Crystal structure of *Escherichia coli* purine nucleoside phosphorylase complexed with acyclovir

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Escherichia coli purine nucleoside phosphorylase (PNP), which catalyzes the reversible phosphorolysis of purine ribonucleosides, belongs to the family I hexameric PNPs. Owing to their key role in the purine salvage pathway, PNPs are attractive targets for drug design against some pathogens. Acyclovir (ACV) is an acyclic derivative of the PNP substrate guanosine and is used as an antiviral drug for the treatment of some human viral infections. The crystalline complex of *E. coli* PNP with acyclovir was prepared by co-crystallization in microgravity using counter-diffusion through a gel layer in a capillary. The structure of the *E. coli* PNP–ACV complex was solved at 2.32 Å resolution using the molecular-replacement method. The ACV molecule is observed in two conformations and sulfate ions were located in both the nucleoside-binding and phosphate-binding pockets of the enzyme. A comparison with the complexes of other hexameric and trimeric PNPs with ACV shows the similarity in acyclovir binding by these enzymes.

1. Introduction

Escherichia coli purine nucleoside phosphorylase (PNP) belongs to the family I purine nucleoside phosphorylases (EC 2.4.2.1) and catalyzes the reversible phosphorolysis of the glycosidic bond in purine nucleosides and 2'-deoxypurine nucleosides to yield the free base and ribose 1-phosphate (Pugmire & Ealick, 2002). PNPs of family I share a single-domain subunit with a common topology but differ in amino-acid sequence, oligomeric state and specificity (Mao *et al.*, 1997). Similar to the majority of prokaryotic enzymes, *E. coli* PNP is hexameric in its biologically active form and is characterized by a broad range of substrate specificity (Jensen & Nygaard, 1975; Jensen, 1978). It is able to cleave the glycosidic bonds in 6-oxopurine and 6-aminopurine ribonucleosides and to a lesser extent in purine arabinosides. The other members of family I, mammalian PNPs, have a trimeric structure and are specific for 6-oxopurine ribonucleosides only. In some microorganisms, such as *E. coli* and *Bacillus subtilis*, both trimeric and hexameric forms of PNP are found (Seeger *et al.*, 1995; Senesi *et al.*, 1976; Hori *et al.*, 1989). An alignment of the amino-acid sequences of several representatives of both types of PNP is shown in Fig. 1. PNPs are able to catalyse transglycosylation, *i.e.* the transfer of a carbohydrate moiety from one purine base to another (Canduri *et al.*, 2004; Galmarini, 2006). Owing to this, PNPs are used in biotechnology for the combined chemical/enzymatic synthesis of analogues of natural nucleosides, many of which are effective antiviral and

anticancer drugs (Krenitsky *et al.*, 1981; Utagawa *et al.*, 1985; Hennen & Wong, 1989; Mikhailopulo & Miroshnikov, 2010, 2011). Transglycosylation is a key reaction in the salvage pathway of nucleoside biosynthesis (Stoeckler, 1984; Montgomery, 1993). The salvage pathway represents an essential cellular process that is crucial for many organisms in which the *de novo* synthesis of purine is not available. The difference in specificity between PNPs from different organisms enables the targeting of PNPs for therapeutic purposes (Takebayashi *et al.*, 1995). PNPs from microorganisms can activate a number of prodrugs, converting them to their toxic forms in mammalian organisms (Bennett, Anand *et al.*, 2003). Potent inhibitors of *E. coli* PNP are promising candidates for tumour-directed gene therapy (Bennett, Anand *et al.*, 2003).

The activation of prodrugs by *E. coli* PNP provides a method for the selective killing of tumour cells (Stoeckler, 1984; Daddona *et al.*, 1986; Montgomery, 1993). A genetic deficiency in PNP in humans leads to profound T-cell-mediated immunosuppression. PNP inhibitors are used to reduce the immune response in the transplantation of organs and tissues or during chemotherapy (Morris *et al.*, 2000; Farutin *et al.*, 1999). Therefore, PNPs remain attractive targets for inhibitor development (Wielgus-Kutrowska *et al.*, 2007). Potent inhibitors of PNP are frequently structural analogues of nucleoside substrates that have been modified in the base or pentose component (Wielgus-Kutrowska *et al.*, 2007). One of these inhibitors that can be used as a lead compound for structure-based drug design is acyclovir [9-(2'-hydroxyethoxymethyl)-

Figure 1
Sequence alignment of *E. coli*, *B. subtilis* and human PNPs. This figure was created using *ESPrpt* (Gouet *et al.*, 2003).

