Biochimica et Biophysica Acta 1850 (2015) 13–21

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Allosteric regulation of the partitioning of glucose-1-phosphate between glycogen and trehalose biosynthesis in *Mycobacterium tuberculosis*

Matías D. Asención Diez [a], Ana M. Demonte [a], Karl Syson [b], Diego G. Arias [a], Andrii Gorelik [b], Sergio A. Guerrero [a], Stephen Bornemann [b], Alberto A. Iglesias [a,*]

[a] Instituto de Agrobiotecnología del Litoral (UNL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Paraje El Pozo, S3000ZAA Santa Fe, Argentina
[b] Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, United Kingdom

ARTICLE INFO

Article history:
Received 16 July 2014
Received in revised form 20 September 2014
Accepted 22 September 2014
Available online 30 September 2014

Keywords:
ADP-glucose pyrophosphorylase
Glycogen synthase
UDP-glucose pyrophosphorylase
Trehalose-6-phosphate synthase
Phospho*enol*pyruvate
Glucose-6-phosphate

ABSTRACT

Background: Mycobacterium tuberculosis is a pathogenic prokaryote adapted to survive in hostile environments. In this organism and other Gram-positive actinobacteria, the metabolic pathways of glycogen and trehalose are interconnected.
Results: In this work we show the production, purification and characterization of recombinant enzymes involved in the partitioning of glucose-1-phosphate between glycogen and trehalose in *M. tuberculosis* H37Rv, namely: ADP-glucose pyrophosphorylase, glycogen synthase, UDP-glucose pyrophosphorylase and trehalose-6-phosphate synthase. The substrate specificity, kinetic parameters and allosteric regulation of each enzyme were determined. ADP-glucose pyrophosphorylase was highly specific for ADP-glucose while trehalose-6-phosphate synthase used not only ADP-glucose but also UDP-glucose, albeit to a lesser extent. ADP-glucose pyrophosphorylase was allosterically activated primarily by phospho*enol*pyruvate and glucose-6-phosphate, while the activity of trehalose-6-phosphate synthase was increased up to 2-fold by fructose-6-phosphate. None of the other two enzymes tested exhibited allosteric regulation.
Conclusions: Results give information about how the glucose-1-phosphate/ADP-glucose node is controlled after kinetic and regulatory properties of key enzymes for mycobacteria metabolism.
General significance: This work increases our understanding of oligo and polysaccharides metabolism in *M. tuberculosis* and reinforces the importance of the interconnection between glycogen and trehalose biosynthesis in this human pathogen.

1. Introduction

Mycobacterium tuberculosis (*Mtb*) is the causative agent of tuberculosis (TB) in humans, which is one of the most serious pathogenic prokaryotes and one of the leading causes of mortality due to a single infectious agent [1]. *Mtb* is very successful as a pathogen that has adapted itself to survive hostile environments [2]. Many of its metabolic processes have not yet been fully described, and even pathways common to other organisms frequently exhibit distinctive characteristics in *Mtb* [3,4], which illustrates a metabolic plasticity that helps the organism to adapt and/or survive in the different microenvironments it is challenged with [4–9]. These particularities in the growth and survival of *Mtb* under nutritionally restrictive conditions (for example in the phagosome) represent attractive targets for new anti-tuberculosis therapies to cope with latent infection of the bacterium [5].

Oligo and polysaccharides are relevant molecules in biology in general, as they are involved in the storage of carbon and energy reserves as well as in establishing cellular structures [10]. Glycogen is a polysaccharide composed of glucose in an α-1,4-linked linear arrangement with α-1,6-branches that serves as a storage molecule in many organisms, including eukaryotes and prokaryotes [11,12]. Although the particular physiological role of glycogen in bacteria has not been clearly established, it was suggested that its accumulation could give advantages during starvation periods, providing a stored source of energy and carbon surplus [11]. In addition to glycogen, other two polysaccharides in *Mtb* are worth mentioning because of their important physiological roles [13]. One is the extracellular α-glucan, a glycogen-like polymer that is a major component of the capsule that surrounds the bacterial cell and participates in pathogenesis by serving to evade the immune response of the host [14]. The second is methyl glucose

