The adenine phosphoribosyltransferase (APRTase) from *Giardia lamblia* was co-crystallized with 9-deazaadenine and sulfate or with 9-deazaadenine and Mg-phosphoribosylpyrophosphate. The complexes were solved and refined to 1.85 and 1.95 Å resolution. *Giardia* APRTase is a symmetric homodimer with the monomers built around Rossmann fold cores, an element common to all known purine phosphoribosyltransferases. The catalytic sites are capped with a small hoo d domain that is unique to the APRTases. These structures reveal several features relevant to the catalytic function of APRTase: 1) a non-proline cis peptide bond (Glu100–Ser101) is required to form the pyrophosphate binding site in the APRTase9dA:MgPRPP complex but is a trans peptide bond in the absence of pyrophosphate group, as observed in the APRTase9dA:SO4 complex; 2) a catalytic site loop is closed and fully ordered in both complexes, with Glu100 from the catalytic loop acting as the acid/base for protonation/deprotonation of N-7 of the adenine ring; 3) the pyrophosphoryl charge is neutralized by a single Mg2+ ion and Arg83, in contrast to the hypoxanthine-guanine phosphoribosyltransferases, which use two Mg2+ ions; and 4) the nearest structural neighbors to APRTases are the orotate phosphoribosyltransferases, suggesting different paths of evolution for adenine relative to other purine PRTases. An overlap comparison of AMP and 9-deazaadenine plus Mg-PRPP at the catalytic sites of APRTases indicated that rotation coordinate motion involves a 2.1-Å excursion of the ribosyl anomic carbon, whereas the adenine ring and the 5-phosphoryl group remained fixed. *G. lamblia* APRTase therefore provides another example of nucleophilic displacement by electrophile migration.

Adenine phosphoribosyltransferase (APRTase) catalyzes the reversible Mg2+-dependent transformation of adenine and 5-phospho-o-o-ribosyl-1-pyrophosphate (PRPP) to AMP and pyrophosphate (Fig. 1; Ref. 1). The primary functions of APRTase, present in bacteria, yeast, plants, and mammals, are adenine salvage and adenine recycling (2–4). In humans, APRTase has the sole metabolic function of recycling adenine formed in the polyamine pathway. A deficiency of APRTase results in the inappropriate oxidation of adenine to 2,8-dihydroxadenine, an insoluble metabolite that causes urolithiasis and is capable of causing kidney failure after several decades of accumulation (5). The symptoms are variable and are reversible upon removal of 2,8-dihydroxadenine. The relatively mild effects of APRTase deficiency in humans make APRTase a possible target for the treatment of parasite infections by disrupting the pathways of adenine salvage and polyamine biosynthesis.

*Giardia lamblia* is a human and animal parasite that causes the most common protozoan infection in North America (6, 7). Protozoan parasites, including *G. lamblia*, are deficient in *de novo* purine synthesis and require purine uptake from the host, as well as efficient purine salvage. The stringent purine economy makes APRTase especially important in these organisms. *Giardia* expresses two distinct purine ribosyltransferases (PRTases), APRTase and GPRTase, for purine base salvage rather than the broad-specificity HGXPRTase found in the *Plasmodium* (8). GPRTase from *Giardia* prefers guanine as the purine substrate; its catalytic mechanism has been well studied by enzyme kinetics, mutagenesis, and x-ray crystallography (9–11). The deduced sequences of APRTases from genome sequences were summarized recently, but only two crystallographic studies have been reported, neither of which exhibit closed catalytic loops (12–14).

