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Crystal structure and molecular dynamics studies of human purine nucleoside phosphorylase complexed with 7-deazaguanine

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ABSTRACT

In humans, purine nucleoside phosphorylase (HsPNP) is responsible for degradation of deoxyguanosine, and genetic deficiency of this enzyme leads to profound T-cell mediated immunosuppression. HsPNP is a target for inhibitor development aiming at T-cell immune response modulation. Here we report the crystal structure of HsPNP in complex with 7-deazaguanine (HsPNP:7DG) at 2.75 Å. Molecular dynamics simulations were employed to assess the structural features of HsPNP in both free form and in complex with 7DG. Our results show that some regions, responsible for entrance and exit of substrate, present a conformational variability, which is dissected by dynamics simulation analysis. Enzymatic assays were also carried out and revealed that 7-deazaguanine presents a lower inhibitory activity against HsPNP (K_i = 200 μM). The present structure may be employed in both structure-based design of PNP inhibitors and in development of specific empirical scoring functions.

1. Introduction

Purine nucleoside phosphorylase (PNP) (EC 2.4.2.1) is a key enzyme of the purine salvage pathway, responsible for the reaction between (deoxy)nucleosides and bases, which can in turn be transformed to uric acid for excretion or reused in nucleic acid biosynthesis (Parks and Agarwal, 1972). This enzyme catalyzes the reversible cleavage, in the presence of inorganic phosphate (P_i), of *N*-glycosidic bonds of purine (deoxy)nucleosides, except adenosine, to produce alpha-ribose 1-phosphate and the equivalent purine base (Kalckar, 1947). The reaction proceeds with inversion of configuration, from beta-nucleosides to alpha-ribose 1-phosphate (Porter, 1992).

Human PNP (HsPNP) participates in the removal of deoxyguanosine (dGuo), and this process is fundamental for T-cell immune functions (Gelfand et al., 1978a,b). The genetic deficiency of HsPNP has been shown to produce specific T-cell immune deficiency (Giblett et al., 1975). The syndrome results from the increased blood levels of dGuo and its conversion to dGTP in dividing T-cells. Altered deoxynucleotide pools allosterically block ribonucleotide diphosphate reductase, insert errors into DNA and activate apoptosis in dividing T-cells (Bzowska et al., 2000; Minkui et al., 2008; Kicska et al., 2001). Since the establishment of the relationship between PNP and T-cell associated autoimmune diseases, an intense search for PNP inhibitors has been carried out (Schramm, 2004; Schramm and Grubmeyer, 2004; Ghanem et al., 2008; Knapp et al., 2006). Although the mechanism by which HsPNP deficiency causes impaired T-cell maturation and discrimination is not completely understood, T-cell leukemia and lymphomas can be impaired by efficient inhibitors that target HsPNP.

The 7-deazaguanine (7DG) (Fig. 1) is an analogue of the HsPNP substrate guanine, which may act as a competitive inhibitor and thus form a binary complex with free enzyme.

In the present work we describe the crystallographic structure of HsPNP associated with 7DG, which was solved at 2.75 Å, using

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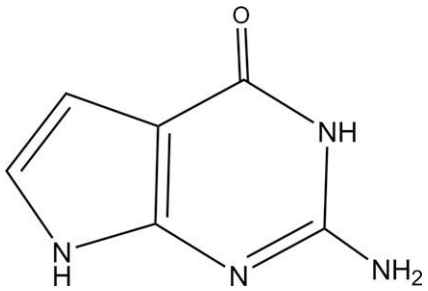

Fig. 1. 7-Deazaguanine chemical structure.

recombinant HsPNP and synchrotron radiation. Molecular dynamics (MD) simulations were applied to verify the stability of the crystal structure in solution and to give detailed information on the dynamics properties of the structure in free form and associated with the ligand. We also present a virtual screening (VS) protocol for analogues of 7DG, which were obtained from ZINC database (Irwin and Shoichet, 2005). In addition, steady-state kinetics and equilibrium fluorescence spectroscopy assays for determination of the K_d value for 7DG confirmed the affinity of this ligand for HsPNP.

