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Biocatalytic production of novel glycolipids with cellodextrin phosphorylase

Hai Giang Tran [a], Tom Desmet [a,*], Karen Saerens [a], Hendrik Waegeman [a], Stéphanie Vandekerckhove [b], Matthias D'hooghe [b], Inge Van Bogaert [a], Wim Soetaert [a]

[a] Centre for Industrial Biotechnology and Biocatalysis, Faculty of Bioscience Engineering, Ghent University, Belgium
[b] Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Belgium

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ABSTRACT

Glycolipids have gained increasing attention as natural surfactants with a beneficial environmental profile. They are typically produced by fermentation, which only gives access to a limited number of structures. Here we describe the biocatalytic production of novel glycolipids with the cellodextrin phosphorylase from *Clostridium stercorarium*. This enzyme was found to display a broad donor and acceptor specificity, allowing the synthesis of five different products. Indeed, using either α-glucose 1-phosphate or α-galactose 1-phosphate as glycosyl donor, sophorolipid as well as glucolipid could be efficiently glycosylated. The transfer of a glucosyl moiety afforded a mixture of products that precipitated from the solution, resulting in near quantitative yields. The transfer of a galactosyl moiety, in contrast, generated a single product that remained in solution at thermodynamic equilibrium. These glycolipids not only serve as a new class of biosurfactants, but could also have applications in the pharmaceutical and nanomaterials industries.

1. Introduction

Surface-active agents (surfactants) are amphiphilic molecules that reduce the interfacial tension between liquids, solids and gases. They constitute one of the most important classes of industrial bulk chemicals, with a total world production exceeding 13 million tons per year (Levinson, 2009). About half of all surfactants are used in household and laundry detergents, and thus inevitably end up in aquatic systems. Due to a growing environmental awareness, the bio-accumulation and eco-toxicity of synthetic surfactants has become an issue of major concern. Consequently, natural surfactants or biosurfactants have gained increasing attention as environmentally-friendly alternatives, because they are readily degraded and can be produced from renewable resources by means of fermentation (Soberón-Chávez, 2010).

Most biosurfactants are composed of a carbohydrate 'head' and a hydroxylated fatty acid 'tail', which are connected through an ether linkage. Unfortunately, the natural diversity of such glycolipids is rather limited, with sophorolipid being one of the few that is produced on industrial scale (Develter and Fleurackers, 2010). To broaden the range of structures that is available for application tests, enzymatic conversions would thus be a worthwhile alternative to fermentation. However, the glycosyltransferases involved in the biosynthetic pathways require nucleotide-activated glycosyl donors, which are very expensive for use in a biocatalytic process (Desmet and Soetaert, 2011). The use of glycoside phosphorylases is, therefore, a very attractive solution to develop a cost-efficient process for the synthesis of glycosylated compounds (Luley-Goedl and Nidetzky, 2010).

Cellodextrin phosphorylase (CDP, EC 2.4.1.49) is classified into family GH-94 and catalyzes the phosphorolysis of cellooligosaccharides into α-glucose 1-phosphate (Glc1P) and cellodextrins of reduced chain length (Reichenbecher et al., 1997). Thanks to the reversible nature of this reaction, CDP can also be employed for glycosylation reactions *in vitro*. Interestingly, the enzyme from *Clostridium stercorarium* has recently been found to display high activity towards alkyl glucosides as acceptors (Tran et al., 2011). In the present work, the production of various glycolipids with CDP has been evaluated. Both the donor and acceptor specificity of the enzyme has been examined, and purification processes for the obtained products have been developed.

2. Methods

2.1. Enzyme and reagents

The expression and purification of the His_6-tagged recombinant cellodextrin phosphorylase from *C. stercorarium* has been described previously (Tran et al., 2011). Deacylated acidic sophorolipid and glucolipid (carbohydrate heads β-linked to 17-hydroxy-9Z-

* Corresponding author. Address: Centre for Industrial Biotechnology and Biocatalysis, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium. Tel.: +32 9 264 9920.
E-mail addresses: haigiang.tran@ugent.be (H.G. Tran), t.desmet@ugent.be (T. Desmet).

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