Biophysical Chemistry 142 (2009) 7–16

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Molecular modeling, dynamics and docking studies of Purine Nucleoside Phosphorylase from *Streptococcus pyogenes*

Luis Fernando Saraiva Macedo Timmers [a], Rafael Andrade Caceres [a], Raquel Dias [a], Luiz Augusto Basso [b], Diogenes Santiago Santos [b], Walter Filgueira de Azevedo Jr [a,*]

[a] *Faculdade de Biociências, Laboratório de Bioquímica Estrutural, Instituto Nacional de Ciência e Tecnologia em Tuberculose, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre-RS, Brazil*

[b] *Centro de Pesquisas em Biologia Molecular e Funcional, Instituto de Pesquisas Biomédicas, Instituto Nacional de Ciência e Tecnologia em Tuberculose, Pontifica Universidade Católica do Rio Grande do Sul, Porto Alegre-RS, Brazil*

ARTICLE INFO

Article history:
Received 3 December 2008
Received in revised form 17 February 2009
Accepted 17 February 2009
Available online 26 February 2009

Keywords:
Molecular dynamics
Molecular modeling
Bioinformatics
Drug design
Streptococcus pyogenes

ABSTRACT

Purine Nucleoside Phosphorylase (PNP) catalyzes the reversible phosphorolysis of *N*-glycosidic bonds of purine nucleosides and deoxynucleosides, except for adenosine, to generate ribose 1-phosphate and the purine base. PNP has been submitted to intensive structural studies. This work describes for the first time a structural model of PNP from *Streptococcus pyogenes* (SpPNP). We modeled the complexes of SpPNP with six different ligands in order to determine the structural basis for specificity of these ligands against SpPNP. Molecular dynamics (MD) simulations were performed in order to evaluate the overall stability of SpPNP model. The analysis of the MD simulation was assessed mainly by principal component analysis (PCA) to explore the trimeric structure behavior. Structural comparison, between SpPNP and human PNP, was able to identify the main features responsible for differences in ligand-binding affinities, such as mutation in the purine-binding site and in the second phosphate-binding site. The PCA analysis suggests a different behavior for each subunit in the trimer structure.

1. Introduction

Streptococcus pyogenes is a member of Group A beta-hemolytic streptococcus gram-positive spherical bacterium that produces a number of somatic constituents like M-protein and capsule, enzymes like streptolysin O, DNase B, and streptoquinase, and toxins like erythrogenic toxins A to C. The pathogen–host interactions play an important role in the development of diseases [1]. In children, *S. pyogenes* is responsible for 30% of pharyngitis cases children aged from 5 to 15 years, and also in 10% of adult cases. However, the most important complications of infection are acute rheumatic fever, acute glomerulonephritis, and peritonsillar abscess. Antimicrobial therapy alleviates pharyngeal symptoms and sometimes prevents some of the sequelae of infection.

Purine Nucleoside Phosphorylase (PNP) has been proposed as target for development of antibacterial drugs [2]. PNP catalyzes the cleavage of *N*-ribosidic bonds of the purine ribonucleosides and 2-deoxyribonucleosides in the presence of inorganic orthophosphate as a second substrate. This reaction generates the purine base and ribose(deoxyribose)-1-phosphate [3,4]. PNP is specific for purine nucleosides in the β-configuration and cleaves the glycosidic bond with inversion of configuration to produce α-ribose-1-phosphate [5]. PNP is a ubiquitous enzyme of purine metabolism that functions in the salvage pathway, including those of Apicomplexan parasites [6]. PNP is classified as belonging to the class I of Nucleoside Phosphorylase (NP-I) [7]. Drugs that inhibit human PNP activity have the potential of being utilized as modulators of the immunological system, to treat leukemia, autoimmune diseases, and rejection in organ transplantation [8,9].

In the present work we modeled the structure of PNP from *S. pyogenes* (SpPNP). Since there is no crystallographic for PNP from *S. pyogenes* we used the crystallographic structures available for human PNPs to model SpPNP. We modeled the complexes of SpPNP with six different ligands in order to determine the structural basis for specificity of these ligands against SpPNP. Comparative analysis of the model of SpPNP and the structure of human PNP allowed identification of structural features responsible for differences in the computational determined ligand affinities. It was analyzed three binding sites present in the structures of human PNP and SpPNP. The analysis was carried out with different ligands in order to identify the structural basis for the specificity of different ligands against PNPs. Furthermore the Molecular Dynamics (MD) simulations was performed to evaluate the overall stability of SpPNP model. The analysis of the MD simulations was assessed mainly by principal component analysis (PCA) to better explore the trimeric structure behavior, because this kind of analysis consider only the significant conformations during the simulation. The understanding of

* Corresponding author. Av. Ipiranga, 6681, CEP 90619-900, Porto Alegre, Rio Grande do Sul, Brazil. Tel.: +55 51 33203500/4529.
E-mail addresses: luis@azevedolab.net (L.F.S.M. Timmers), rafael@azevedolab.net (R.A. Caceres), raquel@azevedolab.net (R. Dias), luiz.basso@pucrs.br (L.A. Basso), diogenes@pucrs.br (D.S. Santos), walter@azevedolab.net (W.F. de Azevedo).

0301-4622/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2009.02.006

the rules governing the specificity of different ligands against PNP could be used to help in the design of more specific inhibitors, and in the case of SpPNP help in the development of new drugs against *S. pyogenes*.