The first APRTase crystal structures were the enzyme from *Leishmania donovani* in complex with adenine, AMP, and citrate-sulfate ions (13). APRTase possesses a Rossmann fold core resembling other purine PRTases (15). It also contains a long catalytic loop that is proposed to close on the active site during catalysis. The catalytic site loop was found in the open conformation and has been well defined as a consequence of crystal packing forces in the *L. donovani* APRTase structure. The *L. donovani* enzyme is classified as a long-APRTase that has N- and C-terminal extensions of over 50 amino acids relative to the amino acid sequences of the short-APRTases, which include the human, yeast, *Escherichia coli*, and *Giardia* enzymes. The structure of the first short-APRTase structure was recently determined.
reported using the enzyme expressed by *Saccharomyces cerevisiae* (12). *Giardia* APRTase is also a short-APRTase and shares a 35% amino acid sequence identity with the enzyme from yeast. The goal of the present work was to establish the crystal structures of an APRTase with a closed catalytic site loop. In other PRPTases, this loop makes contact with substrates in the catalytic sites and is required for catalysis. Resolution of the loop contacts in the APRTases is required to understand the molecular basis for adenine specificity, the atomic excursions during catalysis, as well as to assist in inhibitor design. *Giardia* APRTase crystallized in the presence of 9-deaza-adenine (9dA) or 9dA/PRPP yielded APRTase-9dA/ASO4 and APRTase-9dA-MgPRPP complexes. Both complexes demonstrated ordered catalytic loops closed over the active site. One Mg2+ ion is bound to the pyrophosphate group of PRPP in contrast to the two Mg2+ ions observed in other purine PRPTases including human, *Trypanosoma cruzi* and *Toxoplasma gondii* HGPRPTases and *Plasmodium falciparum* HGXPRTase (16–19). The structures described here are the first APRTase structures to be reported with closed catalytic loops.

### RESULTS AND DISCUSSION

**Overall Fold**—The overall fold of *Giardia* APRTase is closely related to those of the yeast and *Leishmania donovani* APRTase structures (12, 13). The *Giardia* APRTase monomer is a compact single-domain structure composed of six α-helices and nine β-strands (Fig. 2a), which can be divided further into the hood (residues 1–34; α1, β1, β2, and α2 segments), and the core, consisting of the remaining α-helices and β-strands (residues 35–180). As in all type I PRPTases, the core subdomain is a Rossman fold structure consisting of a five-stranded parallel β-sheet (β4, residues 77–84; β3, 55–61; β7, 119–127; β8, 149–158; and β9, 173–180) and four α-helices (α3, residues 35–49; α4, 62–75; α5, 131–143; and α6, 163–169). The hood subdomain of *Giardia* APRTase is composed of two β-strands and two α-helices (β1, residues 12–16; β2, 23–27; α1, 5–11; and α2, 29–33) and is similar to the hoods found in the yeast (short) and *L. donovani* (long) APRTases. In all of these APRTases, the hoods are significantly smaller than those described in other type I PRPTases. The segment consisting of residues 91–109, including β5(91–98) and β6(105–109), forms the flexible catalytic loop that serves to sequester the active site from the solvent. The catalytic mechanisms in related PRPTases are thought to involve a transition state that resembles a highly reactive ribosoxacarbenium ion that must be protected from solvent to prevent hydrolytic reactions (11, 16, 19). The catalytic loops in both of the *Giardia* APRTase complexes, APRTase-9dA-ASO4 and APRTase-9dA-MgPRPP, are well ordered and in the closed conformation (Fig. 3a). Glu100 at the tip of the catalytic loop interacts with N-7 of 9dA (Fig. 4, a and b).

**Quaternary Structure**—*Giardia* APRTase is found as a homodimer in both crystal structures (Fig. 2b). The enzyme crystallized in the trigonal space group P3121 with a monomer in the asymmetric unit. Application of the crystallographic 2-fold symmetry operator generates a dimer that buries a total of 1149 Å2 of solvent (Fig. 3a). The dimer interface for *Giardia* APRTases includes α2–α3 and α4 of the first monomer with β4 and α4 of the second monomer (Fig. 2b). These interactions consist of 4 salt bridges, 20 neutral hydrogen bonds, and 190 van der Waals interactions (Table II). Of the 12 residues involved in hydrogen bonds at the dimer interface, only Asp27 and Arg83 are completely conserved throughout the known short-APRTases (12). The dimer interfaces in *Giardia* and yeast APRTases are similar. In contrast, the long-form *L. donovani* APRTase has an extra 30 amino acid residues at the C terminus that are wrapped around the neighboring monomer to contribute an additional 1250 Å2 buried surface area from each monomer (5300 Å2 total) (13). APRTase-9dA-ASO4 Complex—The adenine binding site is defined by the position of 9dA, which is bound in a cleft formed by the hood and by the loop connecting β8 and α6 in the large.
Diffraction data
Resolution range (Å) 25–1.85 (1.92–1.85)
Completeness (%) 99.1 (98.8)
R_integer (%) 2.7 (35.2)
I/σI 28.8 (2.9)
Number of reflections
Total 73,591
Unique 16,337
Structure refinement
R_cryst (%) 21.9
R_free (%) 26.2
Number of amino acids 181
Number of ligands 1
Number of solvent molecules 90
Average protein B-factor (Å²) 30.1
Average ligand B-factor (Å²) 44.8
Rms deviations from ideal
Bond (Å) 0.007
Angle (°) 1.348

