restriction sites. This plasmid expressed recombinant Aptx with an N-terminal GST and a C-terminal His_8 tag. The mutants $Aptx^{H260A}$, $Aptx^{C319A,C322A}$, and $Aptx^{H260A,C319A,C322A}$ were generated using the QuikChange II site-directed mutagenesis kit (Stratagene). Wild-type and mutant $^{GST}Aprataxin^{His}$ proteins were expressed in *Escherichia coli* BL21-CodonPlus RIL cells at 30 °C for 2 h following induction with 0.2 mM isopropyl-1-thio-β-D-galactopyranoside. Cells were lysed and Aprataxin purified using nickel-nitrilotriacetic acid-agarose (Qiagen) and glutathione-Sepharose (GE Healthcare).

Recombinant human DNA ligase III-XRCC1 complex was a gift from Dr. Tomas Lindahl. T4 DNA ligase was from New England Biolabs and human DNA polymerase β from Trevigen.

DNA Substrates—Oligo 1, 5′-ATTCCGATAGTGACTACA-3′; oligo 2, 5′-TGTAGTCACTATCGGAATGAGGGCGACA-CGGATATG-3′; oligo 3, 5′-CATATCCGTGTCGCCCTC-3′; oligo 4, 5′-CATATCCGTGTCGCCCTCATTCCGATAGTG-ACTACA-3′; oligo 5, 5′-TGTAGTCACTATCGGAAT-3′; and abasic oligo 1 with a 5′-deoxyribosephosphate (dRP) residue, 5′-dRP-TTCCGATAGTGACTACA-3′, were purchased from Sigma and purified by denaturing gel electrophoresis. 5′-^{32}P labeling was carried out using T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP (GE Healthcare). Terminal

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[4] The abbreviations used are: AOA1, ataxia oculomotor apraxia-1; HIT, histidine triad; GST, glutathione *S*-transferase; dRP, deoxyribosephosphate; BER, base excision repair; DTT, dithiothreitol.

deoxynucleotidyl transferase (New England Biolabs) and [α-^{32}P]ddATP (GE Healthcare) were used for 3′-^{32}P labeling.

5′-^{32}P-labeled oligo 4, alone or annealed to oligo 2, was used in DNA binding assays. The nicked substrate was prepared by annealing 5′-^{32}P-labeled oligo 1 with oligos 2 and 3. 5′-^{32}P-labeled oligo 1 was adenylated as described (8), purified and used as AMP-single-stranded DNA, or annealed with oligo 5 to create AMP-double-stranded DNA or oligos 2 and 3 to create AMP-nicked DNA.

For the abasic nicked DNA substrate, abasic oligo 1 was 3′-labeled using [^{32}P]ddATP and annealed with oligos 2 and 3. The abasic nicked DNA was then adenylated with human DNA ligase III-XRCC1, and the adenylated 18^{dRP} oligo1 was repurified and annealed with oligos 2 and 3 to form the final substrate. For the nicked control, oligo 1 (in this case synthesized with a terminal 5′-phosphate) was 3′-^{32}P-labeled and the adenylated nicked substrate was prepared essentially as described (8).

Ligation Reactions—Reactions (5 μl) contained DNA (50 nM) and 5 nM DNA ligaseIII-XRCC1, or 20 nM T4 DNA ligase, in 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$, 5 mM DTT, 25 μg/ml bovine serum albumin, 1 mM ATP. After 1 or 2 min of incubation at 37 °C, reactions were stopped by addition of formamide and heated for 3 min at 90 °C. Products were analyzed by 10% denaturing PAGE, and ^{32}P-labeled DNA products were detected by autoradiography.

DNA Adenylate Hydrolysis—Unless stated otherwise, reactions (10 μl) contained DNA (50 nM) and recombinant Aprataxin in 50 mM Tris-HCl, pH 8.0, 40 mM NaCl, 5 mM EDTA, 1 mM DTT, 100 μg/ml bovine serum albumin, and 5% glycerol. Incubation was for 2 min at room temperature. Reactions were stopped by addition of formamide loading buffer, and denatured DNA was analyzed by 10% denaturing PAGE followed by autoradiography. For time course experiments, reactions (25 μl) contained 50 nM DNA and 0.2 nM Aptx. DNA was analyzed by 10% denaturing PAGE and quantified using a Storm 840 phosphorimaging system (GE Healthcare). Hydrolysis reactions using whole cell extracts were performed as described (8) in a buffer containing 50 mM Tris-HCl, pH 8.0, 40 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 100 μg/ml bovine serum albumin, and 5% glycerol.

