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Characterization of the farnesyl pyrophosphate synthase of *Trypanosoma cruzi* by homology modeling and molecular dynamics

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Abstract

Chagas' disease, caused by the *Trypanosoma cruzi* parasite, is one of the largest public health problems in the Western hemisphere, with 16–18 million people infected, and approximately 100 million people at risk. Many efforts towards the development of targeted antiparasitic agents have recently been described. Of interest, bisphosphonates, pyrophosphate analogs in which the oxygen bridge between the two phosphorus atoms has been replaced by a carbon substituted with different side chains, are able to inhibit the growth of *T. cruzi*. The enzyme *T. cruzi* farnesyl pyrophosphate synthase (TcFPPS) involved in the mevalonate pathway, has been recently identified as the target of bisphosphonates. The protein has 362 amino acids and a molecular mass of 41.2 kDa. Several sequence motifs found in other FPPSs are present in TcFPPS. In this study we have modeled the structure of TcFPPS based on the structure of the avian FPPS. We have characterized the interaction with its substrates, isopentyl pyrophosphate and dimethylallyl pyrophosphate, and the mechanism of inhibition by the potent bisphosphonate risedronate (K_i of 0.032 ± 0.002 μM) by means of molecular dynamics techniques. We propose that homorisedronate, which has an extra methylene and a K_i of 8.17 ± 1.36 μM, does not form strong hydrogen bonds with TYR 211 and THR 208, which may be responsible for its lower activity as compared to risedronate. Moreover, we were able to reproduce the structural changes that occur upon the binding of the third Mg^{2+} to the active site of the protein. Taken together, our results provide a structural model for the design of novel inhibitors that may prove useful for the treatment of Chagas' disease.

Keywords: Chagas' disease; Homology modeling; Molecular dynamics; Bisphosphonates; Farnesyl pyrophosphate synthase

1. Introduction

Chagas' disease (CD) also called *American trypanosomiasis*, is an infection caused by the parasite *Trypanosoma cruzi*. Worldwide, it is estimated that 16–18 million people are infected with CD; of those infected, 50,000 will die each year [1]. CD is the most important parasitic disease in Latin America in terms of its impact on national economies and public health systems [2]. Parasites are transmitted to humans in three ways: (1) by bloodfeeding *Triatomina*e bugs, which live in cracks and crevices of poor-quality houses, usually in rural areas; (2) through transfusion with infected blood; (3) congenitally, from infected mother to fetus [3]. There has been considerable interest in developing novel chemotherapeutic approaches, based on unique aspects of the structure and metabolism of this parasite.

It has been reported that during the proliferative stages of the parasite large quantities of inorganic pyrophosphate are found in *T. cruzi* acidocalcisomes and that pyrophosphate can be even more abundant than ATP [4]. This led to the idea that parasite growth might be inhibited in the presence of a stable analog of diphosphate. Taking this into account, bisphosphonates, pyrophosphate analogs in which the oxygen bridge between the two phosphorus atoms has been replaced by a carbon substituted with different side chains, have been proven to inhibit the growth in vitro and in vivo of the parasite [4–10]. Bisphosphonates represent an important class of drugs currently used to treat osteoporosis, Paget's disease and hypercalcemia due to malignancy. The enzyme farnesyl pyrophosphate synthase (FPPS), involved in the mevalonate pathway, has been identified as the target of bisphosphonates in