Table I
Data collection and refinement statistics

|                  | ARTase·9dA·MgPRPP | APRTase·9dA·SO₄ |
|------------------|-------------------|-----------------|
| Resolution range (Å) | 25–1.95 (2.02–1.95) | 25–1.85 (1.92–1.85) |
| Completeness (%) | 99.5 (99.8) | 99.1 (98.8) |
| R_integer (%) | 3.7 (36.9) | 2.7 (35.2) |
| I/σI | 23.4 (3.0) | 28.8 (2.9) |
| Number of reflections | 70,505 | 73,591 |
| Unique | 14,082 | 16,337 |
| R_cryst (%) | 21.8 | 21.9 |
| R_free (%) | 26.2 | 26.1 |
| Number of amino acids | 179 | 181 |
| Number of ligands | 1 | 1 |
| Number of solvent molecules | 80 | 90 |
| Average protein B-factor (Å²) | 32.4 | 30.1 |
| Average ligand B-factor (Å²) | 39.4 | 44.8 |
| Rms deviations from ideal | 1.416 | 1.348 |

a Values in parentheses are for the highest resolution shell.

Fig. 2. *G. lamblia* APRTase structure. a, stereoview of *Giardia* APRTase monomer. The APRTase monomer consists of six α-helices and nine β-strands. The catalytic loop (residues 91–109, β3 and β6) is closed onto the active site in the *Giardia* APRTase structure. The catalytic site ligands, 9dA and PRPP, are shown in green and red, respectively. b, stereoview of the *Giardia* APRTase dimer. Monomers A and B are depicted in light green and purple. The dimer interface of *Giardia* APRTase includes α3–α6 and α4 of one molecule with β4 and α4 of the other molecule. Figs. 2–6 and 9 were generated using SETOR (35).

β-sheet central to the core (Fig. 4a). The 9dA ring is stacked between the hydrophobic side chains of Phe35 from the hood and Val126 from the core. The backbone carbonyl of Ala24 and the ε-amino of Lys34 from the hood form hydrogen bonds to the exocyclic N-6 amine and to N-1 of 9dA. A carboxyl oxygen of Glu100 from the catalytic loop forms a 2.7 Å hydrogen bond with N-7 of 9dA, a major contact between the substrate and the catalytic loop in its closed conformation. The reactions catalyzed by PRTases have been proposed to involve oxacarbenium transition states in which the purine leaving group is activated.
by protonation or strong hydrogen bond formation to N-7 (19, 25). In human HGPRTase, Glu\textsuperscript{100} is proposed to play the same role as Glu\textsuperscript{100} in \textit{Giardia} APRTase (16, 26). However, the amino acid sequence alignment between APRTases and HGPRTases reveals insufficient homology to predict this role for Glu\textsuperscript{100} in APRTase (12, 14). In the sequence alignment of
Ribosyl Migration in G. lamblia APRTase Closed Site Complexes

APRTase, this Glu is conserved in all enzymes with demonstrated APRTase activity (12, 14). Mutagenesis of this conserved Glu to Leu in yeast APRTase reduced $k_{\text{cat}}/K_m$ by a factor of 10\(^6\) and mutation from Glu to Gln decreased $k_{\text{cat}}/K_m$ by $10^3$, as a Gln residue at this position retains the ability for hydrogen bond formation but is not competent to act as an acid/base (12).