2. Materials and methods

2.1. Crystallization and data collection

Recombinant HsPNP was obtained as previously described (Silva et al., 2003). PNP solution was concentrated to 12 mg/mL against 20 mM Tris–HCl, pH 8.0 and incubated for 2 h with 7DG in a molar ratio between protein and ligand of 1:3. Hanging drops were prepared mixing 1 μL of protein solution and 1 μL of reservoir solution containing 19% ammonium sulfate and 0.5 M sodium citrate pH 5.6. The crystals complexed with 7DG appeared 24 h after the drops were prepared. The crystals were flash frozen at 100 K and cryoprotected with 20% of glycerol. X-ray diffraction data were collected at wavelength 1.431 Å using Synchrotron Radiation Source (Laboratório Nacional de Luz Síncrotron, Campinas, SP, Brazil) and CCD detector (MARCCD) with an exposure time of 60 s per image at a crystal to detector distance of 120 mm. The crystals diffracted at 2.75 Å and the data were processed using MOSFLM and scaled using SCALA (Collaborative Computational Project No. 4, 1994). Cell parameters for HsPNP complexed with 7DG were $a = b = 138.7$ and $c = 159.4$ Å.

2.2. Structure resolution and refinement

The crystal structure of HsPNP complexed with 7DG was determined by standard molecular replacement methods using the program AMoRe (Navaza, 1994), incorporated in the CCP4 program package (Collaborative Computational Project No. 4, 1994), using as search model the structure of HsPNP complexed with 8-azaguanine (PDB access code: 1V41). Structure refinement was performed using the program REFMAC (Collaborative Computational Project No. 4, 1994). The atomic positions acquired from molecular replacement were used to start the crystallographic refinement. The overall stereochemical quality of the final model was assessed by the program PROCHECK (Laskowski et al., 1993). The trimeric structure and atomic models were superposed using the program LSQKAB from CCP4 (Collaborative Computational Project No. 4, 1994).

2.3. Molecular dynamics simulations

The MD simulations were performed with the trimeric structure of the HsPNP to provide a more realistic analysis, since the HsPNP is biologically active as a trimer. The MD simulations were carried out in periodic boundary conditions using the GROMACS 3.3.1 (van der Spoel et al., 2005) package using the Gromos 96.1 (53A6) force field (Oostenbrink et al., 2005). The molecular topology file and force field parameters, except for the charges for the inhibitor 7DG, were generated by the program PRODRG (van Aalten et al.,

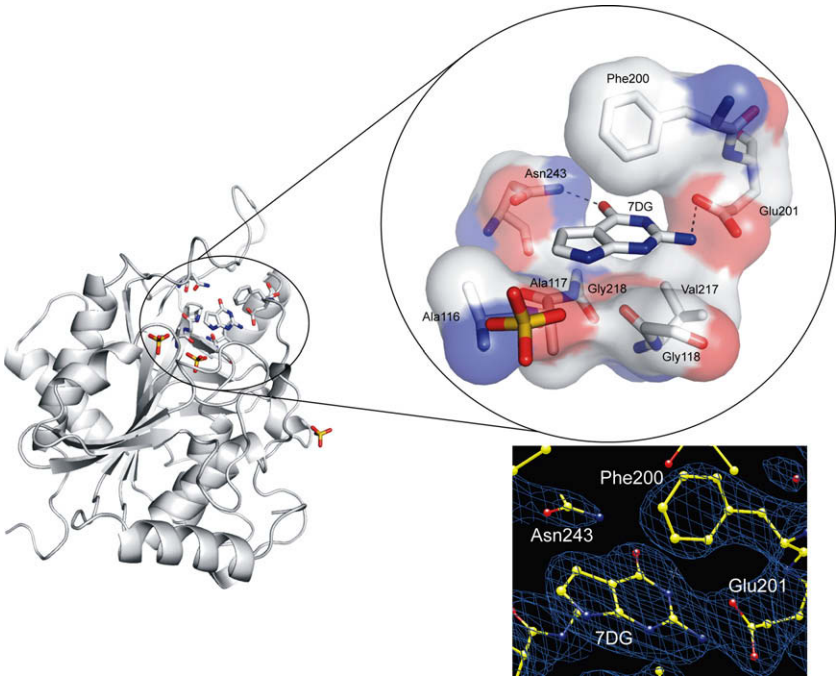

Fig. 2. (A) (top right) Classical binding site of HsPNP; the 7DG is anchored by the two main residues of the binding pocket (Glu201 and Asn243). (B) (bottom right) Electron density ($2F_{obs} - F_{calc}$) map of 7DG in binding pocket.