Table 1
Macromolecule-production information.

Source organism	<i>E. coli</i> BL21(DE3)/pERPUPHOI
DNA source	<i>E. coli</i> BL21(DE3)/pERPUPHOI
PNP-forward primer†	5'-GGTGGTCCATGGCGGACCGCCCTC TTGATCTTC-3'
PNP-reverse primer‡	5'-GGTGGTCTCGAGTGTGAAGAGGCTGGA CACCGAC-3'
Cloning vector	pET-23d+
Expression vector	pET-23d+
Expression host	<i>E. coli</i> BL21(DE3)/pERPUPHOI
Complete amino-acid sequence of the construct produced	MATPHINAEMGDFADVLMPGDPLRAKYIA ETFLEDAREVNNVRGMLGFTGTGTYGKRKI SVMGHGMGIPSCSIYTKELITDFGVKKI IRVGSCGAVLPHVKLRDVIIGMGACTDS KVNRIKFDDHFAAIAFDVMVRNAVDA KALGIDARVGNLFSADLFYSPDGEMFDV MEKYGILGVEMEAAGIYGVAEFGAKAL TICTVSDHIRTHERQTTAERQTTF NDMIKIALESVLLGDK

† The NcoI site is underlined. ‡ The XhoI site is underlined.

guanine; ACV]. Acyclovir is an acyclic analogue of 2'-deoxyguanosine and is used as an antiviral drug to treat herpes virus infections. It has a moderate inhibitory effect on human PNP (Tuttle & Krenitsky, 1984). The structure of the complex with ACV is known for human PNP (dos Santos *et al.*, 2003), *Mycobacterium tuberculosis* PNP (Caceres *et al.*, 2012) and *B. subtilis* PNP (de Giuseppe *et al.*, 2012). Here, we present the three-dimensional structure of the *E. coli* PNP-ACV complex and compare this structure with the previously determined structures of PNP complexes.

2. Materials and methods

2.1. Macromolecule production

The *deoD* gene (GenBank accession No. M609170) encoding PNP was amplified by PCR using the synthetic primers PNP-forward and PNP-reverse (Table 1). The PCR product was purified, digested using the NcoI and XhoI restriction endonucleases and ligated with expression vector pET-23d+ (Novagen) treated with the same endonucleases. The resulting recombinant was designated pETPPHOI (Esipov *et al.*, 2002).

E. coli PNP was produced using the producer strain *E. coli* BL21(DE3)/pERPUPHOI. The producer strain was cultured in YT medium supplemented with ampicillin (100 µg ml⁻¹) at 37°C until an absorbance (*A*₅₉₅) of 0.8 was achieved. Gene expression was induced with 0.4 mM isopropyl β-D-1-thiogalactopyranoside and the culture was grown for a further 4 h at 37°C. The cells were separated by centrifugation at 5180g. The biomass was resuspended in lysis buffer (20 mM Tris-HCl pH 7.5, 5 mM EDTA) and disintegrated by ultrasound. The homogenate was centrifuged at 21 044g. The supernatant was fractionated on a Q Sepharose XL column pre-equilibrated with buffer consisting of 20 mM Tris-HCl pH 7.7, 2 mM EDTA using a NaCl gradient from 0 to 0.5 M. The pooled fractions were salted out and purified on a Q Sepharose HP column pre-equilibrated with buffer consisting of 20 mM Tris-HCl pH 7.5, 2 mM EDTA using a NaCl gradient from 0 to

Table 2
Crystallization.

Method	Liquid diffusion
Plate type	Capillary
Temperature (K)	294
Protein concentration (mg ml ⁻¹)	32
Buffer composition of protein solution	0.02 M Tris-HCl pH 7.5
Composition of reservoir solution	25% ammonium sulfate in 0.05 M sodium citrate buffer pH 4.9, 0.04% sodium azide, 5 mM acycloguanosine
Volume of drop (µl)	7
Volume of reservoir (µl)	180

0.3 M. The eluate was concentrated by ultrafiltration on a YM30 regenerated cellulose membrane (Millipore). The recombinant protein was purified on a HiLoad 16/60 column with Superdex 200 sorbent (GE Healthcare) equilibrated with buffer consisting of 20 mM Tris-HCl pH 7.7, 100 mM sodium chloride, 0.04% sodium azide. The protein was concentrated to 32 mg ml⁻¹ by ultrafiltration and stored at -80°C.