DNA Binding Assays—Binding reactions (15 μl) contained DNA (50 nM) and Aprataxin in 50 mM Tris-HCl, pH 8.0, 30 mM NaCl, 5 mM EDTA, 1 mM DTT, 100 μg/ml bovine serum albumin, and 5% glycerol. Where indicated, reactions contained 300 ng of poly[dI·dC]. Incubation was for 10 min at room temperature. Reactions were then put on ice and analyzed immediately by 4% non-denaturing PAGE using 0.5× Tris borate-EDTA as the running buffer at 4 °C followed by autoradiography.

RESULTS

Substrate Specificity of Aprataxin—It was shown previously that Aprataxin acts upon adenylated nicked duplex DNA to remove covalently bound AMP, leaving a 5′-phosphate at the nick that can be ligated to a proximal 3′-OH group (8). To further characterize the DNA de-adenylation activity of human Aprataxin and to define the specific contribution of each domain of the protein, we used a mutational analysis approach to produce four forms of Aprataxin: 1) the wild-type protein

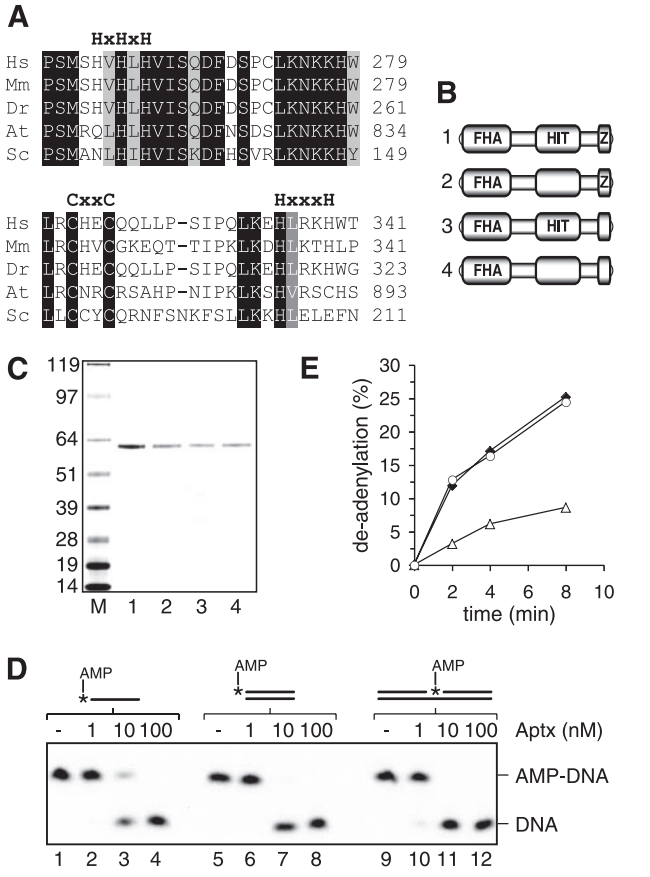

FIGURE 1. **Substrate specificity of Aprataxin.** *A*, ClustalW alignment of the amino acid sequences comprising the conserved active site histidine triad motif (*HXHXH*, *upper panel*) and C-terminal zinc finger motif (C2H2; *lower panel*) of Aprataxin orthologs from human (*Hs*, *Homo sapiens*), mouse (*Mm*, *Mus musculus*), fish (*Dr*, *Danio rerio*), plant (*At*, *Arabidopsis thaliana*), and yeast (*Sc*, *Saccharomyces cerevisiae*). Conserved and similar amino acids are indicated by *black* and *gray shadings*, respectively. *B*, schematic representation of Aptx proteins used in this study highlighting the tripartite domain structure. The wild-type protein (*1*) contains an N-terminal FHA domain, a central histidine triad domain (*HIT*), and a C-terminal domain with a zinc finger motif (*Z*). Mutant proteins were created to disrupt the HIT domain (*2*) (Aptx^{H260A}), the zinc finger motif (*3*) (Aptx^{C319A,C322A}), and both domains simultaneously (*4*) (Aptx^{H260A,C319A,C322A}). *C*, four N-terminal GST- and C-terminal His-tagged Aprataxin proteins (~0.3 μg/lane) were purified using nickel-nitrilotriacetic acid-agarose and glutathione-Sepharose. They were then analyzed by SDS-PAGE followed by Coomassie blue staining. *Lane numbers* correspond to the proteins indicated in *panel B*. *Lane M*, molecular mass markers (kDa). The ^{GST}Aptx^{His} proteins have an apparent molecular mass of ~60 kDa. *D*, activity of Aptx on adenylated single- and double-stranded linear DNA and adenylated nicked duplex DNA. Products were analyzed by denaturing PAGE and detected by autoradiography. 5′-^{32}P labels are indicated with *asterisks*. *E*, quantification of Aptx activity on adenylated single-stranded (*open triangles*), double-stranded linear (*filled triangles*), and nicked duplex (*circles*) DNA under limiting enzyme concentrations.

