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A chemoenzymatic route to synthesize unnatural sugar nucleotides using a novel *N*-acetylglucosamine-1-phosphate pyrophosphorylase from *Camphylobacter jejuni* NCTC 11168

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ABSTRACT

A novel *N*-acetylglucosamine-1-phosphate pyrophosphorylase was identified from *Campylobacter jejuni* NCTC 11168. An unprecedented degree of substrate promiscuity has been revealed by systematic studies on its substrate specificities towards sugar-1-P and NTP. The yields of the synthetic reaction of seven kinds of sugar nucleotides catalyzed by the enzyme were up to 60%. In addition, the yields of the other nine were around 20%. With this enzyme, three novel sugar nucleotide analogs were synthesized on a preparative scale and well characterized.

Unnatural sugar nucleotides, analogs of common sugar nucleotides equipped with functional groups or its derivatives, are indispensable materials for deciphering structure–function relationships in carbohydrate-associated pathways and discovering carbohydrate-based drugs. In addition to presenting nine common sugar nucleotides identified in mammalian cells, several unnatural sugar nucleotides have been discovered from different organisms, for example, UDP-2-acetamindo-2,6-dideoxy-α-D-xylo-hexos-4-ulose,[1] UDP-4-keto-6-deoxyl-GlcNAc,[2] UDP-2,4-diacetamido-Bac, ADP-Glc,[3] dTDP-Glc, dTDP-4-keto-L-rhamnose, dTDP-L-rhamnose.[4] Recent studies show that those unnatural sugar nucleotides are considered to have functional impact on biological activity, selectivity and pharmacokinetic properties of glycoconjugates.

Uridine 5′-diphosphate *N*-acetylglucosamine (UDP-GlcNAc) is a ubiquitous and essential cytoplasmic amino sugar nucleotide, plays an important role in the biosynthetic pathway of peptidoglycan,[5,6] the core and lipid A moieties of the lipopolysaccharides,[7] enterobacterial common antigen, some O antigens of Gram-negative bacteria and the teichoic acid of Gram-positive bacteria. Chemical and/or enzymatic approaches have been thoroughly studied to synthesize UDP-GlcNAc.[8,9] Given the potential roles of unnatural sugar nucleotides and the functional roles of UDP-GlcNAc, the development of chemical and/or enzymatic methods to derivatize UDP-GlcNAc with diverse moieties has attracted much attention, however, for enzymatic methods, the major bottleneck is the limited availability of functional enzymes which have broad substrate specificity. *N*-acetylglucosamine-1-phosphate pyrophosphorylase (GlmU) is a cytoplasmic enzyme involved in prokaryotic biosynthesis pathway and an attractive target for antibiotic drug discovery.[10] Our previous work on *Escherichia coli* K12 GlmU (EcGlmU) demonstrated that EcGlmU could be used to prepare UDP-GlcNAc derivatives.[11] Unfortunately, the relatively strict substrate specificity and low yields of products have restricted the application of this enzyme.

To gain insight into chemoenzymatic synthesis of unnatural sugar nucleotides, especially those UDP-GlcNAc derivatives, we focus on *Campylobacter jejuni* glycosylation system[12–15] to find a feasible and effective approach to synthesize unnatural sugar nucleotides which can greatly benefit synthetic, biological and medicinal chemistry. Herein, we provide a chemoenzymatic method to synthesize multiple unnatural sugar nucleotides using a novel *N*-acetylglucosamine-1-phosphate pyrophosphorylase (CjGlmU) from *C. jejuni* NCTC 11168 (Scheme 1).

According to our previous work on *Escherichia coli* K12 GlmU,[11,16] we cloned and overexpressed CjGlmU. The catalytic activity of CjGlmU was detected under standard condition in a total 100 μL solution system containing 20 mM Tris–HCl (pH 7.5), 5 mM

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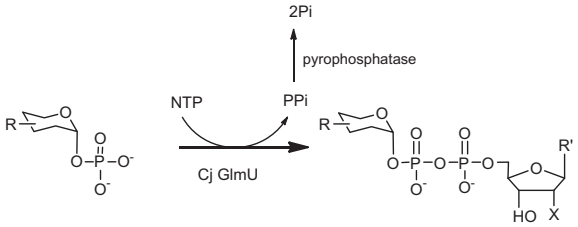
$MgCl_2$, 5 mM GlcNAc-1-P, 5 mM UTP, 1 U/mL yeast inorganic pyrophosphatase and 10 μL purified protein (about 6 μg). Yeast inorganic pyrophosphatase could drive the reaction forward by



Scheme 1. Enzymatic synthesis of unnatural sugar nucleotides using CjGlmU from sugar-1-Ps and NTPs.

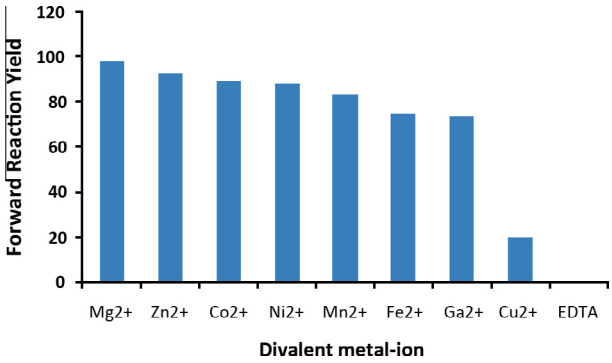
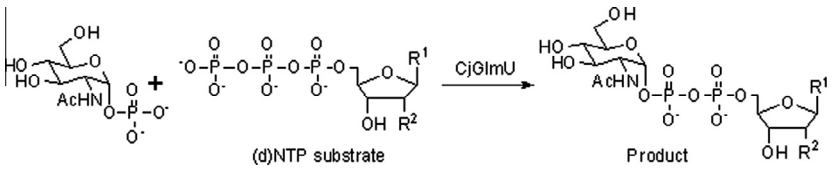
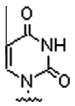
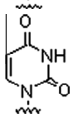
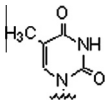
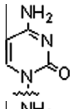
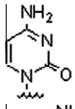
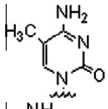
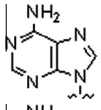
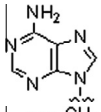
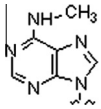
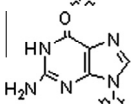


Figure 1. Divalent metal-ion preference.

Table 1
Yields of NDP-GlcNAc analogs synthesized by CjGlmU



Entry	R^1	R^2	(d)NTP substrate	Product	Exact mass	Reaction yield[a] (%)
1		–OH	UTP	UDP-GlcNAC	607.1	97.9
2		–H	dUTP	dUDP-GlcNAC	591.1	94.2
3		–H	dTTP	dTDP-GlcNAC	605.1	97.7
4		–OH	CTP	CDP-GlcNAC	606.1	64.6
5		–H	dCTP	dCDP-GlcNAC	590.1	3.6
6		–H	dm^5CTP	dm^5CDP-GlcNAC	614.1	N/A[b]
7		–OH	ATP	ADP-GlcNAC	630.1	3.3
8		–H	dATP	dADP-GlcNAC	614.1	N/A[b]
9		–H	dm^6ATP	dm^6ADP-GlcNAC	628.1	10.8
10		–OH	GTP	GDP-GlcNAC	646.1	N/A[b]

[a] Yield from profiles of capillary electrophoresis.
[b] No product detected by MS or capillary electrophoresis.