* Corresponding author: Laboratorio de Enzimología Molecular. Instituto de Agrobiotecnología del Litoral, FBCB, UNL, S3000ZAA Santa Fe, Argentina. Fax: +54 342 4575209x217.
E-mail address: iglesias@fbcb.unl.edu.ar (A.A. Iglesias).

http://dx.doi.org/10.1016/j.bbagen.2014.09.023
0304-4165/

lipopolysaccharide (MGLP), an intracellular polymer taking part in modulating the elongation of fatty acids [15–17]. On the other hand, trehalose (Tre) is also a key carbohydrate in actinobacteria, and its synthesis in mycobacteria was found to be critical because the disaccharide acts as an energy reserve compound and also has structural relevance [18]. Tre is found esterified with different fatty acyl groups in the mycobacterial cell envelope, forming acyltrehaloses [18,19]. For example, Tre esterified at positions 6 and 6′ by mycolates constitutes the compound known as cord factor, which is a determinant for virulence and survival of *Mtb* in host cells [20]. The synthesis of cord factor has therefore attracted a lot of attention in the development of new anti-TB therapies.

Partitioning of Glc-1P into different metabolic pathways occurs at the point of incorporation of the glycosidic moiety into nucleoside-diphospho-Glc (NDP-Glc) by specific pyrophosphorylases. Subsequently, different glycosyl transferases lead the monosaccharide to the multifaceted routes of carbohydrate anabolism. For the production of storage and structural polysaccharides in bacteria, the synthesis of ADP-Glc and UDP-Glc is most relevant. UDP-Glc is synthesized in a reaction catalyzed by UDP-Glc PPase (EC 2.7.7.9), an enzyme ubiquitously distributed in organisms with a critical role in carbohydrates metabolism [21]. Many important nucleotide sugars such as UDP-xylose, UDP-glucuronic acid and UDP-galactose derive from UDP-Glc [22]. Some of these activated sugars are used to build the glycosidic structure of the bacterial cell wall and capsule or more complex oligo and polysaccharides [22,23]. UDP-Glc PPases from prokaryotes are not known to be allosterically regulated [24], sharing less than 10% identity with their eukaryotic counterparts [21].

Glycogen synthesis in prokaryotes involves the elongation of an α-1,4-glycosidic chain by glycogen synthase (EC: 2.4.1.21; GSase), using ADP-glucose (ADP-Glc) as the glucosyl donor [11,12]. In Gram-negative bacteria and cyanobacteria, a key regulatory step in this metabolic route occurs at the level of ADP-Glc synthesis, in the reaction catalyzed by allosteric ADP-Glc pyrophosphorylase (EC: 2.7.7.27; ADP-Glc PPase) [11,25]. Much less is known concerning what happens in Gram-positive bacteria, with recent reports showing important differences in allosteric regulation [26,27]. ADP-Glc PPase and GSase are respectively coded by *glgC* and *glgA* which, with the addition of *glgB* (the gene coding for branching enzyme), establish the classical GlgCA pathway for bacterial glycogen synthesis [28].

In *Mtb*, the OtsAB pathway is essential in synthesizing Tre with the use of NDP-Glc by Tre-6P synthase [29–31]. It has been recently demonstrated [28,32] that in mycobacteria Tre constitutes a glycogen precursor via a novel pathway (GlgE route), where the dissacharide is converted to maltose and activated to maltose-1P, the latter being transferred to an α-polyglucan molecule. The GlgE pathway thus establishes a metabolic link between Tre and polysaccharides, whose coordinated function and regulation are of relevance for the physiology of the microorganism. GlgE is known to be negatively regulated by phosphorylation [33] and has been genetically validated as a potential drug target [34]. To what extent each of the GlgE and GlgCA pathways contribute to cytosolic glycogen and capsular α-glucan is not yet known.

In general, efforts devoted to the characterization of enzymes related to glycogen metabolism in Gram-positive bacteria are scarce [11]. Recently, our group approached this issue in *Streptomyces coelicolor* [27] and *Streptococcus mutans* [26], where Glc-1P partitioning was understood to be controlled by the allosteric regulation of ADP-Glc PPase. In this work we extend this analysis to the metabolism in *Mtb*, studying the enzymes directing monosaccharides to glycogen and Tre synthesis. We report the molecular cloning and expression of *Mtb* genes coding for ADP-Glc PPase, UDP-Glc PPase, GSase and Tre-6P Sase and characterization of their recombinant products. Kinetic parameters were determined and ADP-Glc PPase regulatory properties were analyzed in detail. Biochemical data are discussed in the context of the metabolism of Tre, glycogen and α-glucan synthesis in mycobacteria, revealing how such a central metabolic node in the production of NDP-Glc is regulated in an important human pathogen.