The initial crystallization of Giardia APRTase occurred in the presence of 0.2 M (NH\(_4\))\(_2\)SO\(_4\), resulting in a total of three sulfate ions in the APRTase-9dA-SO\(_4\) structure. One sulfate ion is bound at the site typically occupied by the 5'-phosphate of AMP or PRPP molecules (Fig. 4, a and b). This sulfate ion binds to the turn formed by residues 128-132, which is defined as the 5'-phosphate binding loop. The peptide backbone amide groups of these residues orient toward the sulfate and contribute four direct hydrogen bonds. The hydroxyl groups of Thr129 and Thr132 provide two additional hydrogen bonds to this sulfate ion, thus stabilizing the crystallization buffer with NH\(_4\)Ac allowed Mg\(^{2+}\) to occupy any site involved in substrate or product binding. It participates in hydrogen bonds with backbone and side-chain atoms of Glu\(^{61}\)-Ser\(^{62}\). Residues Glu\(^{61}\), Ser\(^{62}\), and Arg\(^{63}\) are highly conserved throughout all APRTases and are essential elements of the pyrophosphate binding site. In the Giardia APRTase-9dA-SO\(_4\) complex has Arg\(^{63}\) located near the sulfate ion site. However, when the catalytic site is full, Arg\(^{63}\) swings across the active site to bind pyrophosphate (Fig. 4, a and b; Fig. 5).

A second sulfate ion is bound near the active site but does not occupy any site involved in substrate or product binding. It participates in hydrogen bonds with backbone and side-chain atoms of Glu\(^{61}\)-Ser\(^{62}\). Residues Glu\(^{61}\), Ser\(^{62}\), and Arg\(^{63}\) are highly conserved throughout all APRTases and are essential elements of the pyrophosphate binding site. In the Giardia APRTase-9dA-SO\(_4\) complex, the peptide segment connecting Glu\(^{61}\) and Ser\(^{62}\) is in trans conformation in contrast to a cis conformation found in the APRTase-9dA-MgPRPP complex (Fig. 5). The equivalent peptide bond in related HGPRTases has been proposed to shift from trans to cis conformation to bind the β-phosphoryl moiety in phosphoribosyltransferase reactions; however, both trans and cis conformations have been observed in high resolution crystal structures of type I PRTases when the pyrophosphate binding site is empty (11, 12, 18). The sulfate ion in the Giardia APRTase-9dA-SO\(_4\) complex is bound in a location opposite to the β-phosphoryl binding site, stabilizing the Glu\(^{61}\)-Ser\(^{62}\) peptide segment in a trans conformation and preventing formation of the pyrophosphate binding site.

The third sulfate ion is bound near the C terminus and forms hydrogen bonds with two of the histidine residues in the Histag. This sulfate ion is a result of the His tag expression system and is not relevant to catalysis; however, it may have facilitated the crystallization of the enzyme.

**Table II**

| Atom 1\(^a\) | Atom 2\(^b\) | Distance A |
|---|---|---|
| **Salt bridge** | | |
| Asp\(^{61}\), OD2 | Arg\(^{112}\), NH2 | 2.9 |
| Asp\(^{61}\), OD1 | Arg\(^{112}\), NE | 3.0 |
| **H-bond** | | |
| Asp\(^{61}\), OD2 | Gln\(^{110}\), OE1 | 3.2 |
| Ser\(^{29}\), OG1 | Gln\(^{110}\), NE2 | 2.8 |
| Ser\(^{29}\), OG1 | Gln\(^{110}\), NE2 | 2.9 |
| Ser\(^{29}\), OG1 | Arg\(^{112}\), NH2 | 3.1 |
| Asp\(^{61}\), OD1 | Gln\(^{110}\), NE2 | 2.8 |
| Leu\(^{24}\), O | Phe\(^{73}\), N | 2.7 |
| Ser\(^{62}\), OG | Gln\(^{110}\), OE1 | 2.8 |
| Asp\(^{61}\), OD1 | Asn\(^{73}\), ND2 | 2.9 |
| Arg\(^{63}\), NH1 | Asn\(^{73}\), OD1 | 2.7 |
| Ser\(^{62}\), OG | Arg\(^{63}\), NH2 | 3.1 |

\(^a\) From molecule A or B.  
\(^b\) From molecule B or A.