2.2. Crystallization

The crystallization conditions described previously for apo PNP (Timofeev *et al.*, 2016) were modified and optimized for co-crystallization of the enzyme with acyclovir. The crystals used for X-ray data collection were grown in microgravity by the counter-diffusion technique through a gel layer (Tanaka *et al.*, 2004). The experiments were performed as described in Kuranova *et al.* (2011). Protein solution (7 µl) was placed into a glass capillary 0.5 mm in diameter. One end of the capillary was hermetically sealed and the other end was plugged with a 0.5 mm silicone tube filled with 1% agarose gel. The silicone tube was dipped into a cylinder containing the reservoir solution. In the experiments employing the capillary counter-diffusion method, the protein and precipitant concentrations and the length of the silicone tube containing agarose gel were varied. Single crystals were obtained using a protein solution at a concentration of 32 mg ml⁻¹ in 0.02 M Tris-HCl buffer pH 7.5 containing 0.1 M sodium chloride, 0.04% sodium azide and 5 mM acycloguanosine. The precipitant solution consisted of 25% ammonium sulfate in 0.05 M sodium citrate buffer pH 4.9, 0.04% sodium azide and 5 mM acycloguanosine. Crystallization information is summarized in Table 2.

2.3. Data collection and processing

Diffraction data sets were collected at the BL41XU station of the SPring-8 synchrotron, Japan at a temperature of 100 K. A Dectris PILATUS3 6M device was used as a detector. The diffraction data were obtained by rotation using a single crystal. The wavelength was 0.8 Å, the crystal-to-detector distance was 100 mm, the oscillation angle was 0.5° and the angle of rotation was 180°. The experimental intensities were processed using *iMosflm* (Battye *et al.*, 2011) to 2.32 Å resolution. Data-collection and processing statistics are summarized in Table 3.

Table 3

Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	BL41XU, SPring-8
Wavelength (Å)	0.8
Temperature (K)	100
Detector	PILATUS3 6M
Crystal-to-detector distance (mm)	100
Rotation range per image (°)	0.5
Total rotation range (°)	180
Exposure time per image (s)	0.1
Space group	<i>P</i> ₆ 122
<i>a</i> , <i>b</i> , <i>c</i> (Å)	120.03, 120.03, 238.14
α , β , γ (°)	90, 90, 120
Mosaicity (°)	0.34
Resolution range (Å)	30.0–2.32 (2.45–2.32)
No. of unique reflections	44040
Completeness (%)	98.66 (93.72)
Multiplicity	5.55 (3.68)
$\langle I/\sigma(I) \rangle$	7.0040 (3.45)
<i>R</i> _{int}	0.107 (0.328)
Overall <i>B</i> factor from Wilson plot (Å ²)	27.4

Table 4

Structure solution and refinement.

Values in parentheses are for the outer shell.

Resolution range (Å)	29.95–2.32 (2.38–2.32)
Completeness (%)	98.5
No. of reflections, working set	41823 (2759)
No. of reflections, test set	2152 (127)
Final <i>R</i> _{cryst}	0.176 (0.227)
Final <i>R</i> _{free}	0.217 (0.277)
No. of non-H atoms	
Protein	5391
Ion	25
Ligand	48
Water	134
Total	5598
R.m.s. deviations	
Bonds (Å)	0.010
Angles (°)	1.495
Average <i>B</i> factors (Å ²)	
Protein	33.2
Ion	41.2
Ligand	69.2
Water	26.7
Ramachandran plot	
Most favoured (%)	98
Allowed (%)	2

2.4. Structure solution and refinement

The structure was solved by the molecular-replacement method using *Phaser* (McCoy *et al.*, 2007) with the coordinates of *E. coli* PNP at 0.99 Å resolution (PDB entry 4rg2) as a search model. Structure refinement was carried out using *REFMAC5* (Murshudov *et al.*, 2011). Manual rebuilding of the model was performed using the *Coot* interactive graphics program (Emsley *et al.*, 2010), and electron-density maps were calculated with $2|F_o| - |F_c|$ and $|F_o| - |F_c|$ coefficients. In the electron-density map calculated with $|F_o| - |F_c|$ coefficients at the 2.0σ level, clear electron density was observed in the active site of each subunit and could be interpreted as an acyclovir molecule. Clear electron densities for sulfate ions, which are present at high concentration in crystallization solution, were also found (Fig. 2a). A number of water molecules were

located in the difference electron-density maps. The atomic coordinates of the PNP–ACV complex have been deposited in the Protein Data Bank as entry 5i3c. Refinement statistics are summarized in Table 4.