(Aptx), 2) an active site mutant Aptx^{H260A} with a non-functional HIT domain, 3) Aptx^{C319A,322A}, which is mutated for the critical cysteine residues in the putative C-terminal zinc finger motif, and 4) Aptx^{H260A,319A,322A}, in which the mutation in the active site HIT domain is combined with mutations in the zinc finger motif (Fig. 1, *A* and *B*). For direct comparison, all proteins carried an N-terminal GST tag and a C-terminal His_8 tag, which allowed their purification to near homogeneity (Fig. 1*C*). They will hereafter be referred to by their protein name and respective mutation(s). Control experiments have shown that the specific activity of GST- and His-tagged wild-type Aprataxin on

adenylated DNA is comparable with that of untagged Aprataxin (data not shown).

First, we compared the activity of wild-type Aprataxin on single- and double-stranded DNA. In these experiments, each substrate contained the same ^{32}P-labeled adenylated 18-mer oligonucleotide, and de-adenylation was monitored by its increased mobility through a denaturing gel following removal of covalently bound AMP. We found that Aprataxin acted preferentially on duplex DNA containing an adenylate residue at the 5′ terminus of a nick (Fig. 1*D*, *lanes 9–12*) or double-strand break (*lanes 5–8*) compared with adenylated single-stranded DNA (*lanes 1–4*). Time course experiments using limiting enzyme concentrations showed that the de-adenylation activity with double-stranded DNA was ~3× greater than that observed with single-stranded DNA (Fig. 1*E*).

To determine whether Aprataxin exhibits a different binding affinity for the various DNAs, we carried out electrophoretic mobility shift assays. Aprataxin bound linear (Fig. 2, *lanes 6–8*) or nicked (*lanes 10–12*) duplex DNA more tightly than single-stranded DNA (*lanes 2–4*), consistent with previous studies (11).

When electrophoretic mobility shift assays were carried out using nicked duplex DNA substrates, we observed three distinct protein-DNA complexes (designated I, II, and III). These complexes were observed with both nicked duplex (Fig. 3, *lanes 2–4*) and adenylated nicked duplex (*lanes 15–17*) DNA. Given that Aprataxin is catalytically active under the conditions of this assay and de-adenylation occurs (data not shown), it was necessary to carry out similar reactions with Aptx^{H260A}, containing an inactivating mutation in the HIT domain. Using Aptx^{H260A} we observed enhanced complex formation with the nicked adenylated substrate (compare *lanes 8–10* with *lanes 21–23*). Furthermore, in contrast to complexes formed by wild-type Aptx, we found that the protein-DNA complexes formed between Aptx^{H260A} and the adenylated substrate were resistant to competition with a 30-fold excess of poly[dI·dC] (*lanes 24–26*). We also found that the poly[dI·dC] converted complexes III and II to the least retarded complex I. Control reactions with catalytically active Aptx, and with Aptx^{H260A} and unadenylated nicked duplex DNA, showed that complex formation was competed away by the presence of poly[dI·dC] (*lanes 5–7*, *11–13*, and *18–20*). Based on these results, we suggest that complex I represents Aprataxin bound at the site of the nick, an interaction that is stabilized by the adenylate moiety, and that complexes II and III are formed by the binding of Aprataxin to flanking regions of DNA. Poly[dI·dC] competes away the general DNA binding but is unable to disrupt the specific complex formed between catalytically inactive Aptx^{H260A} and its target, the adenylated residue.

