European Journal of Medicinal Chemistry 128 (2017) 274–286

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Rational design of reversible inhibitors for trehalose 6-phosphate phosphatases

Chunliang Liu, Debra Dunaway-Mariano*, Patrick S. Mariano**

Department of Chemistry and Chemical Biology, University of New Mexico, Albuquerque, NM 87131, USA

A R T I C L E I N F O

Article history:
Received 3 January 2017
Received in revised form
1 February 2017
Accepted 3 February 2017
Available online 4 February 2017

Keywords:
HAD phosphatase
Trehalose 6-sulfate
Trehalose 6-phosphate phosphatase
T6PP inhibitor
Antihelminthic

A B S T R A C T

In some organisms, environmental stress triggers trehalose biosynthesis that is catalyzed collectively by trehalose 6-phosphate synthase, and trehalose 6-phosphate phosphatase (T6PP). T6PP catalyzes the hydrolysis of trehalose 6-phosphate (T6P) to trehalose and inorganic phosphate and is a promising target for the development of antibacterial, antifungal and antihelminthic therapeutics. Herein, we report the design, synthesis and evaluation of a library of aryl D-glucopyranoside 6-sulfates to serve as prototypes for small molecule T6PP inhibitors. Steady-state kinetic techniques were used to measure inhibition constants (K_i) of a panel of structurally diverse T6PP orthologs derived from the pathogens *Brugia malayi*, *Ascaris suum*, *Mycobacterium tuberculosis*, *Shigella boydii* and *Salmonella typhimurium*. The binding affinities of the most active inhibitor of these T6PP orthologs, 4-*n*-octylphenyl α-D-glucopyranoside 6-sulfate (**9a**), were found to be in the low micromolar range. The K_i of **9a** with the *B. malayi* T6PP ortholog is 5.3 ± 0.6 μM, 70-fold smaller than the substrate Michaelis constant. The binding specificity of **9a** was demonstrated using several representative sugar phosphate phosphatases from the HAD enzyme superfamily, the T6PP protein fold family of origin. Lastly, correlations drawn between T6PP active site structure, inhibitor structure and inhibitor binding affinity suggest that the aryl D-glucopyranoside 6-sulfate prototypes will find future applications as a platform for development of tailored second-generation T6PP inhibitors.

© 2017 Elsevier Masson SAS. All rights reserved.

1. Introduction

The (α-1,α′-1)-glucose dimer trehalose is produced by specialized bacteria, fungi, plants and nematodes in response to environmental stress [1–6]. Trehalose biosynthesis is catalyzed, collectively, by trehalose 6-phosphate synthase (T6PS), which catalyzes the condensation of glucose 6-phosphate and uridine 5′-diphosphoglucose (UDP-glucose) to trehalose 6-phosphate (T6P), and trehalose 6-phosphate phosphatase (T6PP), which catalyzes the hydrolysis of T6P to trehalose and inorganic phosphate (see Fig. 1) [7,8]. Gene silencing experiments, carried out with *Mycobacterium tuberculosis*, *Cryptococcus neoformans* and *C. gattii*, *Aspergillus fumigatus*, *Candida albicans*, *Caenorhabditis elegans* and *Brugia malayi*, demonstrated the sensitivity of microbial and nematodal human pathogens to the disruption of the trehalose pathway [9–16]. T6PP, the subject of our work, is required both for trehalose production and for the prevention of T6P accumulation and associated toxicity [11,15]. Despite the considerable discussion given to T6PP as a platform for the development of antibacterial, antifungal and antihelminthic therapeutics [9,11,12,15,16], T6PP inhibitors have not yet been reported. In this article, we report the synthesis and evaluation of a library of first generation T6PP inhibitors designed using a modular approach.

2. Results and discussion

2.1. Inhibitor design

The T6PP inhibitor design was guided by the X-ray crystal structure of the T6PP ortholog from the archaeon *Thermoplasma acidophilum* (Ta-T6PP) [17]. The Ta-T6PP backbone fold conforms to that of the type C2 Haloalkanoic Acid Dehalogenase (HAD)

* Corresponding author. Department of Chemistry and Chemical Biology, University of New Mexico, MSC03 2060, 300 Terrace St. NE, Albuquerque, NM 87131-0001, USA.