3. Results and discussion

The PNP crystals belonged to space group *P*₆122 and contained three subunits in the asymmetric unit. The hexameric PNP molecule that corresponds to the biologically active form of the enzyme was built by the application of symmetry elements x , $x - y - 1$, $-z + 1/6$ to the asymmetric unit. The hexameric molecule can be considered as a trimer of dimers, where each dimer contains two complete active sites (Fig. 2b). The polypeptide chain in the subunit has a typical fold for the type I PNP family (Mao *et al.*, 1997). Each monomer consists of a central core comprising mixed β -sheet surrounded by α -helices with an extended C-terminal α 7 helix.

The active sites are located within the deep cavity between the dimer subunits and comprise amino-acid residues from both subunits. The active-site cavity is predominantly lined by hydrophobic amino acids from strands β 5, β 7, β 8, β 9 and β 10 and helix α 7. The adjacent dimer subunit contributes the side chains of His4* and Arg43* to the active site.

Some regions in the polypeptide chain of the PNP–ACV complex are poorly defined by electron density and are characterized by high temperature factors. Upon the structural alignment of crystallographically independent subunits on C ^{α} atoms, the largest deviations are between the segments of the polypeptide chain that limit the active-site cavity and contain some invariant residues that are essential for activity, such as Asp204 and Arg217. A significant divergence is observed between amino-acid residues 214 and 217, which form the N-terminal part of helix α 7. It has been suggested that the distortion of helix α 7 accompanies transition of the enzyme active site from the open to the closed conformation (Koellner *et al.*, 2002).

The ACV molecule occupies the nucleoside-binding pocket in the active-site cavity, where it adopts two conformations (Fig. 3a). The purine bases are shifted by 20° relative to each other; however, they are both stabilized by the same hydrophobic interactions and hydrogen bonds. One side of the guanine ring is close to the hydrophobic wall of the pocket lined by the side chains of Val178 and Ile206 from strands β 9 and β 10. The opposite side of the guanine base interacts with the side chains of Met180 and Phe159. There is a stacking interaction between the guanine and Phe159 rings. Several direct and water-mediated hydrogen bonds exist between the O and N atoms of the guanine base and the surrounding amino acids. In subunit *A* the O6, N1 and N2 atoms of the guanine base form water-mediated hydrogen bonds to the main-chain O atoms of Leu158 and Phe159. One of these water molecules is incorporated into the conserved water cluster observed in family I PNPs (Bennett, Li *et al.*, 2003). The chain of water molecules extends from the amino group of the guanine base to amino-acid residues Asp112 and Asp157. It has been suggested that this chain of water molecules takes part in

proton translation during the reaction (Bennett, Li *et al.*, 2003). In subunits *A* and *C* the guanine base is distant from Asp204, which is essential according to Bennett, Li *et al.* (2003), allowing proton transfer and stabilizing the transition state during the reaction. In subunit *B* a direct hydrogen bond exists between the O6 and N7 atoms of the guanine moiety and the OD1 and OD2 atoms of Asp204. It is known that the aspartic acid residue in the nucleoside-binding pocket plays an important role in catalysis and is responsible for the substrate specificity of PNP. Its replacement by asparagine, as is typical

in trimeric PNPs, narrows the specificity (Ealick *et al.*, 1990; Erion *et al.*, 1997; Stoeckler *et al.*, 1997; Tomoike *et al.*, 2013).

The acyclic moiety in one conformation is stabilized by a hydrogen bond between the O3' atom and His4* NE2 from an adjacent subunit. The O3' atom in the second conformation forms a hydrogen bond to Glu181 OE1 and to an O atom of the adjacent sulfate ion. In other complexes of hexameric PNPs, sulfate ion, which is present in excess owing to the crystallization conditions, occupies a phosphate-binding site and forms hydrogen bonds to Ser90 OG, the main-chain N atom of Gly20 and the guanidine groups of Arg87 and of Arg43* from an adjacent subunit. The sulfate ion binds to the

Figure 2

(a, b) Acyclovir in the active site of the *E. coli* PNP subunit. The electron-density map was calculated without ligands with coefficients $2|F_o| - |F_c|$ and is contoured at 2σ for the two conformations of acyclovir. (c) A hexameric molecule of *E. coli* PNP with an acyclovir molecule and a sulfate ion in the active sites. These figures were created using PyMOL (<https://pymol.org/2/>).