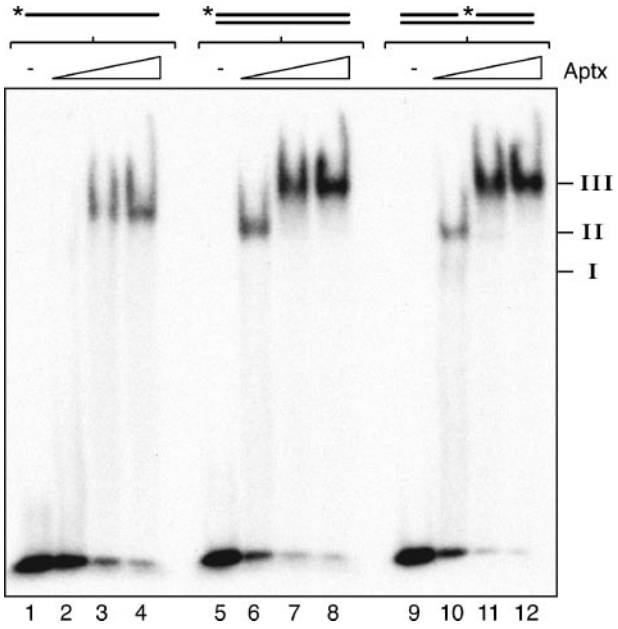

FIGURE 2. **DNA binding by Aprataxin.** Reactions contained single-stranded DNA, double-stranded DNA, or nicked DNA and Aprataxin (50, 250, and 500 nM) as indicated. Protein-DNA complexes were analyzed by electrophoretic mobility shift assay and detected by autoradiography. *Roman numerals* mark the position of three distinct protein-DNA complexes.

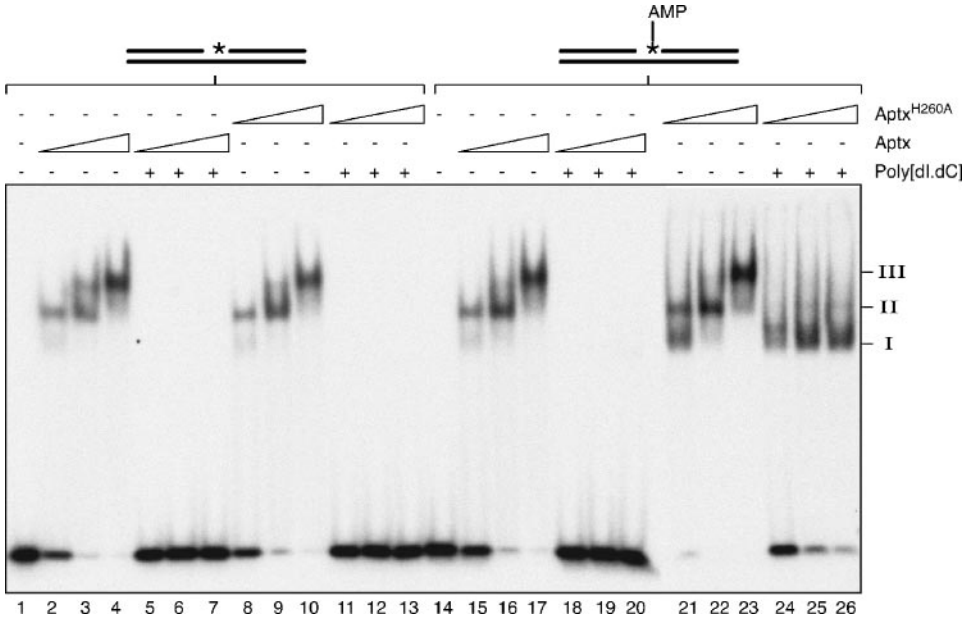

FIGURE 3. **Specific interaction of Aprataxin with adenylated residues.** Reactions contained the indicated nicked or adenylated nicked substrates and either wild-type or catalytically inactive Aptx^{H260A} (50, 250, and 500 nM). Poly[dI·dC] was present where indicated. Protein-DNA complexes were analyzed as described in Fig. 2 legend.

These results show that Aprataxin has the ability to bind duplex DNA, possibly enabling the protein to scan the genome for sites of adenylation. When such a site is encountered, Aprataxin forms a high affinity complex that (with wild-type protein) leads to rapid de-adenylation.

Binding of Aptx to Duplex DNA Is Mediated by the C-terminal Zinc Finger—To date, the precise role of the putative zinc finger motif of Aprataxin has not been established. We therefore assessed the contribution of the zinc finger