**Fig. 5.** Peptide conformation of Glu\(^{61}\)-Ser\(^{62}\). **a,** stereoview of trans conformation of Glu\(^{61}\)-Ser\(^{62}\) in the Giardia APRTase-9dA-SO\(_4\) complex. The peptide segment connecting Glu\(^{61}\) and Ser\(^{62}\) is stabilized in the trans conformation by a sulfate ion. **b,** stereoview of the cis conformation of the same peptide in APRTase-9dA-MgPRPP complex. The unusual cis conformation in the peptide link between Glu\(^{61}\) and Ser\(^{62}\) orients the amide nitrogen atoms of Ser\(^{62}\) and Arg\(^{63}\) to bind PRPP.

APRTase-9dA-MgPRPP Complex—Replacing (NH\(_4\))\(_2\)SO\(_4\) in the crystallization buffer with NH\(_4\)Ac allowed Mg\(^{2+}\) and PRPP to fill the active site in the APRTase-9dA-MgPRPP complex (Fig. 3b). The overall structures of APRTase in the two complexes are very similar, with root-mean-square deviations of 0.35 Å for all Ca atoms. Binding of 9dA is in a similar hydrophobic and hydrogen bond network as observed in the APRTase-9dA-SO\(_4\) complex (Fig. 4, a and b). Beneath the 9dA, PRPP binds in the active site with C-1 of the ribose ring 3.4 Å away from C-9 of 9dA. The monophosphate group of PRPP is bound in the highly conserved 5-phosphate binding loop. The O-2 and O-3 hydroxyl oxygens of ribose are anchored by hydrogen bonds (2.6 and 2.7 Å) to carboxyl groups of Glu\(^{124}\) and Asp\(^{125}\), the two highly conserved acidic residues found at the catalytic sites of all known purine PRTases. The Mg\(^{2+}\) ion is octahedral-coordinated, making contact with O-2 and O-3 (both at 2.5 Å), O-2A and O-3B (2.3 and 2.2 Å, respectively) of PRPP, and two water molecules (both at 2.1 Å; Fig. 6). The Mg\(^{2+}\) ion orients the ribose ring and neutralizes part of the negative charge from the pyrophosphate moiety. Both APRTase and OPRTase utilize a single Mg\(^{2+}\) ion at the catalytic site, whereas other purine PRTases sandwich the pyrophosphate group between two Mg\(^{2+}\) ions (Fig. 4, a and b). OPRTase and APRTase solve the problem of pyrophosphate charge stabilization by replacing the second Mg\(^{2+}\) ion with Arg or Lys groups.

Residues Glu\(^{61}\), Ser\(^{62}\), and Arg\(^{63}\) provide the major amino acid contacts in the pyrophosphate binding site. Peptide Glu\(^{61}\)-
Ser62 exhibits the unusual cis conformation that orients the amide nitrogen atoms of Ser62 and Arg63 to participate in hydrogen bonds with the β-phosphoryl of PRPP (2.9 and 3.0 Å, respectively). The side chain of Arg63 is also involved in hydrogen bonds with the β-phosphoryl and the N-3 of 9dA (3.0 and 2.8 Å, respectively). Arg63 is completely conserved throughout all APRTases, and mutation of this Arg residue to Ala in yeast APRTase resulted in a 105-fold decrease in cat/Km (12). Tyr101 from the catalytic loop hydrogen bonds to the α-phosphoryl group of PRPP and is also completely conserved throughout APRTases. Mutation of the corresponding Tyr to Phe in the yeast enzyme reduced the cat/Km by 10-fold (12). The hydrogen bond network at the catalytic site of the Giardia APRTase-9dA-MgPRPP complex indicates at least one hydrogen bond or metal-chelate interaction at every donor/acceptor pair on the ligands, except for O-3A (Fig. 7).

Comparison of Giardia APRTase with Other APRTases—
Giardia APRTase shares 33–35% sequence identity with the APRTases from S. cerevisiae and L. donovani. The overall structures are similar, with root-mean-square deviations of 1.3 and 1.7 Å, respectively, over the conserved 163 Cα atoms with the catalytic loop excluded from the comparison. The present structure of Giardia APRTase departs from both the yeast and L. donovani structures reported previously by the closed catalytic site loop and from the L. donovani structure by the absence of the C-terminal extension (12, 13). Despite the missing residues at the C terminus, Giardia APRTase still forms an extensive dimer interface.

