Carbohydrate Research 345 (2010) 586–597

Contents lists available at ScienceDirect

Carbohydrate Research

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

Synthesis of acceptor substrate analogs for the study of glycosyltransferases involved in the second step of the biosynthesis of O-antigen repeating units

John G. Riley, Changchang Xu, Inka Brockhausen *

Department of Medicine, Division of Rheumatology, Queen's University, Kingston, Ontario, Canada K7L 3N6
Department of Biochemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6

ARTICLE INFO

Article history:
Received 25 September 2009
Received in revised form 21 December 2009
Accepted 22 December 2009
Available online 29 December 2009

Keywords:
Chemical synthesis
Undecaprenol-pyrophosphate-sugar
Glycosyltransferases
Substrate specificity
Acceptor substrate analogs
O-antigen repeating unit

ABSTRACT

O-antigens of Gram negative bacteria are polysaccharides covalently attached to lipopolysaccharides (LPS) that have roles as virulence factors. Due to the lack of defined substrates for in vitro assays only a few of the enzymes involved in the biosynthesis of O-antigens have been studied. Many O-antigens have GlcNAc at the reducing end of the oligosaccharide chain linked to pyrophosphate-lipid. We therefore designed and synthesized a series of GlcNAc-pyrophosphate-lipid analogs of the natural GlcNAc-pyrophosphate-undecaprenol acceptor substrate for studies of the acceptor specificities of O-antigen biosynthetic enzymes. We synthesized analogs with modifications of the pyrophosphate bond as well as the lipid chain. These compounds will be useful for the specificity studies of many bacterial glycosyltransferases. Knowledge of the substrate specificities is the basis for the development of specific glycosyltransferase inhibitors that could block O-antigen biosynthesis.

1. Introduction

Lipopolysaccharides (LPS) of Gram negative bacteria are important components of the outer bacterial membrane where they function to support the structural integrity of the organism and protect the cell from external chemical attack.[1,2] LPS are antigenic, act as pro-inflammatory agents, and are considered to be important virulence factors. The outermost portion of the LPS is the O-antigenic polysaccharide (O-antigen) which is of crucial importance, since it determines the pathogenicity by controlling host–bacterial interactions.[3] The O-antigen is thought to be assembled as repeating units of shorter oligosaccharides linked to pyrophosphate-lipids.[1,2] The natural acceptor substrate for the first step of repeating unit biosynthesis is undecaprenol-phosphate which is enzymatically converted to sugar-pyrophosphate-undecaprenol by the reversible transfer of sugar-phosphate from nucleotide sugar. The biosynthetic enzymes required for the transfer of sugars to form the repeating unit are thought to reside on the cytosolic side of the inner bacterial membrane. Many of the O-antigens contain GlcNAc as the first sugar at the reducing end of the repeating unit which has been transferred as GlcNAcα-phosphate from UDP-GlcNAc. Subsequently, glycosyltransferases add a second sugar residue to the nonreducing end of GlcNAc, followed by other transferases that complete the synthesis of the repeating unit. In the biosynthetic pathway of heteropolymeric O-antigens, dependent on polymerase Wzy, the repeating unit is flipped to the periplasm, polymerized, and then transferred from undecaprenol-phosphate to the outer core oligosaccharide of lipid A to form LPS. The completed LPS is then translocated to the outer membrane.

A number of proteins are involved in the regulation of this process, and several different mechanisms of assembly have been proposed.[1] Many of the genes encoding biosynthetic enzymes have been identified in O-antigen gene clusters. The location of the genes and the structures of O-antigens suggest the pathways that are involved in the assembly of O-antigen repeating units. Genes that potentially encode glycosyltransferases have been assigned mainly by comparison of the sequences and overall folds of the gene products to those of other glycosyltransferases. However, very few of the glycosyltransferases have been assayed and biochemically characterized.[4–9]

We have recently synthesized GlcNAcα-PO_3-PO_3-$(CH_2)_{11}$-*O*-phenyl (GlcNAc-PP-PhU)[4] as a natural substrate analog and have shown that the GlcNAc derivative was an excellent acceptor substrate for enzymes from *Escherichia coli*, *Salmonella*, and *Shigella* that catalyze the second reaction of the repeating unit assembly by transferring a sugar residue to GlcNAc. This synthetic substrate allowed the characterization of these enzymes. In another synthetic method, UDP-GlcNAc which has GlcNAc in the α-configuration can be conveniently used as the starting material for the synthesis of GlcNAcα-pyrophosphate substrate analogs.[10]

* Corresponding author. Tel.: +1 613 533 2927; fax: +1 613 549 2529.
E-mail address: brockhau@queensu.ca (I. Brockhausen).