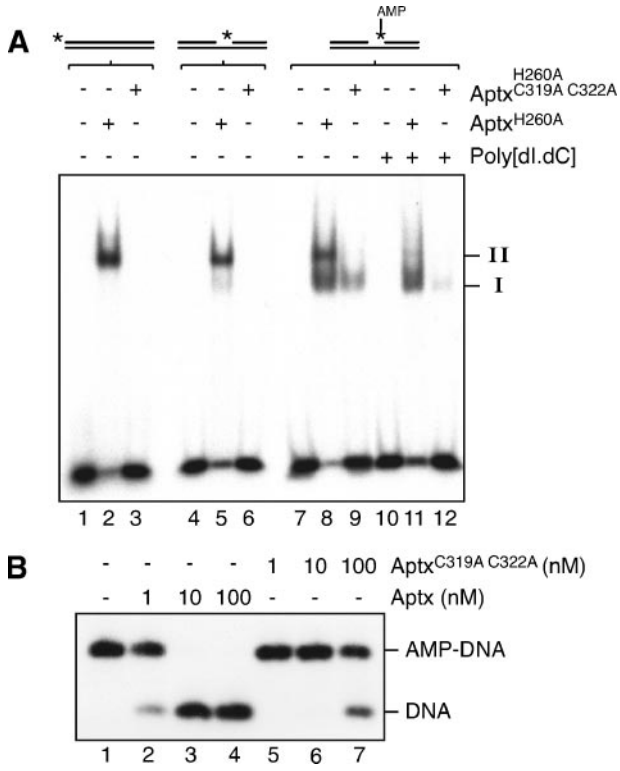

FIGURE 4. **The zinc finger motif of Aptx is required for substrate recognition and hydrolysis.** *A*, electrophoretic mobility shift assay reactions contained wild-type or mutant Aptx and linear, nicked, or adenylated nicked DNA as indicated. Poly[dI·dC] was included in the reactions of *lanes 10–12*. Protein-DNA complexes were analyzed as described in the Fig. 2 legend. *B*, DNA de-adenylation reactions contained wild-type Aptx or the zinc finger mutant $Aptx^{C319A,C322A}$ with adenylated nicked duplex DNA. Products were analyzed by denaturing PAGE and detected by autoradiography.

to DNA binding by carrying out site-directed mutagenesis to convert the zinc-coordinating cysteines (residues Cys-319 and Cys-322) to alanine. A comparison of $Aptx^{H260A}$ with $Aptx^{H260A,C319A,C322A}$ showed that disruption of the zinc finger resulted in a significant loss of DNA binding activity (Fig. 4*A*, *lanes 3* and *6*). Similar results were obtained with $Aptx^{H260A,C319A,C322A}$ and the nicked DNA adenylate, although in this case some residual binding was observed (*lanes 9* and *12*). Residual binding to the nicked adenylated DNA resulted in the formation of complex I only, indicating that this complex results from a specific interaction with the adenylated residue at the nick.

Taken together, the results shown in Figs. 3 and 4 show that Aprataxin forms a stable complex with nicked adenylated DNA that is resistant to competition when challenged by excess duplex DNA. The stability of this complex is dependent upon the C-terminal zinc finger of the protein.

Efficient De-adenylation Requires the Zinc Finger Motif—When the zinc finger mutant $Aptx^{C319A,C322A}$ was analyzed for its ability to promote de-adenylation, we observed a near 100-fold reduction in activity compared with wild-type Aprataxin (Fig. 4*B*). This result demonstrates that the zinc finger domain is important for efficient hydrolysis of DNA adenylates. Even though the catalytic domain of $Aptx^{C319A,C322A}$ is intact, the loss of its zinc finger DNA binding module renders Aptx inefficient.

Adenylated Abasic Nicks Constitute a Novel Substrate for Aptx—Previously, we showed that Aprataxin acts upon nicked adenylated DNA substrates such as those that might be produced by abortive ligation reactions that take place at sites of oxidative damage (8). An additional and important target substrate might also arise from premature ligation of nicked abasic sites (AP sites) resulting from the removal of damaged bases by monofunctional DNA glycosylases such as human UNG, SMUG-1, MPG, TDG, and MYH, followed by AP endonuclease 1 incision. To test this hypothesis, we first determined whether abortive ligation reactions occurred at base excision repair (BER) intermediates and therefore synthesized a model substrate with a dRP residue at the 5′ terminus of the nick. When incubated with human DNA ligase III-XRCC1 complex, we found that ligation of the abasic nick took place (Fig. 5*A*, *lanes 2–3*) with an efficiency that was ~5–10 times below that observed with normal nicked DNA (*lanes 7–8* and data not shown). Similar results were observed with T4 DNA ligase (*lanes 4–5* and *9–10*) as described previously (12, 13). However, in reactions containing human DNA ligase III-XRCC1 and the abasic substrate, we also observed abortive ligation reactions culminating in the accumulation of DNA adenylates (indicated as $AMP\text{-}18^{dRP}$ in Fig. 5*A*, *lanes 2–3*).

To test whether these DNA adenylates lacking a 5′-terminal base at the nick serve as a substrate for Aptx, ligation reactions with human DNA ligase III-XRCC1 were supplemented with Aptx (Fig. 5*B*). In these reactions, we did not observe the presence of any DNA adenylates but instead observed an increase in ligation products (*lane 3*). These results indicate that without the actions of Aprataxin, abortive ligation events at BER intermediates could lead to a relatively high incidence of DNA adenylate formation.