** Corresponding author. Department of Chemistry and Chemical Biology, University of New Mexico, MSC03 2060, 300 Terrace St. NE, Albuquerque, NM 87131-0001, USA.

E-mail addresses: dd39@unm.edu (D. Dunaway-Mariano), mariano@unm.edu (P.S. Mariano).

http://dx.doi.org/10.1016/j.ejmech.2017.02.001
0223-5234/© 2017 Elsevier Masson SAS. All rights reserved.

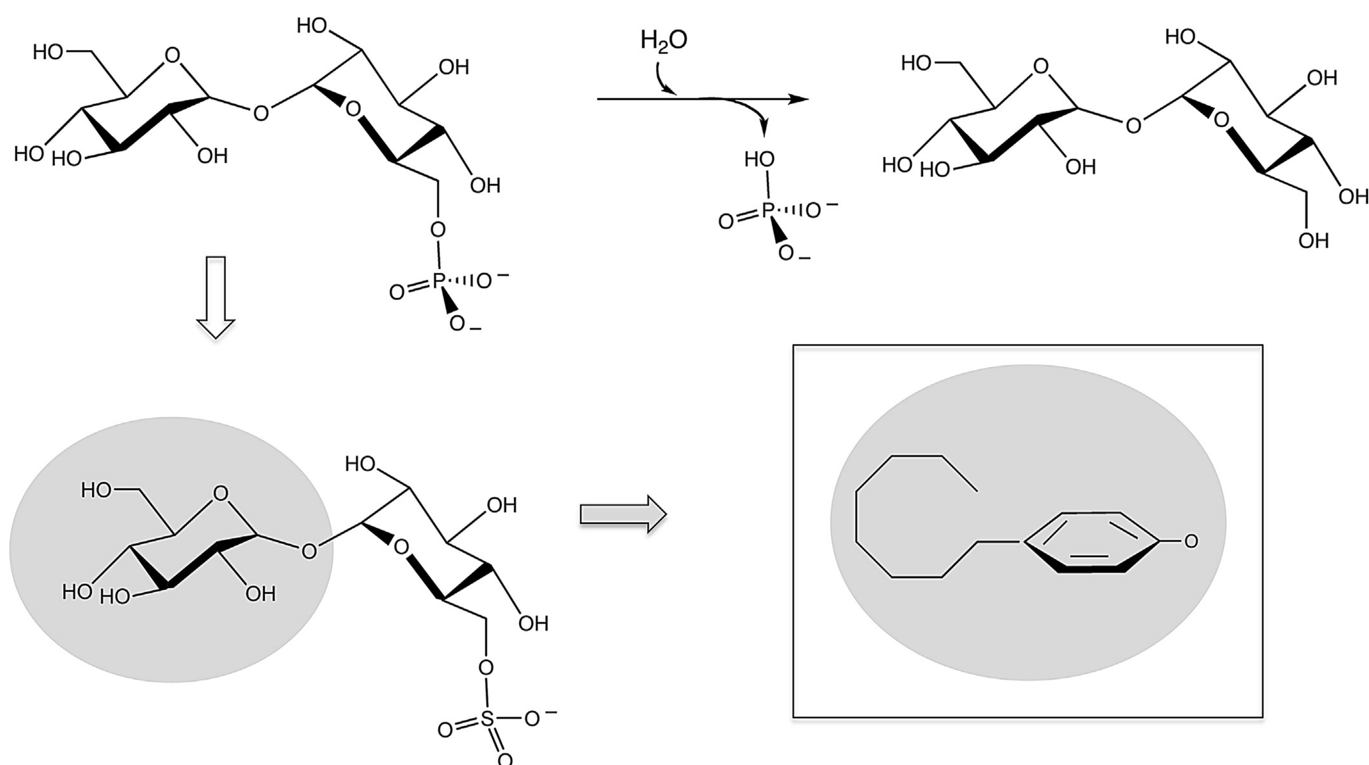

Fig. 1. An illustration of the trehalose 6-phosphate phosphatase-catalyzed hydrolysis of trehalose 6-phosphate to trehalose and inorganic phosphate and the sequential modifications made to the substrate structure in forming the first generation inhibitor 4-*n*-octylphenyl α-D-glucopyranoside-6-sulfate.

