Chemical Synthesis Community
Lecture Series

NDP-sugar biosynthesis and glycorandomization

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## Glycoslated Biomolecules and their functions

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significance of Glycoslated Bimolecules

some human diseases are associated with protein glycosylation patterns.

viral infections often involves recognition of specific cell surface protein glycoforms

bacterial virulence is related to cell-surface polysaccharides

glycosylated small molecules are clinically useful for the treatment of bacterial and fungal infections, cancer, and other human diseases
Glycoslated secondary metabolites

Cororubicin
respinomycin
rubradirin
kijanimicin
calicheamicin
everninimicin

Current Opinion in Chemical Biology 2008, 12:297–305
Sugar Activation

F-6-p → G-6-p → TDP-sugars → CDP-sugars → UDP-sugars → GDP-sugars

PMI → GlmS → mutase → thymidylyl-transferase → cytidylyl-transferase

mutase → uridylyl-transferase

guanidlylyl-transferase → Leloir Pathway

Thymidine diphosphate-α-D-glucose: precursor for sugar modifications

- Enzymatic recognition
- Good leaving group for glycoslation
Entry point into TDP-sugar metabolism

Naturally occurring TDP-sugars

Group I

6-deoxysugars

4-amino-4,6-dideoxysugar

3-amino-3,6-dideoxysugar

Naturally occurring TDP-sugars
Group II

TDP-D-desosamine
TDP-D-chalcomycin
actinospectose

TDP-4,6-dideoxysugars

3-amino-2,3,6-trideoxysugars

Naturally occurring TDP-sugars
Group III

TDP-2,6-dideoxysugars

TDP-2,3,6-trideoxysugars

TDP-4-amino-2,3,6-trideoxysugars

Other Naturally occurring nucleotide-sugars

UDP-sugars

GDP-sugars

CDP-sugars

General Reaction Types of Sugar Biosynthetic Enzymes

Reduction

Epimerization/isomerization

Transamination

Methylation

Deoxygenation
4,6-dehydratase

Adrian D. Hegeman,‡ Jeffrey W. Gross,‡ and Perry A. Frey Biochemistry, Vol. 40, No. 22, 2001
3,5-epimerase

TDP-4-keto-6-deoxy-L-mannose

4-aminotransferase

external aldimine intermediate

3-C-methyltransferase
2-dehydratase

Unusual modification of NDP-sugars

fortimycin, and moenomycin

Unusual modification of NDP-sugars

In vitro sugar biosynthesis (TDP-L-epivancosamine)

Huawei Chen*, Michael G. Thomas, Brian K. Hubbard, Heather C. Losey, Christopher T. Walsh†, and Michael D. Burkart*
Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115

Chen et al. PNAS October 24, 2000 vol. 97 no. 22 11947
Production of TDP-L-epivancosamine via azide Reduction

Extent of reduction > 95%

Nitrososynthase Oxidation Reaction: Time Course

Nitrososynthase Substrates

Glycosyltransferase structure

(a) The GT-A fold is represented by the inverting enzyme SpsA from *Bacillus subtilis*, (b) the GT-B fold, by bacteriophage T4 β-glucosyltransferase

Glycosyltransferase

\[ \text{retaining GT} \]

\[ \text{inverting GT} \]

\[ \alpha-D\text{-glycoside} \]

\[ \beta-L\text{-glycoside} \]

Glycosyltransferase mechanism

Glycosyltransferase

aryl-C-glycosidic bond formation

Glycosyltransferase

in vitro and in vivo Glycorandomization

Expensive cofactors
Protein purification
Optimization of rxn conditions

Provided by host

TDP16 (glycosyltransferase) substrates

OleD active site residues

To maximize the efficiency of OleD for glycosylation within the cytoplasm of *E. coli*

8 residues were chosen for mutation by saturation mutagenesis creating 8 mutant libraries

Activity of prototype glycoside producing strains

4-methylumbelliferone (3)  novobiocin (4)

urdamycin
Engineering of nucleotidyltransferase

E. coli galactokinase (GalK)

Chemoenzymatic glycorandomization

Library of reducing sugars

Library of sugar-1-phosphates

Library of nucleotide diphosphate sugars

GalK

21 vancomycin analogues
N3-289  39 additional vancomycin derivatives

CuI-catalyzed Huisgen [3+2]cycloaddition

d-glucose and l-vancosamine

Summary

- Structural diversity with a handful of enzyme activities
- Unique arrangements of catalytic residues, coenzymes, and cofactor
- Deoxysugars can be further modified by unusual enzymes
- Sugar modifying enzymes and glycosyltransferases exhibit a degree of substrate flexibility
- Metabolic pathway engineering approaches resulted in new glycoforms
- Characterizing and modifying the sugar biosynthetic enzymes will expand glycodiversification leading to generation of new compounds of interesting clinical use