When the efficiency of AMP hydrolysis by Aprataxin at normal and abasic nicks was compared, we found that the abasic DNA adenylate served as a substrate for Aptx and was hydrolyzed with an efficiency that was ~5 times lower than that observed with normal adenylated nicked DNA (Fig. 5*C*, compare *lanes 2–4* with *6–8*). Taken together, these data lead us to suggest that abortive ligation reactions that take place at BER intermediates may require Aprataxin for efficient repair and restoration of the DNA backbone. This notion is supported by the observation that adenylated abasic nicks persist in whole cell extracts prepared from the AOA1 patient cell lines Ap1 and Ap3 (Fig. 5*D*, *lanes 3* and *4*), while AMP hydrolysis can be observed in normal extracts (*lane 2*). In agreement with this, we found that human DNA polymerase β, which is known to add dNTPs to 3′ termini at dRP sites (14), is unable to utilize its lyase activity to remove adenylated dRP termini (Fig. 5*E*, *lane 2*).

DISCUSSION

In this work we have shown that Aprataxin forms specific complexes with nicked adenylated DNA and promotes the repair and restoration of 5′ termini that are adenylated by abortive ligation reactions. The actions of Aprataxin are essential in non-replicative cells, such as neuronal cells, which otherwise as a consequence of oxidative damage would accumulate adenylates that may pose a block to the transcriptional machinery. Analyses of the DNA binding capacity of Aprataxin revealed that the protein binds undam-