superfamily phosphatase [18]. As with other phosphatases of this structural class, T6PP is comprised of a Rossmann-fold catalytic domain and the inserted α/β-fold cap domain. Ensuing structure determinations, first on the T6PP ortholog from the pathogenic nematode *B. malayi* (Bm-T6PP) [19], and most recently on the T6PP orthologs from fungal pathogens *C. albicans*, *C. neoformans* and *A. fumigatus* [20] and the bacterial pathogen *M. tuberculosis* [21], have illuminated the structural determinants of substrate recognition.

A comparative analysis of the T6PP X-ray structures identifies the active site formed at the cap domain-catalytic domain interface and provides insight into the domain dynamics associated with catalytic cycling. To illustrate, the superposition of the structures of the T6P complex of the T6PP-D24 N (site-directed mutant) from *C. neoformans* (colored cyan) and the (unbound) *apo*-TPP from *A. fumigatus* (green) is shown in Fig. 2A. From the outset, we had assumed that T6PP, like other HAD phosphatases possessing a mobile cap domain, would undergo changes in domain-domain association as required to open the catalytic site for substrate binding, close it for catalysis, and open it for product release. Indeed, the structures of the fungal T6PP orthologs define two cap domain-catalytic domain orientations, one in which the active site is open to enable ligand exchange (hereafter referred to as the "open conformer") and the other in which the active site is closed for catalysis (the "closed conformer").

Although the suggested T6PP substrate induced-fit mechanism (depicted by the cartoon in Fig. 2B) confers substrate specificity, it also subtracts from the intrinsic binding energy, hence binding affinity [22]. Furthermore, the carbohydrate portion of the substrate is an unlikely source of binding energy because its partitioning between solvent and the T6PP binding site relies primarily on the entropic advantage gained from intramolecular multi-site binding created by the active site [23]. Weak T6P binding is consistent with the large steady-state K_m values (200–1600 μM) observed for T6PP orthologs [19–21,24–28], and confirmed by using substrate-fragment analysis [28]. The analysis showed that phosphate, glucose 6-phosphate and trehalose do not generate sufficient binding energy to overcome the entropy penalty intrinsic to protein-ligand association. Thus, stable complexes are not formed [28].

With this information in hand and the original apo-TaT6PP structure to guide inhibitor design, we pursued the divide-and-conquer strategy depicted in Fig. 1. The plan was to retailor the substrate by first replacing the labile phosphate group with a stable mimetic, and then substitute the carbohydrate portion with a structural module tailored to complement the substrate binding site. Because the T6P phosphate group binds to a polar pocket, where it favorably interacts with the Mg^{2+} cofactor and several stringently conserved electropositive amino acid residues (Fig. 2B), we limited our search to tetrahedral oxyanions. This led to the ultimate discovery that the sulfate group is best suited for this purpose [28]. Trehalose 6-sulfate (T6S) thus became the platform for tailoring the trehalose unit for enhanced binding affinity.

Inspection of the T6PP structure given in Fig. 2A, reveals that the two substrate glucosyl units are bound in starkly different environments. Whereas the hydroxyl groups of inner glucosyl moiety (the one that is phosphorylated) collectively form multiple hydrogen bonds with the charged side chains of a cap domain Glu-Lys-Glu triad, the outer glucosyl unit (the one that is not phosphorylated) is bound through interactions with the main chain amide groups of an encompassing loop from the catalytic domain. For the purpose of inhibitor design, the inner glucosyl unit was left intact and used for tethering a structural motif designed to extract binding energy through optimized interaction with the active site. Because the "cap-closing" interactions between the T6P inner glucosyl hydroxyl groups are retained, the T6PP inhibitor will

Download English Version:

https://daneshyari.com/en/article/5158530

Download Persian Version:

https://daneshyari.com/article/5158530

Daneshyari.com