0008-6215/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2009.12.022

Table 1
O-antigen structures of different serotypes of Gram negative bacteria, terminating in GlcNAc at the reducing end of the O-antigen repeating unit (ECODAB), and the putative enzymes expected to add a second sugar to GlcNAc-PP-R in the synthesis of the repeating unit

Serotype	Linkage	Putative enzyme	Short name	Starting NTP	Donor
Escherichia coli					
O7	D-Galβ1-3	β3-Gal-T	WbbD	UDP-Glc	UDP-Gal
O55	D-Galβ1-3	β3-Gal-T	WbgM	UDP-Glc	UDP-Gal
O64; O114; O153	D-Galβ1-3	β3-Gal-T		UDP-Glc	UDP-Gal
O83; O136	D-Galβ1-4	β4-Gal-T		UDP-Glc	UDP-Gal
O1; O1B; O3; O10; O18A1; O18ac; O18B; O18B1; O18A, O18A1; O69; O113; O126	D-Galα1-3	α3-Gal-T		UDP-Glc	UDP-Gal
O111	D-Galα1-3	α3-Gal-T	WbbP	UDP-Glc	UDP-Gal
O116	D-GalAα1-3	α3-D-GalA-T		UDP-GlcA	UDP-GalA
O65	D-GalAβ1-3	β3-D-GalA-T		UDP-GlcA	UDP-GalA
O23A; O121	D-GalNAcα1-3	α3-GalNAc-T		UDP-GlcNAc	UDP-GalNAc
O138	D-GalNAcAα1-3	α3-GalNAcA-T		UDP-GlcNAc	UDP-GalNAcA
O56	D-Glcβ1-3	β3-Glc-T	WfaP	UDP-Glc	UDP-Glc
O152	D-Glcβ1-3	β3-Glc-T	WfgD	UDP-Glc	UDP-Glc
O173	D-Glcβ1-3	β3-Glc-T		UDP-Glc	UDP-Glc
O148	D-Glcα1-3	α3-Glc-T	WbbG	UDP-Glc	UDP-Glc
O126	D-GlcNAcα1-3	α3-GlcNAc-T		UDP-GlcNAc	UDP-GlcNAc
O98	L-QuiNAcα1-3	α3-L-QuiNAc-T	WbwW	UDP-D-GlcNAc	UDP-L-QuiNAc
O6; O44; O66; O77	D-Manβ1-3	β3-Man-T		GDP-Man	GDP-Man
O78; O88	D-Manα1-3	α3-Man-T		GDP-Man	GDP-Man
O58	D-Man2Acβ1-3	α3-Man2Ac-T		UDP-GlcNAc	UDP-Man2Ac
O141	D-Man6Acα1-3	α3-Man6Ac-T		UDP-GlcNAc	UDP-Man6Ac
O159	L-Fucα1-3	α3-Fuc-T		GDP-Fuc	GDP-L-Fuc
O4; O25; O26	L-FucNAcα1-3	α3-FucNAc-T		UDP-GlcNAc	UDP-L-FucNAc
O105	L-Rhaβ1-3	β3-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
O1A; O2; O149	L-Rhaβ1-4	β4-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
O16; O31; O75; O119; O139	L-Rhaα1-3	α3-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
O16	L-Rha2Acα1-3	α3-L-Rha2Ac-T		dTDP-L-Rha	dTDP-L-Rha
Salmonella					
O35 (23)	D-Galβ1-4	β4-D-Gal-T		UDP-Glc	UDP-Gal
O43	D-GalNAcα1-3	α3-D-GalNAc-T	WfbG	UDP-GlcNAc	UDP-GalNAc
O145 (105)	L-FucNAmα1-3	α3-L-FucNAm-T		UDP-GlcNAc	UDP-L-FucNAc
O111 (88)	D-Galα1-3	α3-D-Gal-T		UDP-Glc	UDP-Gal
Shigella					
O7 (83); O114 (85); O40	D-Galβ1-3	β3-D-Gal-T		UDP-Glc	UDP-Gal
D9	D-Galβ1-3	β3-D-Gal-T	WbdH	UDP-Glc	UDP-Gal
O136 (B14)	D-Galβ1-4	β4-D-Gal-T	WfeD	UDP-Glc	UDP-Gal
O167 (32)	D-Galfβ1-3	β3-D-Galf-T		UDP-Glc	UDP-Galf
O121 (78)	D-GalNAcAα1-3	α3-D-GalNAcA-T		UDP-GlcNAc	UDP-GalNAcA
O152 (D12)	D-Glcβ1-3	β3-D-Glc-T		UDP-Glc	UDP-Glc
O148	D-Glcα1-3	α3-D-Glc-T	WbbG	UDP-Glc	UDP-Glc
O143 (43)	D-GlcAβ1-3	β3-D-GlcA-T		UDP-Glc	UDP-GlcA
B5; B9	D-GlcAα1-3	α3-D-GlcA-T		UDP-GlcA	UDP-GlcA
B16	D-Manβ1-3	β3-D-Man-T		UDP-Man	UDP-Man
O58 (84)	D-Man2Acα1-3	α3-D-Man2Ac-T		UDP-GlcNAc	UDP-Man2Ac
O159 (15); O168(D4)	L-Fucα1-3	α3-L-Fuc-T		GDP-Fuc	GDP-L-Fuc
O29 (D11)	L-FucNAcβ1-3	β3-L-FucNAc-T		UDP-GlcNAc	UDP-L-FucNAc
O172 (4)	L-FucNAcα1-3	α3-L-FucNAc-T		UDP-GlcNAc	UDP-L-FucNAc
B11; O150(D13)	L-Rhaβ1-3	β3-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
O149 (B1); B4	L-Rhaβ1-4	β4-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
D10	L-Rhaα1-4	α4-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
B13	L-QuiNAcα1-3	α3-L-QuiNAc-T		UDP-GlcNAc	UDP-L-QuiNAc
Yersinia					
O98 (86)	L-QuiNAcα1-3	α3-L-QuiNAc-T		UDP-D-GlcNAc	UDP-L-QuiNAc

(*continued on next page*)