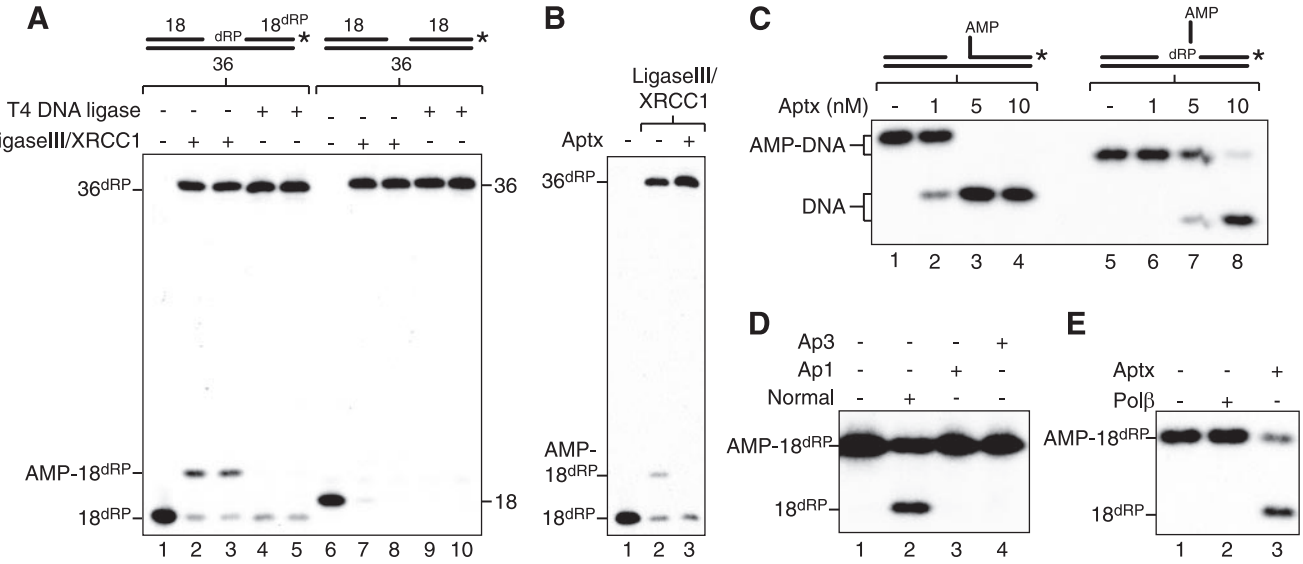

FIGURE 5. **Actions of Aprataxin with adenylated abasic nick substrates.** *A*, human DNA ligase III-XRCC1 complex promotes the ligation of abasic nicked DNAs, but abortive ligation reactions give rise to adenylated products. Ligation reactions, containing 3′-^{32}P-labeled abasic nicked or control nicked DNA substrate, with T4 DNA ligase or human DNA ligase III-XRCC1, were incubated for 1 min (*lanes 2*, *4*, *6*, and *8*) or 2 min (*lanes 3*, *5*, *8*, and *10*). Products were analyzed by denaturing PAGE and detected by autoradiography. The abasic 18-mer and the ligation product containing the abasic 18-mer are marked with *dRP*. *B*, ligation reactions containing human DNA ligase III-XRCC1 and abasic nicked DNA substrate were incubated for 1 min. Wild-type Aptx (10 nM) was present in the reaction shown in *lane 3*. Products were analyzed by denaturing PAGE and detected by autoradiography. *C*, hydrolysis reactions contained wild-type Aptx and adenylated nicked or abasic nicked DNA as indicated. Products were analyzed by denaturing PAGE and detected by autoradiography. *D*, adenylated abasic nicks (50 nM) were treated with whole cell extracts (1 μg of total protein) from normal and AOA1 lymphoblastoid human cell lines (*Ap1* and *Ap3*) as indicated. Incubation was for 5 min at 37 °C, and the products were analyzed by denaturing PAGE and detected by autoradiography. *E*, reactions contained adenylated abasic nicked DNA (50 nM) with wild-type Aptx (2 nM) or polymerase β (20 nM) as indicated in 50 mM Tris-HCl, pH 8.8, 10 mM $MgCl_2$,10 mM KCl, 1 mM DTT, 1% glycerol, 20 μM each of dCTP, dGTP, dATP, and dTTP. Incubation was for 15 min at 37 °C, and the products were analyzed by denaturing PAGE and detected by autoradiography.

aged duplex DNA in agreement with previous observations (11). However, competition studies using catalytically inactive Aptx^{H260A} showed that the protein forms a specific and stable complex with nicked adenylated duplex DNA. The binding and de-adenylation of single-stranded DNA was less than that observed with duplex substrates.

As far as we are aware, Aprataxin is the only enzyme capable of de-adenylating AMP-DNA substrates. However, like other members of the HIT superfamily, Aprataxin can act upon AMP-NH_2 or AppppA, but the catalytic activity observed with these substrates is 3–4 orders of magnitude less than that observed with adenylated DNA (8, 11). It is therefore our opinion that the neurological disorder (AOA1) associated with Aprataxin loss relates to the loss of DNA de-adenylation activity.

Taken together with our previous observations (8), the data presented here indicate that adenylates present in duplex DNA are the physiological substrates for Aprataxin. The specific action of Aptx on damaged duplex DNA is consistent with observations showing that Aptx associates with the DNA ligase III-XRCC1 and DNA ligase IV-XRCC4 complexes (4, 6). Our finding that Aptx hydrolyzes adenylated double-strand breaks strengthens the possibility of a role in double-strand break repair, in agreement with the observed mild sensitivity to ionizing radiation in patient-derived Aptx-deficient cell lines (4).

Aprataxin is a member of the HIT superfamily of nucleotide hydrolases and transferases (15). Mutation of the histidine triad motif completely abolishes the ability of Aprataxin to hydrolyze AMP-DNA (8), and inactivating disease-associated mutations of Aptx are largely confined to the HIT domain (10). Other domains of Aprataxin appear to play important, though non-critical roles, in protein association, substrate recognition, and catalytic activity. First, the N-terminal FHA domain is necessary for interaction with XRCC1 and XRCC4, but not for hydrolase activity (11). Second, we found that mutation of the C-terminal zinc finger motif led to reduced AMP-DNA hydrolase activity, and this defect was shown to be due to inefficient binding of the DNA adenylates. Consistent with these results, disease-associated Aptx truncations that delete the C-terminal domain exhibit an AOA1 phenotype, although this is somewhat milder than that observed with mutations in the HIT domain (3). We suggest that the Aprataxin zinc finger domain provides stabilizing contacts that lock the enzyme onto a high affinity AMP-DNA target site.

It is known that abortive ligation reactions at dirty DNA breaks produced by reactive oxidative species lead to the accumulation of adenylated DNA intermediates in the absence of Aptx activity (8). Here, we show that abortive ligation intermediates that might arise during base excision repair can also serve as substrates for Aptx. Abasic nicks with 3′ OH and 5′ deoxyribosephosphate termini are created by the action of monofunctional DNA glycosylases, followed by AP endonuclease 1 incision at the 5′-side of the excised base (14). We found that while abasic nicks can be ligated by DNA ligase III-XRCC1, a significant fraction were converted into adenylated abasic nicks through abortive ligation. Aptx was able to resolve these adenylated abasic nicks, whereas the dRP lyase activity DNA polymerase β could not. Furthermore, we were unable to detect any AMP hydrolysis activity in Aptx-deficient cell extracts. Thus, Aptx may be important to channel abortive ligation intermediates back into the scheduled repair mechanism in which DNA

