Carbohydrate Research 344 (2009) 869–880

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Competitive inhibitors of type B ribose 5-phosphate isomerases: design, synthesis and kinetic evaluation of new D-allose and D-allulose 6-phosphate derivatives

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ARTICLE INFO

Article history:
Received 26 November 2008
Received in revised form 20 February 2009
Accepted 23 February 2009
Available online 3 March 2009

Keywords:
Sugar phosphates
Enzyme inhibitor
Hydroxamic acids
Ribose 5-phosphate isomerase
Allose 6-phosphate isomerase
Mycobacterium tuberculosis

ABSTRACT

This study reports syntheses of D-allose 6-phosphate (All6P), D-allulose (or D-psicose) 6-phosphate (Allu6P), and seven D-ribose 5-phosphate isomerase (Rpi) inhibitors. The inhibitors were designed as analogues of the 6-carbon high-energy intermediate postulated for the All6P to Allu6P isomerization reaction (Allpi activity) catalyzed by type B Rpi from *Escherichia coli* (EcRpiB). 5-Phospho-D-ribonate, easily obtained through oxidative cleavage of either All6P or Allu6P, led to the original synthon 5-dihydrogenophospho-D-ribono-1,4-lactone from which the other inhibitors could be synthesized through nucleophilic addition in one step. Kinetic evaluation on Allpi activity of EcRpiB shows that two of these compounds, 5-phospho-D-ribonohydroxamic acid and *N*-(5-phospho-D-ribonoyl)-methylamine, indeed behave as new efficient inhibitors of EcRpiB; further, 5-phospho-D-ribonohydroxamic acid was demonstrated to have competitive inhibition. Kinetic evaluation on Rpi activity of both EcRpiB and RpiB from *Mycobacterium tuberculosis* (MtRpiB) shows that several of the designed 6-carbon high-energy intermediate analogues are new competitive inhibitors of both RpiBs. One of them, 5-phospho-D-ribonate, not only appears as the strongest competitive inhibitor of a Rpi ever reported in the literature, with a K_i value of 9 μM for MtRpiB, but also displays specific inhibition of MtRpiB versus EcRpiB.

1. Introduction

Ribose 5-phosphate isomerase (Rpi, E.C. 5.3.1.6), a key enzyme of the pentose phosphate pathway and of the Calvin cycle of plants, is the metal-independent aldose–ketose isomerase that catalyzes the reversible conversion of D-ribose 5-phosphate (R5P) into D-ribulose 5-phosphate (Ru5P). As observed in the case of triosephosphate isomerase (TIM),[1] phosphoglucose isomerase (PGI),[2,3] and phosphomannose isomerase (PMI),[3,4] the isomerization reaction catalyzed is thought to proceed through a proton transfer mechanism between the two carbon atoms C-1 and C-2 of the substrates, concomitant with a proton transfer between the two oxygen atoms O-1 and O-2, and thus involves a 1,2-*cis*-enediol(ate) high-energy intermediate (HEI) as depicted in Chart 1.

Two unrelated forms of the enzyme, with no amino acid sequence similarity and different structures, are known to catalyze the isomerization. RpiA is the most common form and is found in almost all organisms including humans, *Spinacia oleracea*, and *Escherichia coli* (Ec). The second type, RpiB, is found mainly in bacteria, including *Mycobacterium tuberculosis* (Mt) and *E. coli*. Due to important differences in the two active sites including a change in the sequence position and nature of the catalytic base, *M. tuberculosis* and *E. coli* RpiBs were proposed to be representative of two distinct sub-families.[5] Indeed, we recently demonstrated, through kinetic analyses and structural studies, that EcRpiB, but not MtRpiB is also a functional allose 6-phosphate isomerase (Allpi), catalyzing the reversible isomerization of the 6-carbon sugars D-allose 6-phosphate (All6P) and D-allulose (also called D-psicose) 6-phosphate (Allu6P).[6,7] A proton transfer mechanism similar to that proposed for the Rpi activity would involve the 6-carbon HEI depicted in Chart 1.

While a number of strong competitive inhibitors designed as mimics of the HEI proposed to be involved in the isomerization of the 5-carbon sugars R5P and Ru5P have been reported in the literature, such as 4-phospho-D-erythronate,[8,9] 4-deoxy-4-phosphonomethyl-D-erythronate,[10] and 4-phospho-D-erythronohydroxamic acid,[9] to date no corresponding inhibitors based on the All6P to Allu6P isomerization reaction have been kinetically evaluated. Competitive inhibitors of Allpi activity would be

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doi:10.1016/j.carres.2009.02.024