2. Materials and methods

2.1. Molecular modeling

When the crystallographic structure of a protein target is not available the homology modeling could be a potential method to build its tertiary structure. To use the atomic coordinates of a crystallographic structure (template) the sequences of protein target and template need to share at least 30% of identity. For modeling of the SpPNP complexed with acyclovir, guanine, 7-methyl-6-thio-guanosine, 3-deoxyguanosine, guanosine and inosine the following crystallographic structures were used as templates, 1PWY [10], 1V2H [11], 1YRY [12], 1V45 [13], 1RFG [14] and 1RCT [13], respectively. The web server PARMODEL was used to model the binary complexes [15]. PARMODEL is a parallelized version of the MODELLER [16]. The modeling procedure begins with alignment of the sequence to be modeled (target) with related known three-dimensional structures (templates). This alignment is usually the input to the program and the output is a three-dimensional model for the target sequence containing all main-chain and side-chain non-hydrogen atoms [17].

The high degree of primary sequence identity between SpPNP (target) and of Human PNP(HsPNP) indicates that these crystallography structures are good models to be used as templates for SpPNP enzyme (target). The alignment of the SpPNP (target) and human PNP is shown in Fig. 1 [18].

A total of 1000 models were generated for each binary complex and the final models were selected based on stereochemical quality. All optimization process was performed on a Beowulf cluster with 16 nodes (BioComp, AMD Athlon XP 2100+, BioComp, Brazil).

2.2. Evaluation of binding affinity

The affinity and specificity between a ligand and its protein target depend on directional hydrogen bonds and ionic interactions, as well as on shape complementarity of the contact surfaces of both partners [19–21]. We used the program XSCORE [22] to evaluate the binding affinity of the ligands against HsPNP and SpPNP. According to this method, the binding affinity of the ligand can be decomposed to the contribution of individual atoms. Each ligand atom obtains a score, called the atomic binding score, indicating its role in the binding process. The program reads the structure, assigns atom types and parameters, performs the calculation, and gives the dissociation constant of the given protein–ligand complex. The computational results are fed into a text file in which the detailed information of each ligand atom, including the atomic binding score, is tabulated. This data were used to evaluate the correlation coefficient between the affinities against both PNPs to verify possible resemblance in the structural basis for specificity against these enzymes.

2.3. Analysis of the models

The overall stereochemical quality of the final models for each enzyme of the SpPNP was assessed by the program PROCHECK [23]. Atomic models were superposed using the program LSQKAB from CCP4 [24] and the intermolecular hydrogen bonds were assessed by the program LIGPLOT [25].

2.4. Molecular docking protocol

Rigid-body docking simulations (RDS) were performed using ZDOCK 2.3 [26], which is used for the prediction of the three-dimensional (3D) structure of a protein–protein complex from the coordinates of its components structures, it is classified as bound docking or unbound docking [27]. This method is important for the development of new drugs, as summarized in previous works [28–38] because RDS is based on three basic tasks, which are: (1) characterization of the binding site; (2) positioning of the ligand into the binding site; and (3) evaluating the strength of interaction for a specific ligand–receptor complex [39].

The RDS was performed with 3-deoxyguanosine (3DG) against SpPNP. Before running this simulation, it was carried out a validation with the HsPNP crystallographic structure (PDB access code: 1PF7) [40]. In the validation, the ligand of the HsPNP crystallographic structure, was rotated 180° along *z* axis and translated 1 (fractional coordinates) along the three axis, using the program LSQKAB [24]. After this, the protein was kept fixed and the binding site was restricted. The residues Gly115,

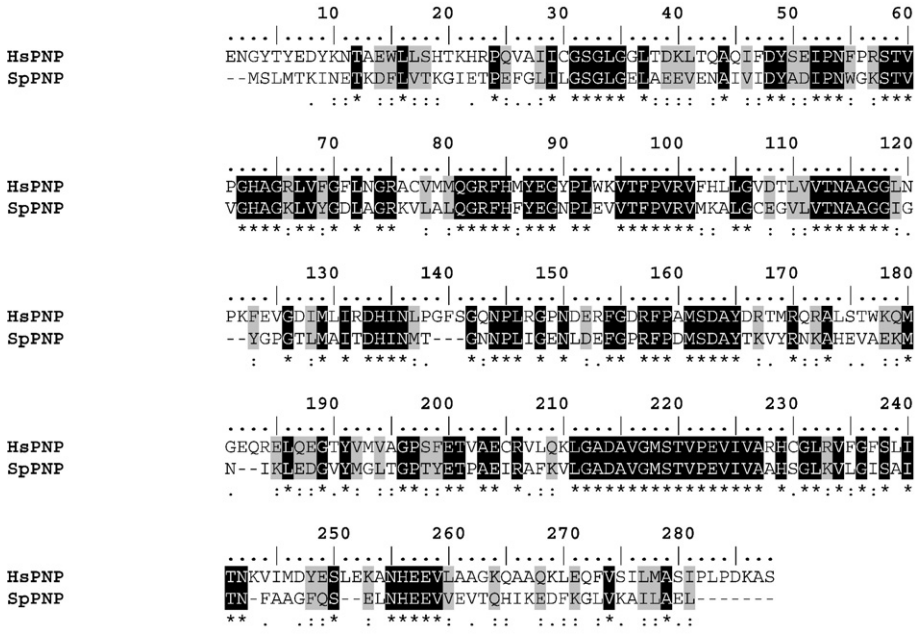

Fig. 1. Sequence alignment for Human Purine Nucleoside Phosphorylase and PNP from *Streptococcus pyogenes*. The multiple alignment was performed using ClustalW [17] and edited with BioEdit [16]. (*) indicates positions which have a single, fully conserved, (:) indicates that one of the following 'strong' groups is fully conserved, and (.) indicates that one of the following 'weaker' groups is fully conserved.