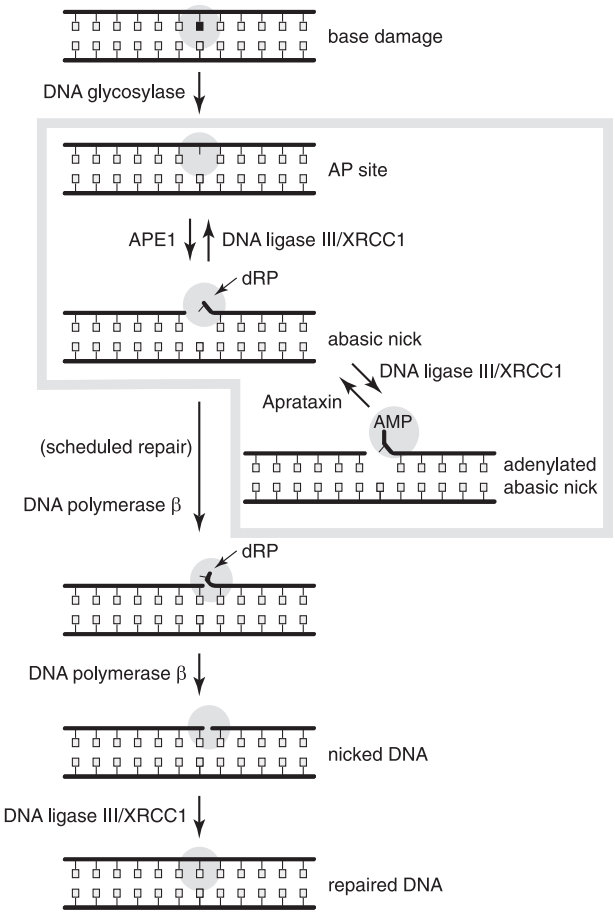

FIGURE 6. **Schematic diagram of base excision repair and role of Aprataxin.** The damaged base (*black*) is removed by a monofunctional glycosylase to produce an abasic site (*AP site*). AP endonuclease 1 incises at the 5′-side of the AP site to generate an abasic nick with a deoxyribosephosphate residue (marked *dRP*) at the 5′ terminus. Untimely ligation reactions may be successful or could be abortive, leading to the formation of an adenylated abasic nick. Aprataxin can efficiently remove the adenylate to restore the abasic nick and allow subsequent re-entry into the BER pathway.

polymerase β will promote gap filling and 5′-dRP removal. Finally, DNA ligase III-XRCC1 will seal the processed nick (Fig. 6). The fact that Aptx-deficient cell lines have been shown to be sensitive to MMS (6), which leads to the formation of lesions that are acted upon by the monofunctional DNA glycosylase MPG, lends support to an involvement in BER.

The repair pathway described above is referred to as "short patch repair", as it leads to a repair patch of a single nucleotide and is the preferred mechanism of BER. Alternatively, repair may involve "long patch repair" mechanisms in which DNA polymerases δ or ϵ take over from polymerase β at the gap-filling step and continue DNA synthesis, thereby displacing the strand carrying the dRP residue (14, 16). In this subpathway, a 5′-flap is created and subsequently removed by the structure-specific endonuclease FEN1. Long patch repair mechanisms may be utilized to remove any blocking 5′-adenylates, and provide an alternative to Aptx actions. However, long patch repair is thought to be active mainly during S-phase (16–18) and involves proliferating cell nuclear antigen, DNA polymerases δ and ϵ, DNA ligase I, and FEN1, all of which function during DNA replication. This cell cycle bias could be significant with regard to AOA1, as long patch repair may not be readily available in non-proliferating neuronal cells. The dependence on Aptx may be further exacerbated by the high rate of oxygen metabolism in the brain, given that reactive oxygen species contribute to base damages that can lead to the formation of abasic nicks (19). It is therefore likely that in the absence of Aptx the additional accumulation of DNA adenylates at incised AP sites will contribute to the genotoxic load faced by the cell.

In conclusion, our results provide new insight into the substrate specificity of Aptx. We have shown that DNA adenylates in duplex DNA are the primary substrates for Aptx and that the C-terminal zinc finger is a key determinant for the binding of adenylated DNA. Furthermore, our biochemical studies lead us to suggest that Aptx activity may provide a proofreading mechanism during ligation that is utilized by several distinct DNA repair pathways, including single-strand break repair, double-strand break repair, and BER.

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Actions of Aprataxin in Multiple DNA Repair Pathways

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