Figure 3

(a) Active site of *E. coli* PNP with the acyclovir molecule bound in two conformations and a sulfate ion. One subunit is shown in green and the second subunit is shown in blue. Conformational changes of the apoenzyme upon binding acyclovir and sulfate ion. (b) The superposition on C^α atoms of apo PNP and PNP complexed with ACV and sulfate ion is shown. These figures were created using PyMOL (<https://pymol.org/2/>).

main-chain O atom of Asp21 through a water molecule, indicating that the ACV-binding and phosphate-binding sites are connected to each other.

The binding of ACV does not induce large conformational changes. A superposition of the trimers of apo PNP and PNP-ACV gives an average r.m.s.d. of 0.498 Å. The largest divergence between the structures is in the region of residues 206–219 (r.m.s.d. of 6.609 Å for Glu211 in subunit A) comprising the loop between strand β 10 and helix α 7 and the N-terminal part of helix α 7. These parts of the polypeptide chain restrict the active-site area and contain several invariant residues. Significant structural changes are observed in the N-terminal part of helix α 7. In the complex structure containing ACV this helix shortens by three amino acids, which results in breakage of the Arg217–Asp204 interaction

that is essential for activity (Fig. 3*b*). It has been shown that Arg217 should be closer to Asp204 in the catalytically active closed conformation (Koellner *et al.*, 2002). The side chain of Ser90 is shifted and its OG atom forms a hydrogen bond to an O atom of the sulfate ion.

The structure of the homologous hexameric PNP from *B. subtilis* complexed with ACV has been determined at 2 Å resolution (de Giuseppe *et al.*, 2012). The degree of sequence homology between the *B. subtilis* and *E. coli* PNPs (56% identity) is rather high. Superposition on C α atoms of both complexes with ACV shows that the mode of ACV binding is rather similar in these enzymes and that the majority of binding positions are occupied by homologous amino-acid residues (Fig. 4*a*). There is a difference in the number of water molecules around the guanine base of ACV. In the *E. coli*

Figure 4

Comparison of the active sites of several PNPs complexed with different guanosine derivatives. Acyclovir is shown (*a*) in the active sites of *E. coli* PNP (green) and *B. subtilis* PNP (pink) and (*b*) in the active sites of *E. coli* PNP (green) and human PNP (pink). (*c*) ACV in the active site of *E. coli* PNP (green) and 2'-deoxyguanosine bound in the active site of *B. subtilis* PNP (blue). (*d*) ACV (N9-acyloguanosine) in the active site of *E. coli* PNP (green) and N7-acyloguanosine in the active site of calf spleen PNP (grey). The corresponding structures are superimposed on the C α atoms of amino acids in the active site. These figures were created using PyMOL (<https://pymol.org/2/>).

PNP–ACV complex only two water molecules mediate the binding of the guanine moiety to the enzyme. In the *B. subtilis* PNP–ACV complex four water molecules mediate the binding of the guanine moiety and the fifth water molecule participates in the binding of the acyclic part to the enzyme. The O6 and N7 atoms of the guanine base in the *B. subtilis* complex form water-mediated hydrogen bonds to Asp203 (Asp204 in *E. coli*) and the N1 and N2 atoms form water-mediated hydrogen bonds to the main-chain O atom of Gln158 (Leu158 in *E. coli*). The O3' atom of the acyclic chain forms a hydrogen bond to the OE2 atom of Glu180 (Glu181 in *E. coli*). There is no hydrogen bond between Asp204 and the guanidine moiety in subunits A and C in the *E. coli* PNP–ACV complex. Thus, the region surrounding the ligand in *B. subtilis* PNP is less hydrophobic and the bound ligand is more solvent-accessible.