The structure of L. donovani APRTase was solved with AMP and citrate ion (a component in the crystallization buffer) in the active site (Fig. 4c). The location of the adenine group of AMP is similar to that of 9dA observed in Giardia APRTase, which stacks Phe27 and Val148 corresponding to Phe42 and Val148 in the L. donovani enzyme. The 5′-phosphate binding pocket is highly conserved for all type I PRTases, and the complexes of Giardia ARTase-9dA-MgPRPP and L. donovani APRTase-AMP-cit provide no exception, except for the hydrogen bond from the amide Glu100 (Glu107 in L. donovani), which does not form in the L. donovani complex because of the open catalytic loop.

The ribose groups of AMP and PRPP in the APRTase complexes show substantial differences in sugar pucker near C-1. The C-1 ribosyl atoms are positioned -2.1 Å apart in the superimposed structures, consistent with the enzymatic mechanism of nucleophilic substitution by electrophile migration that has been recognized for several sugar transferases (27–29). The PRRTase reactions are nucleophilic substitutions in which both attacking and leaving group nucleophiles are held tightly in their respective binding pockets, and the C-1 carbon

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**Fig. 6. Mg⁡²⁺ binding sites in APRTase and HGPRTase.**

*a.* Stereoview of the Mg⁡²⁺ binding site in Giardia APRTase. Giardia APRTase utilizes a single Mg⁡²⁺ ion, and the side chain of Arg⁡⁶⁵ occupies the second site observed in HGPRTases. *b.* Stereoview of the two Mg⁡²⁺ ion binding sites in malarial HGPRTase (19) (PDB ID code 1cjb). Immaculina HP (ImmHP) (LS)-1-(9-deazahypoxanthin-9-y)-1,4-dideoxy-1,4-imino-d-ribitol-5-phosphate) is a transition state analogue for the HGPRTase reaction and a nanomolar inhibitor for malarial HGXPRTase. Mg⁡²⁺ ions are shown in light blue and the water molecules in red.

**Fig. 7. Hydrogen bond network in Giardia APRTase-9dA-MgPRPP complex.** Distances (<3.2 Å) are in angstroms.

**Fig. 8. Surface-exposed catalytic site ligands and disposition of the catalytic loop in complexes of APRTases.** The closed catalytic site complex of Giardia APRTase with 9dA (in red) and PRPP (in blue) is shown in the left panel with the catalytic site loop (yellow) in the closed position. None of the atomic surface of 9dA is visible, indicating complete burial at the catalytic site. The yeast APRTase (right panel) shows a molecular model of the same complex but with the catalytic site loop in the open position. Note the solvent exposure of 9dA in this complex. This figure was generated using GRASP (36).
of AMP translocates between the relatively immobile nucleophiles during catalysis (27, 28). This distance is also 2.1 Å in the HGPRTases, and the 2.1 Å distance observed between the C-1 atom of PRPP and C-1' of AMP provides supporting evidence that *Giardia* APRTase uses this same catalytic strategy (see below).

The electron density assigned as a Mg$^{2+}$ ion and reported in the *L. donovani* APRTase-AMP-cit complex is ~1.5 Å distant from that observed here for *Giardia* APRTase-9dA-MgPRPP. However, this atomic assignment is questionable for the *L. donovani* complex because the proposed Mg$^{2+}$ ion has only three ligands in the coordination sphere, and these are all at 2.7–2.9 Å (13). Magnesium ions in crystallographic complexes typically demonstrate six coordinated ligands at distances from 2.0–2.5 Å (30), suggesting that the electron density assigned as a Mg$^{2+}$ ion in the *L. donovani* complex may represent a solvent molecule. Despite the molecular differences between citrate and pyrophosphate ions, the amino acids that form the pyrophosphate binding sites are in similar positions in the *Giardia* and *L. donovani* complexes with bound IPP and citrate, respectively.

