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Chemoenzymatic synthesis of the sialyl Lewis X glycan and its derivatives

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ABSTRACT

A combination of recombinant FKP and α-(1→3)-fucosyltransferase allows the facile synthesis of the sialyl Lewis X tetrasaccharide glycan and its derivatives in excellent yield. In this system, the universal fucosyl donor, guanidine 5′-diphosphate-β-L-fucose (GDP-fucose), or its analogues can be generated in situ by cofactor recycling using pyruvate kinase.

1. Introduction

The kaleidoscopic functions of cell-surface glycans are governed by their diverse structures and their dynamic interactions with glycan-binding proteins (i.e., lectins).[1] Multivalent glycan–lectin interactions either convey changes in the extracellular environment or modulate cell–cell/cell–pathogen communications. For example, sialyl Lewis X (sLe^x), a tetrasaccharide glycan constitutively expressed on leukocytes, mediates the recruitment of these cells from the bloodstream into surrounding tissues during the early stage of inflammation.[2–4] SLe^x binds to E- and P-selectins, upregulated on the surface of endothelial cells, leading to leukocyte tethering and extravasation.[5] In addition, the sLe^x–E-selectin interaction is also crucial for directing the migration of hematopoietic stem cells through the blood, across the endothelial vasculature to different organs and to their bone marrow niches, the first and essential step in clinical stem cell transplantation.[6]

As revealed by NMR spectroscopy and X-ray crystallography, the Le^x domain of the sLe^x tetrasaccharide assumes a very rigid structure, displaying two surfaces along the NeuAc-Gal-Fuc axis of opposite hydrophilicity.[7] The stability of this highly compact, bipolar structure stems from the stacking of its fucose ring on top of the galactose residue with its exocyclic C-5 methyl group forming key van der Waals contacts with the hydrophobic surface of the galactose. Studies have shown the formation of this stack to be important in the binding of sLe^x to target lectins. Removal of the methyl group leads to a fivefold decrease in binding affinity of sLe^x to its target protein, E-selectin.[8]

In inflammatory diseases (i.e., rheumatoid arthritis, asthma and transplant rejection), the body's immune system inappropriately triggers an inflammatory signal and causes damage to its own tissues. One approach to treat these diseases is to disrupt the recruitment of leukocytes, a process mediated by sLe^x–selectin interactions. The possibility that inhibitors of selectin-mediated cell adhesion could serve as broad-spectrum anti-inflammatory agents has sparked significant efforts in both the pharmaceutical industry and academic laboratories to design sLe^x-based small-molecule inhibitors as selectin antagonists.[5] In order to evaluate the therapeutic value of sLe^x-based selectin antagonists, it is necessary to develop efficient methods for the synthesis of sLe^x and its derivatives bearing unnatural functionalities in each of the monosaccharide building blocks for structure–activity relationship studies.

Chemical syntheses of sLe^x have been pursued intensively,[5,9] and have permitted the elucidation of the key functional groups and structural features that contribute to the sLe^x–selectin interaction. Despite recent advances in glycosylation methodologies, the chemical synthesis of complex fucosides is still complicated by tedious protecting-group manipulations, and the use of harsh reagents and stringent anhydrous conditions.[10] This problem has been elegantly addressed by several groups using alternative approaches based on enzymatic glycosylation. Pioneering studies on the chemoenzymatic synthesis of the sLe^x tetrasaccharide were performed by Wong and co-workers who employed a recombinant human α-(1→3)-fucosyltransferase produced in eukaryotic cells.[7,11] In this process, the universal fucosyl donor, guanidine