The structure of the complex with acyclovir is known for the trimeric PNPs from human (dos Santos *et al.*, 2003) and *M. tuberculosis* (Caceres *et al.*, 2012). As mentioned above, ACV is a moderate inhibitor of human PNP (Tuttle & Krenitsky, 1984). The degree of sequence homology between the hexameric and trimeric members of the family I PNPs is very low (11% identity between *E. coli* and human PNP; Devereux *et al.*, 1984). Nevertheless, these enzymes retain a similar overall fold and their secondary structures correlate well. Even though the overall topology of the active site of *E. coli* PNP is similar to that of human PNP, the subunit interactions are strikingly different. Structural alignment of the ACV complexes of human and *E. coli* PNP (PDB entries 1pwy and 5i3c, respectively) on secondary structure reveals that the arrangement of ACV in the active site and the overall pattern of enzyme–ACV interactions are similar in both enzymes despite the replacement of some amino-acid residues (Fig. 4b). In human PNP, Asn243 is analogous to the essential active-site residue Asp204 found in *E. coli* PNP that is essential for the activity and specificity of the enzyme (dos Santos *et al.*, 2003; Mahor *et al.*, 2016). The O6 and N7 atoms of acyclovir form direct bonds to OD1 and ND2 of Asn243 in the human enzyme, whereas in the *E. coli* enzyme the bonds between these atoms and Asp204 are absent (at least in two subunits). The N1 and N2 atoms of the guanine base in the human PNP–ACV complex structure are bound directly to the OE1 and OE2 atoms of Glu201. The interaction between the NH2 group of ACV and the carboxylic group of Glu201 can be considered to be an electrostatic interaction. There are differences between the structures and the numbers of water molecules in the region of the guanine base. The acyclic tail of ACV in human PNP has a different conformation to that observed in the *E. coli* enzyme and is stabilized by hydrophobic interactions with the main chains of Ala116 and Ala117 and the side chain of Phe200. These residues are structurally equivalent to Ser90, Cys91 and Phe159 in the *E. coli* enzyme.

Acyclovir is the acyclic analogue of the PNP substrate 2'-deoxyguanosine. The three-dimensional structure of the complex with 2'-deoxyguanosine is known for *B. subtilis* PNP (PDB entry 4da0; de Giuseppe *et al.*, 2012). Superposition of the structures of the *E. coli* PNP–ACV and *B. subtilis* PNP–

2'-deoxyguanosine complexes shows that the guanine base is accommodated in a similar manner in both complexes (Fig. 4c). The O5' atom of ribose moiety of 2'-deoxyguanosine is located in nearly the same position as the O3' atom of ACV in the first conformation, while the position of the O3' atom of ACV in the second conformation coincides with the O3' atom of 2'-deoxyguanosine (Fig. 4c). This match between the O3' atom of ACV and the O5' atom of ribose was also found in other PNP–ACV complexes (Ealick *et al.*, 1991; dos Santos *et al.*, 2003; de Giuseppe *et al.*, 2012).

It is known that acycloguanosine derivatives with an acyclic moiety bound to the N7 atom have a higher affinity for PNP than their N9 analogues (Bzowska *et al.*, 1994). The three-dimensional structure of a trimeric PNP with N7-acycloguanosine was solved at 2.15 Å resolution for calf spleen PNP (PDB entry 1fxu; Luić *et al.*, 2001). A comparison of the positions of ACV in the *E. coli* enzyme and of N7-acycloguanosine in calf spleen PNP was carried out by superimposition of the structures on six homologous active-site residues (Fig. 4d). These are Ser90, Glu92, Val178, Met180, Ser203 and Asp204 in *E. coli* PNP and the homologous residues Ala116, Glu118, Val217, Met219, Thr242 and Asn243 in calf spleen PNP. The guanine base of N7-acycloguanosine in calf spleen PNP has an inverted orientation of the purine base compared with the natural substrate (Luić *et al.*, 2001) and binds to the essential Asn243 (Asp204 in *E. coli* PNP) through the N2 and N3 atoms, whereas the N1 atom interacts with Glu201 and the N9 atom binds to the OG atom of Thr242 (Ser203 in *E. coli*). The number of direct interactions between the guanine base and the enzyme in the N7-acycloguanosine–PNP complex is greater than in the N9 analogue. This is consistent with the higher affinity of N7-acycloguanosine for PNP compared with the N9 derivative. It is remarkable that although the guanine base is bound in the inverted orientation, the acyclic moiety of N7-acycloguanosine occupies the same position as in the *E. coli* enzyme and is accommodated in the ribose-binding site in a similar way in both structures. This suggests that both factors, the higher affinity of the acyclic group of N7-acycloguanosine for the ribose-binding site and the stronger binding of the N7-substituted guanine base, promote inversion of the orientation of the base. A similar inversion of the pyrimidine base was observed in the binding of 3'-azidothymidine in the nucleoside-binding site of thymidine phosphorylase, where the presence of the azido group in the pyrimidine ring triggers the inversion (Timofeev *et al.*, 2014).

The data on the reorientation of the ligand in the active sites of nucleoside phosphorylases can be used for the development of new enzyme inhibitors.

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