Available online at www.sciencedirect.com
SCIENCE DIRECT
Archives of Biochemistry and Biophysics 450 (2006) 39–49
ABB
www.elsevier.com/locate/yabbi

Structural analogues of reactive intermediates as inhibitors of glucosamine-6-phosphate synthase and phosphoglucose isomerase

Sławomir Milewski *, Agnieszka Janiak, Marek Wojciechowski

Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, 11/12 Narutowicza St., 80-952 Gdańsk, Poland

Received 27 January 2006, and in revised form 15 March 2006
Available online 5 April 2006

Abstract

The active centers of phosphoglucose isomerase (PGI) and the hexose phosphate isomerase domain (HPI) of glucosamine-6-P (GlcN-6-P) synthase demonstrate apparent similarity in spatial arrangement of critical amino acid residues, except Arg272 of the former and Lys603 and Lys485 of the latter. Ten derivatives of D-hexitol-6-P, 5-phosphoarabinoate, or 6-phosphogluconate, structural analogues of putative *cis*-enolamine or *cis*-enolate intermediates, were tested as inhibitors of fungal GlcN-6-P synthase and PGI. None of the investigated compounds demonstrated equally high inhibitory potential against both enzymes. 2-Amino-2-deoxy-D-mannitol 6-P was found to be the strongest GlcN-6-P synthase inhibitor in the series, with an inhibition constant equal to $9.0\ (\pm 1.0) \times 10^{-6}$ M. On the contrary, 5-phosphoarabinoate (5PA) exhibited specificity for PGI, with $K_i = 2.2\ (\pm 0.1) \times 10^{-6}$ M. *N*-acetylation substantially lowered the GlcN-6-P synthase inhibitory potential of 2-amino-2-deoxy-D-glucitol-6-P but strongly enhanced inhibitory potential of this compound towards PGI. Molecular modeling studies revealed that interactions of the C1–C2 part of transition state analogue inhibitors with the respective areas demonstrating different distribution of molecular electrostatic potential (MEP) inside HPI and PGI active centers determined enzyme:ligand affinity. In *Escherichia coli* HPI, a patch of the negative potential created by Glu488 aided by Val399, supposed to stabilize a putative positively charged intermediate, especially attracts ligands containing 2-amino function. The Arg272, Lys210, and Gly271 peptide bond nitrogen system, present in the corresponding space of rabbit PGI, creates an area of positive MEP, stabilizing *cis*-enolate intermediate and attracting its structural mimics, such as 5PA.

Keywords: Glucosamine-6-phosphate synthase; Phosphoglucose isomerase; Transition state intermediate; Molecular modeling

L-Glutamine:D-Fructose-6-phosphate amidotransferase (hexose isomerizing), EC 2.6.1.16, known as glucosamine-6-phosphate synthase (GlcN-6-P[1] synthase) is an enzyme that catalyzes the first committed step in the biochemical pathway leading to the formation of an activated form of *N*-acetyl-D-glucosamine, namely uridine 5′-diphospho-*N*-acetyl-D-glucosamine. This sugar nucleoside provides *N*-acetyl-D-glucosamine for biosynthesis of lipopolysaccharides, peptidoglycan and teichoic acids in bacteria; chitin in fungi, insects and crustaceans; as well as glycoproteins, glycosaminoglycans and mucopolysaccharides in mammals. The aminosugar-containing biomacromolecules play an important role in both prokaryotic and eukaryotic cells. In fungi and bacteria, deletion of the respective gene encoding the enzyme is lethal [1,2]. In mammals, a temporary depletion of enzyme activity is acceptable, due to the slow turnover of aminosugar-containing macromolecules and a rapid turnover of the mammalian gene encoding the enzyme, known as *GFAT* [3,4]. Features mentioned above

* Corresponding author. Fax: +48 58 3471144.
E-mail address: milewski@chem.pg.gda.pl (S. Milewski).

[1] *Abbreviations used:* DTT, dithothreitol; GAT, glutamine amide hydrolase domain; GlcNAc, *N*-acetyl-D-glucosamine; GlcN-6-P, D-glucosamine-6-phosphate; HPI, hexose phosphate isomerase domain; MEP, molecular electrostatic potential; PGI, phosphoglucose isomerase; ADGP, 2-amino-2-deoxy-D-glucitol-6-phosphate; AADGP, *N*-acetyl-2-amino-2-deoxy-D-glucitol-6-phosphate; ADGPE, 2-amino-2-deoxy-D-glucitol-6-phosphate dimethyl ester; ADMP, 2-amino-2-deoxy-D-mannitol-6-phosphate; DGP, 2-deoxy-D-glucitol-6-phosphate; GP, D-glucitol-6-phosphate; 5PAO, D-arabinose-5-phosphate oxime; 5PA, 5-phospho-D-arabinoate; 5PAA, 5-phospho-D-arabinoamide; 5PAH, 5-phospho-D-arabinohydroxamate; 6PG, 6-phospho-D-gluconate; 6PGA, 6-phospho-D-gluconoamide.

0003-9861/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2006.03.019

make GlcN-6-P synthase a potential target for antimicrobial chemotherapy, especially its antifungal branch [5]. This issue is of crucial importance due to the emerging challenge of disseminated fungal infections and multi-drug resistance. A very limited repertoire of antifungal chemotherapeutics makes the situation even worse. Exploitation of novel targets, including GlcN-6-P synthase, seems therefore an attractive option. Intensive efforts are thus being continued to design GlcN-6-P synthase inhibitors that may become effective antifungals.