2. Materials and methods

2.1. Chemicals

Restriction enzymes were purchased from Promega. All protein standards, antibiotics, isopropyl-β-thiogalactoside (IPTG) and oligonucleotides were obtained from Sigma-Aldrich (Saint Louis, MO, USA). All the other reagents were of the highest quality available.

2.2. Bacteria and plasmids

Escherichia coli Top 10 F′ cells (Invitrogen) and the pGEM®-T Easy vector (Promega) were used for cloning procedures. Expression of *otsA* was performed in *E. coli* BL21 (DE3) using pRSETA vector (Invitrogen). On the other hand, *glgA*, *glgC* and *galU* genes were expressed in *M. smegmatis* mc^{2}155 using the shuttle vector pMIP12 (from Pasteur Institute, Paris, France). Previously, this plasmid was used to obtain a number of proteins from different organisms for immunological purposes [35]. DNA manipulations, *E. coli* and *M. smegmatis* cultures as well as transformations were performed according to standard protocols [36,37].

2.3. Gene amplification

Sequences encoding GSase (*Rv1212c*; *glgA*), ADP-Glc PPase (*Rv1213*; *glgC*), UDP-Glc PPase (*Rv0993*; *galU*) and Tre-6P Sase (*Rv3490*; *otsA*) from *Mtb* H37Rv were amplified by PCR using genomic DNA as the template. Genomic DNA was kindly provided by Drs. Marisa Romano and Fabiana Bigi, from INTA Castelar (Argentina). Primers are listed in Supplemental Table I and were designed for each gene using available genomic information [38,39] in the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/index.html). PCR reaction mixtures (50 μl) contained 100 ng of genomic DNA, 2 pg of each primer; 0.2 mM of each dNTP; 2.5 mM Mg^{2+}, 5% (v/v) DMSO and 1 U *Pfu* DNA polymerase (Fermentas). Standard conditions of PCR were used for 30 cycles: denaturation at 94 °C for 1 min; annealing at 74 °C for *glgC*, 71 °C for *glgA* and 70 °C for *galU* and *otsA*, for 30 s, and extension at 72 °C for 3 min, with a final extension of 10 min at 72 °C. PCR reaction mixtures were resolved in 1% (w/v) agarose gels and PCR products were purified by means of Wizard SV gel & PCR Clean Up kits (Promega). The amplified genes [previously treated with *Taq* polymerase (Fermentas) and dATP] were cloned into the T-tailed plasmid pGEM-TEasy.

2.4. Cloning procedures

Gene identities were confirmed by DNA sequencing (Macrogen, Korea). Afterwards, pGEM-TEasy plasmids harboring *glgC* or *glgA* coding sequences were digested with *KpnI* and *PstI* and the released genes were cloned into pMIP12 to obtain the expression vectors pMIP12/*glgC* and pMIP12/*glgA*. Similarly, pMIP12/*galU* was constructed inserting the gene in the pMIP12 *Bam*HI and *Pst*I sites. Also, pGEM-TEasy/*otsA* plasmid was treated with *Bam*HI and *Hind*III restriction enzymes and subcloned to obtain the pRSET/*otsA* expression vector. In the mycobacterial expression system employed, the recombinant proteins were produced with a C-term His-tag; whereas the Tre-6P Sase was expressed in *E. coli* as a N-terminal His-tagged protein following a strategy similar to that previously reported with slight modifications [40].

2.5. Production of ADP-Glc PPase, GSase and UDP-Glc PPase in *M. smegmatis mc^{2}155*

Competent *M. smegmatis* mc^{2}155 cells were transformed with pMIP12/*glgA*, pMIP12/*glgC* or pMIP12/*galU* according to established protocols [41]. Briefly, competent cells in 200 μl of glycerol 10% (v/v) were mixed with 200 ng of plasmidic DNA in a 2 mm cuvette (HYBAID). Electroporation was performed in a Thermo CelljecT Duo (HYBAID; set