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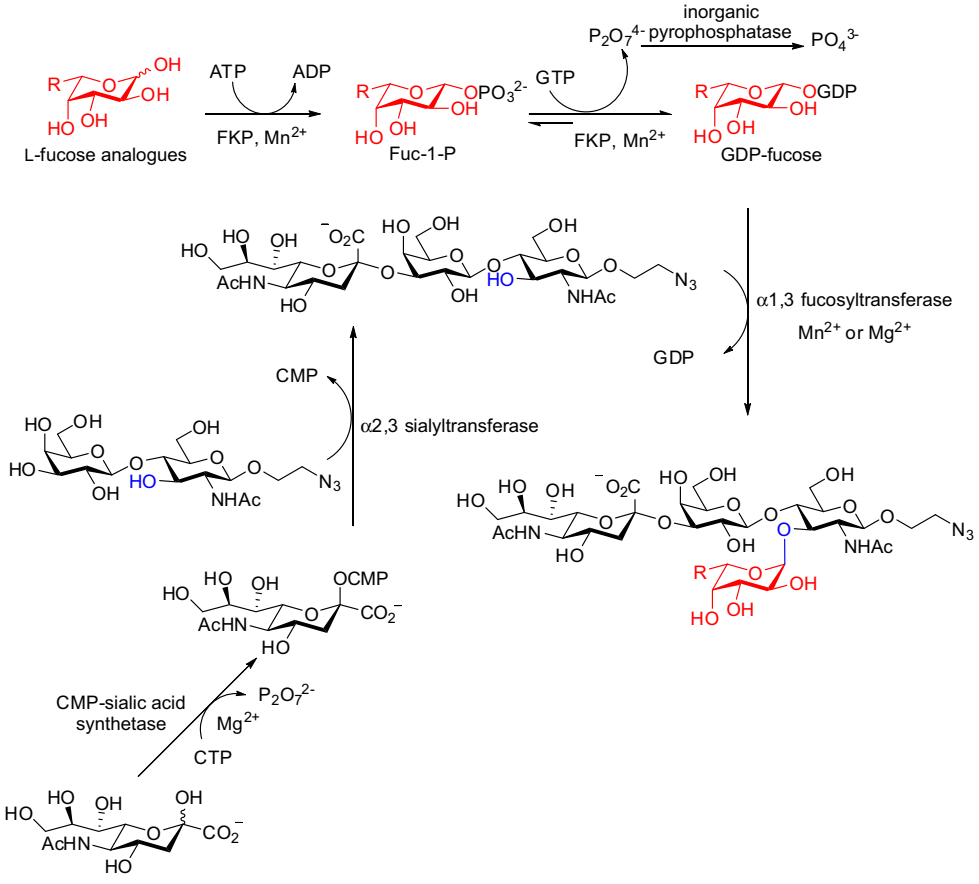
5′-diphosphate-β-L-fucose (GDP-fucose), was generated from mannose-1-phosphate (Man-1-P) via the combination of three enzymes: GDP-mannose pyrophosphorylase, GDP-mannose 4,6-dehydrase (GMD), and GDP-keto-6-deoxymannose 3,5-epimerase/4-reductase (GMER). The latter two enzymes are found in the de novo biosynthetic pathway of GDP-fucose.[12] The human α-(1→3)-fucosyltransferase, being a type II transmembrane glycoprotein, is difficult to produce in large quantities. Additionally, Man-1-P, although commercially available, is prohibitively expensive for large-scale synthesis.

We recently reported a facile and cost-effective method for the chemoenzymatic synthesis of GDP-fucose and Le^x derivatives.[13] This method exploits FKP (L-fucokinase/GDP-fucose pyrophosphorylase), a bifunctional enzyme isolated from *Bacteroides fragilis* 9343, which converts L-fucose to fucose-1-phosphate (Fuc-1-P) and thence to GDP-fucose.[14,15] This transformation is found in the salvage pathway of *B. fragilis* 9343 GDP-fucose production and is conserved in all *Bacteroides* species. We found that a His$_6$-tagged recombinant FKP, expressed in *Escherichia coli*, has relaxed specificity toward fucose analogues bearing unnatural substituents at the C-5 position and is capable of generating GDP-fucose derivatives in vitro with high efficiency. Furthermore, we demonstrated that the activities of FKP can be combined with a *Helicobacter pylori* α-(1→3)-fucosyltransferase for preparative-scale syntheses of Le^x trisaccharide glycans and its structurally related derivatives.[13] Herein, we report a novel chemoenzymatic method for the synthesis of the sLe^x tetrasaccharide glycan and its derivatives on a preparative scale using the recombinant FKP and the α-(1→3)-fucosyltransferase (Scheme 1). Importantly, this approach regiospecifically incorporates fucose or its synthetic analogues to the acceptor glycan, sialyl *N*-acetyllactosamine (sLacNAc). Moreover, we demonstrate that the atom economy of this synthetic process can be improved by simply introducing a biologically inspired cofactor recycling system, in which both ATP and GTP are formed in situ using a commercially available pyruvate kinase.

2. Results and discussion

Our synthetic route is based on previous reports that bacterial fucosyltransferases act efficiently on sialylated glycans with good activity.[16] To confirm the activity of the recombinant *H. pylori* α-(1→3)-fucosyltransferase toward sLacNAc, we prepared this acceptor trisaccharide glycan using the chemoenzymatic approach developed by Chen and co-workers for synthesizing α-(2→3)-linked sialosides.[17] In this method, sialic acid or its analogues are converted to the corresponding sialylated trisaccharides in a one-pot reaction using a combination of two enzymes: CMP-sialic acid synthetase and α-(2→3)-sialyltransferase. We cloned a *Neisseria meningitides* CMP-sialic acid synthetase[18] and a *Pasteurella multocida* α-(2→3)-sialyltransferase[19] and expressed them in *E. coli*. With these recombinant enzymes in hand, we successfully synthesized sLacNAc using the system shown in Scheme 1, in which CMP-sialic acid was generated in situ by the *N. meningitides* CMP-sialic acid synthetase.

To compare the activity of the α-(1→3)-fucosyltransferase toward LacNAc and sLacNAc, we measured the k_{cat} and K_m of the fucosylation reaction for both acceptor substrates using a coupled enzyme assay (Supplementary data). Both these substrates bear a short 2-azidoethyl spacer that allows fast copper-free click modification via azide–alkyne cycloaddition[20] to fabricate glycan microarrays for high-throughput screening of sLe^x–lectin interactions.[21]



Scheme 1. A chemoenzymatic approach for the synthesis of the sLe^x tetrasaccharide derivatives.