Prokaryotic GlcN-6-P synthase is a homodimer, while its eukaryotic counterpart supposedly has a tetrameric structure [6]. A 3D structure of the prokaryotic enzyme [7] but not of the eukaryotic one is known. Each subunit of the *Escherichia coli* enzyme is composed of two domains: glutamine amide hydrolase (GAT) and hexose phosphate isomerase (HPI) [8]. This arrangement seems to be a common feature of all GlcN-6-P synthases. The catalyzed reaction is complex and involves hydrolysis of glutamine, ammonia transfer to fructose-6-phosphate and isomerization of the formed fructosimine-6-phosphate to glucosamine-6-phosphate. The reaction is initiated upon D-fructose-6-phosphate (Fru-6-P) binding to HPI, followed by binding of L-glutamine to GAT and hydrolysis of glutamine amide. The released ammonia is transferred to HPI, where the resulting iminosugar phosphate is isomerized to GlcN-6-P [9]. The mechanism of fructosimine-6-phosphate isomerization postulated by Teplyakov et al. [10] assumes formation of *cis*-enolamine as a transition state intermediate. This is analogous to the general mechanism of other ketose/aldose isomerizations, where a *cis*-endiol is formed, for example, as known for phosphoglucose isomerase, EC 5.3.1.9/PGI/ [11]. In this respect it is worth mentioning that both a native GlcN-6-P synthase and its separately expressed HPI domain exhibit PGI-like activity [12].

A few compounds that mimic putative intermediates of the GlcN-6-P synthase-catalyzed sugar isomerization have been described. Structures of some of them are presented in Fig. 1. D-Arabinose-5-phosphate oxime (5PAO), its methylenephosphate analogue and 2-amino-2-deoxy-D-glucitol-6-phosphate (ADGP) are considered structural mimics of *cis*-enolamine [13,14], while 5-methylphosphono-D-arabinohydroximolactone is an analogue of an fructosimine-6-P intermediate [15]. In their extensive studies Bearne and Blouin [16] suggested a crucial role of the 2-amino function for enzyme inhibitory potency of *cis*-enolamine mimics. In addition, 5-phospho-D-arabinoate (5PA), 6-phospho-D-gluconate (6PG), 5-phospho-D-arabinoamide (5PAA) and 5-phospho-D-arabinohydroxamate (5PAH), considered structural mimics of *cis*-endiol, have been reported as strong inhibitors of PGI [17–20].

In our present studies, we have determined enzyme inhibitory potential of 10 structural mimics of *cis*-enolamine or *cis*-endiol intermediates against *Candida albicans* GlcN-6-P synthase and yeast phosphoglucose isomerase. Comparative analysis of the obtained data, supported by the results of the molecular modeling, has led to the identification of the structural factors determining inhibitory potency of reactive intermediate analogues in regard to both enzymes.

Materials and methods

Reagents and analytic methods

2-Amino-2-deoxy-D-glucose-6-phosphate, D-gluconic acid 6-phosphate (6PG), 2-amino-2-deoxy-D-mannose, D-glucitol 6-phosphate (GP), 2-deoxy-D-glucose 6-phosphate, D-glucosamine-6-phosphate and sodium borohydride were purchased from Sigma Aldrich, St. Louis, MO. All other chemicals were of the highest purity

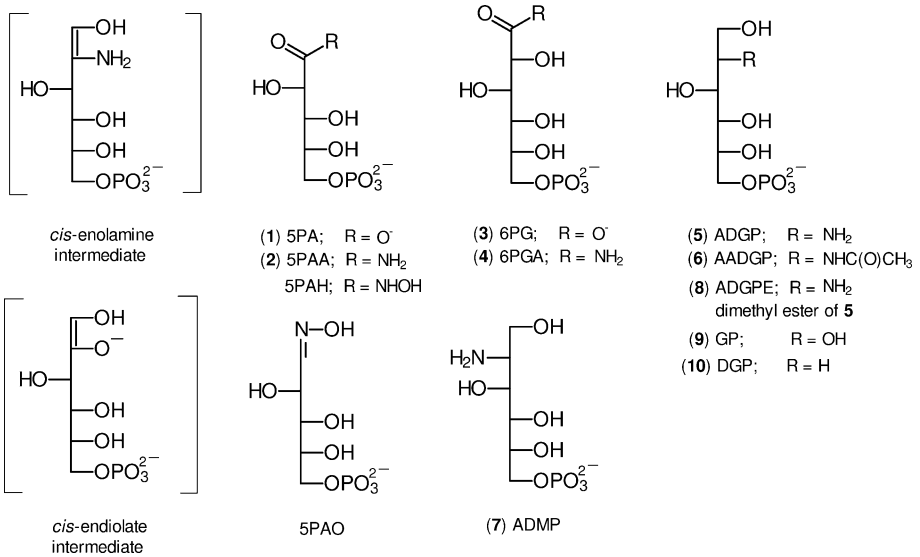

Fig. 1. Structures of putative transition state intermediates in HPI- and PGI-catalyzed reactions and their structural analogues.