The closed-loop structure at the active site of *Giardia* APRTase-9dA-MgPRPP demonstrates closer contacts between the protein and catalytic site contents than those observed in the *L. donovani* APRTase-AMP-cit complex (Fig. 8). This loop motion moves the tip of the loop (Glu100) by >10 Å to exclude solvent access to the otherwise exposed adenine in its binding site and also to cover a portion of the PRPP site. Motion of this loop is required for entry and release of components to the catalytic site. It is proposed that catalysis occurs only in the closed-loop complex because of the contacts to substrate. N-7 deprotonation/protonation of the adenine is an important feature of APRTase catalysis and the 2.7 Å carboxyl group of Glu100 interaction to N-7 of the 9dA is seen only in the closed-loop complex. This interaction provides the general acid/base catalyst, and in the other purine PRTases, forms a novel downfield hydrogen bond in complexes with transition state analogues (19, 31). As discussed above, the E100L mutation in the yeast APRTase reduced catalytic efficiency to $10^{-6}$ of the parental enzyme.

**Comparison of APRTase with OPRTase**—Despite the closely related chemistry found in other purine PRTases, APRTase shows no near relatives in sequence homology except for the phosphate binding loop regions. The closest homologue to APRTase is OPRTase, found in the type I PRTase family. *Giardia* APRTase shares 17% sequence identity with *Salmonella* OPRTase, and the core structures of these enzymes are similar, with a root-mean-square deviation of 3.1 Å between *Giardia* APRTase and *Salmonella* OPRTase for 148 Ca atoms (32). As might be expected for the difference between purine and pyrimidine substrate specificities, the catalytic hoods that determine substrate specificity differ in APRTase and OPRTase. The hood in OPRTase includes two α-helices from the C terminus (residues 1–36, 184–213: α1, 2–15; β1, 17–25; β2, 29–36; α6, 184–192; α7, 197–212) and is twice as large as that observed in APRTase (residues 1–34: α1, 5–11; β1, 12–16; β2, 23–27; α2, 29–33).

The dimer interfaces of APRTase and OPRTase are also similar but differ from those of other members in the type I PRTases. However, the catalytic site loops of APRTase and OPRTase differ in that the catalytic loop in *Salmonella* OPRTase closes onto the active site of the adjacent monomer, whereas the catalytic loop in APRTase closes onto a self-contained active site (32). *Giardia* APRTase crystallized to give a monomer in the asymmetric unit, thus the two active sites in the dimeric structure are related by a crystallographic 2-fold axis and are identical to each other. The recently reported *Salmonella* OPRTase-ORotate-MgPRPP complex showed asymmetric properties between monomers in its homodimer (32). One active site is fully occupied and is closed with the catalytic loop from the adjacent monomer. The other active site is open and disordered, suggesting a single-site binding sequential catalysis mechanism for OPRTase. The catalytic sites of APRTase are identical and equally filled. The structural simi-
larity between OPRTase and APRTase suggests that these enzymes share a common evolutionary progenitor, which is distinct from other PRTases.

**Reaction Coordinate Motion in APRTases**—The availability of APRTase structures with bound substrate and product analogs (compare Fig. 4, panels b and c), permits comparison of these molecules in relation to portions of the proteins that are constant through the reaction. This approach has been used previously for the N-ribosyltransferases: purine nucleoside phosphorylase and hypoxanthine-guanine phosphoribosyltransferase (27, 28). When the AMP from the catalytic site of *L. donovani* is overlaid with 9dA and PRPP in Giardia, it is apparent that the major atomic motion involves translation of the C-1 portion of the ribosyl ring, whereas the purine base, 5-monophosphate, and the pyrophosphate remain relatively fixed (Fig. 9). The 2.1-Å excursion of C-1 of the ribosyl groups is within the range of 1.5 to 2.1 Å observed in the other N-ribosyltransferases (28). Thus, the APRTases represent another example in which nucleophile displacements occur with inversion of configuration by migration of the electrophile between two fixed nucleophiles.

**Summary and Conclusions**—The structures of Giardia APRTase-9dA-SO₄ and APRTase-9dA-MgPRPP are the first APRTase complexes with a closed flexible catalytic site loop. Glu¹⁰⁰ at the tip of the catalytic loop has been identified as an acid/base for catalysis. Similar to OPRTase, APRTase utilizes only one Mg²⁺ ion in the active site with Arg⁹⁰ replacing the second Mg²⁺ found in HGPRTases. A comparison of APRTases with substrate and product analog complexes in the catalytic sites supports a reaction mechanism of ribosyl group migration between relatively fixed adenine and Mg²⁺-pyrophosphate groups. This mechanism has been recognized in other N-ribosyltransferases.

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