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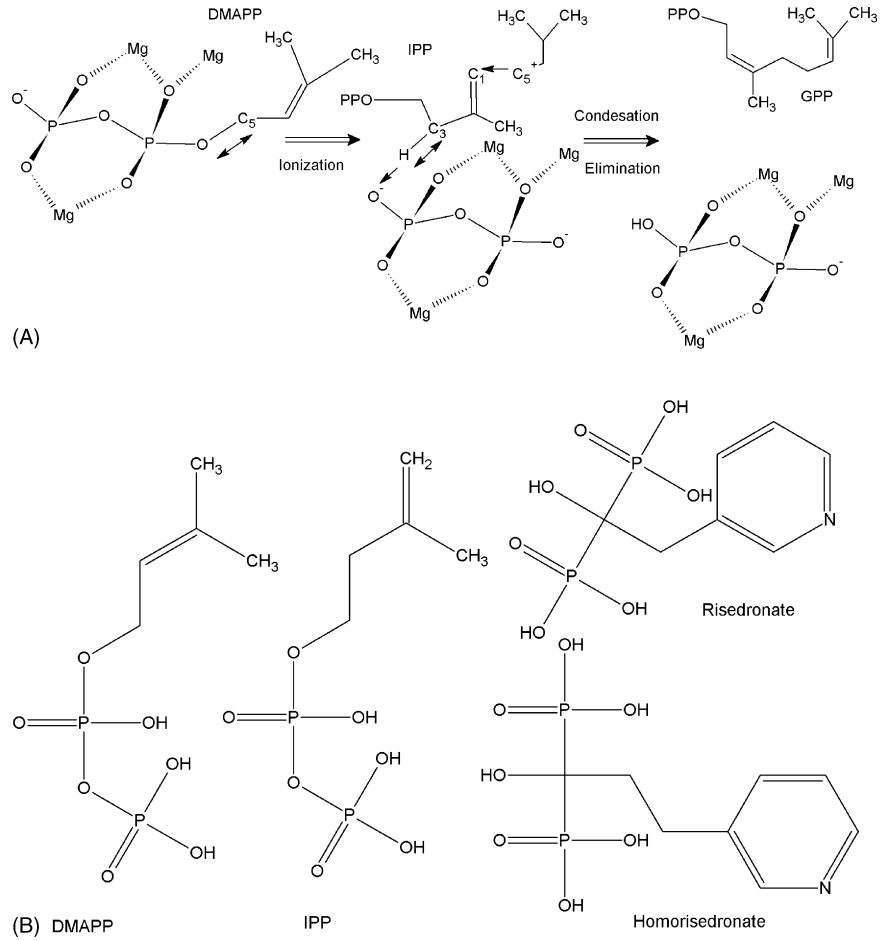

Scheme 1. (A) The proposed reaction mechanism for FPPS [9]. The substrate binds to the active site with 3 Mg^{2+} that labilize the C5–O bond of DMAPP generating an allylic carbocation that alkylates the C1 atom of IPP. In a second step, a DMAPP pyrophosphate oxygen serves as the catalytic base removing the IPP C3 pro-R hydrogen and thus allowing the double bond formation in the final product. (B) Chemical structure of DMAPP, IPP, RIS and HRIS.

different organisms [11–13]. This biosynthetic route leads to the formation of several products, among the most relevant ones are cholesterol and the nonsteroidal isoprenoids farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) of which the latter two can be used as substrates for post-translational protein prenylation. FPPS catalyzes the 1′-4 condensation of dimethylallyl pyrophosphate (DMAPP) with two molecules of isopentenyl pyrophosphate (IPP) to form first geranyl pyrophosphate (Scheme 1A) and in a subsequent step FPP [14]. The structure of apo- and ligand-bound avian FPPS have been solved [15,16] and recently the structures of unliganded *Staphylococcus aureus* FPPS (FPPS-Sa), as well as two *Escherichia coli* FPPS (FPPS-Ec) ternary complexes has been determined [9]. One of these ternary complexes contains IPP and the noncleavable DMAPP analogue dimethylallyl *S*-thiolodiphosphate (DMSPP) [17] and the other a bisphosphonate-bound structure containing IPP and the osteoporosis drug 1-hydroxy-2-(3-pyridinyl)ethylidene bisphosphonic acid, risedronate (RIS) [9]. RIS (Scheme 1B) is an aromatic nitrogen-containing bisphosphonate that inhibits *T. cruzi* FPPS (TcFPPS) with a K_i of 0.032 ± 0.002 μM [11]. Interestingly, the similar homorisedronate (HRIS) (Scheme 1B), which has an extra methylene, has a K_i of 8.17 ± 1.36 μM [11]. The active site of FPPS includes Mg^{2+} cations that are probably involved in ligand binding and catalysis. However, the different experimental structures exhibit 1, 2 or 3 Mg^{2+} ions [9,15,16], and the real role of these ions in the enzyme function is still an open question.

We report a thorough computational study of the structural characteristics of TcFPPS. We have developed a model of TcFPPS with its two substrates, IPP and DMAPP, docked in the active site based on the structure of the avian FPPS. We have evaluated the differences arising from binding 2 or 3 Mg^{2+} cations and propose a specific role for the third Mg^{2+} in the closing of the protein active site based on molecular dynamics simulations (MD). Moreover, we have modeled the interaction with risedronate and homorisedronate and provide structural information that may clarify the molecular basis of the difference in activity of these two similar compounds.

2. Methods

2.1. Sequence alignment

The amino acid sequence of TcFPPS was obtained from the protein database from NCBI (http://www.ncbi.nlm.nih.gov/protein) (gi:14647139). The PSI-BLAST [18] algorithm was used to identify homologous structures for TcFPPS by