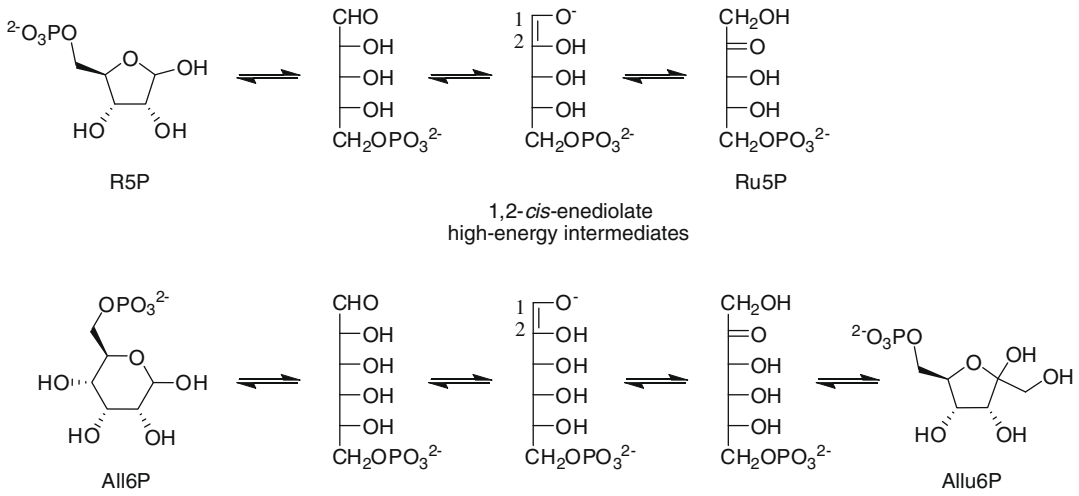

Chart 1. Isomerization reactions of D-ribose 5-phosphate (R5P) to D-ribulose 5-phosphate (Ru5P) and D-allose 6-phosphate (All6P) to D-allulose 6-phosphate (Allu6P).

important tools for future structural and mechanistic studies, as well as promising compounds for the design of species-specific therapeutic agents. Indeed, Allpi activity was suggested to be a characteristic of certain type B Rpis,[11,12] which are mainly found in bacteria, but not of type A Rpis (like human Rpi), which display only Rpi activity. Thus, specific inhibition of enzymes possessing Allpi activity while leaving unaffected those that have only Rpi activity could lead to a new type of highly selective antibiotics. For these reasons, we have actively sought inhibitors that are able to distinguish between enzymes catalyzing the two types of reactions.

A knockout study in *M. tuberculosis* has not yet been performed, but some evaluation of *rpiB* essentiallity is still possible.[6] Using transposon site hybridization experiments, Sassetti et al. identified *rpiB* as being essential for optimal growth of *M. tuberculosis* under some, but not all, circumstances.[13] Further, an Rpi of either type A or B (or both) is present in every complete genome, indicating that the activity is likely to play an important role in an organism's ability to maintain the correct balance of different nutrients in the cell. This is supported by a recent medical study that revealed that a human deficiency in RpiA causes destruction of myelin sheaths (leukoencephalopathy), which in turn leads to extensive brain abnormalities.[14] In the pathogens *Trypanosoma cruzi* and *M. tuberculosis*, the fact that an RpiB is the only Rpi present, and that such enzymes have no homologues in upper eukaryotic organisms, opens up the possibility of considering them as potential targets for the treatment of Chagas disease and tuberculosis.[15]

In this study, we report the synthesis of five new potential Rpi inhibitors, and an improved preparation of two previously described (one potential and one known) Rpi inhibitors, designed as analogues of the HEI postulated for the mechanism of the All6P to Allu6P isomerization (Chart 2). We also report a kinetic evaluation of these compounds on the Allpi activity of EcRpiB, as well as on the Rpi activities of both EcRpiB and MtRpiB. One of them appears as the strongest Rpi inhibitor reported to date.

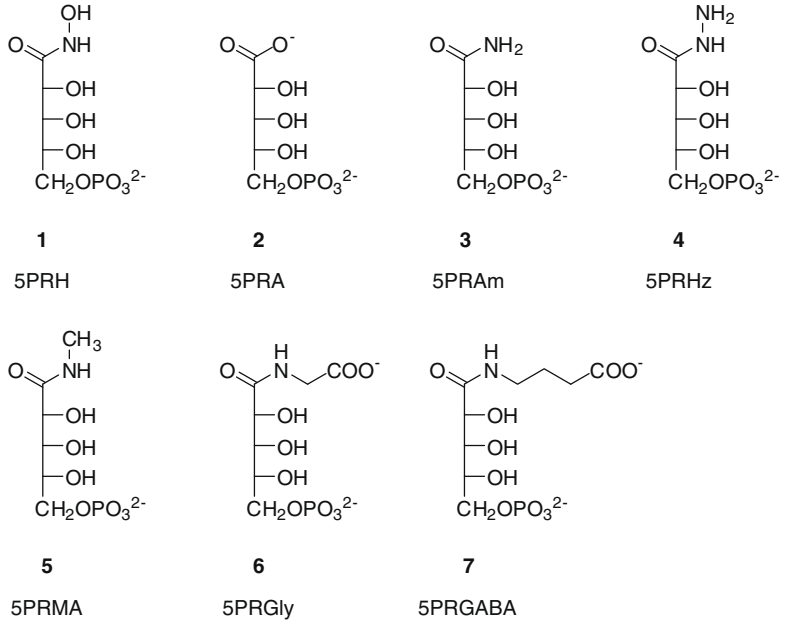

Chart 2. Structure of the inhibitors evaluated in this study: 5-phospho-D-ribonohydroxamic acid (5PRH, **1**), 5-phospho-D-ribonate (5PRA, **2**), 5-phospho-D-ribonamide (5PRAm, **3**), *N*-(5-phospho-D-ribonoyl)-hydrazine (5PRHz, **4**), *N*-(5-phospho-D-ribonoyl)-methylamine (5PRMA, **5**), *N*-(5-phospho-D-ribonoyl)-glycine (5PRGly, **6**), *N*-(5-phospho-D-ribonoyl)-γ-aminobutanoate (5PRGABA, **7**).