Molecular Aspects of Medicine 51 (2016) 125–143

Contents lists available at ScienceDirect

Molecular Aspects of Medicine

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

Exploring human glycosylation for better therapies

Larissa Krasnova [a], Chi-Huey Wong [a,b,*]

[a] Department of Chemistry, The Scripps Research Institute, La Jolla, California, USA
[b] The Genomics Research Center, Academia Sinica, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 1 March 2016
Revised 28 April 2016
Accepted 6 May 2016
Available online 10 May 2016

Keywords:
Glycan antigens
Glycan arrays
Glycosylation probes
Homogeneous antibodies
Cancer vaccines

ABSTRACT

Glycosylation of lipids and proteins is not encoded by genes directly and depends on many factors including the origin of cell-lines, differential expression of carbohydrate enzymes and availability of substrates, as well as environmental conditions. Individual cells from different tissues produce each glycoprotein as heterogeneous mixtures of glycoforms with distinct biological activities in response to different conditions and disease states. As the result, the study of glycosylation could not rely purely on biochemical methods; instead it requires a multidisciplinary approach utilizing a variety of methods including genetic manipulation and glycosylation pathway engineering, structural and functional proteomic analysis, chemical and enzymatic synthesis, development of glycosylation probes and glycan microarrays. This review highlights recent progress and demonstrates how the availability of structure-defined oligosaccharides enables development of new and improved therapies, such as therapeutic homogeneous antibodies and carbohydrate-based vaccines against cancer.

Contents

* Corresponding author. Department of Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA; The Genomics Research Center, Academia Sinica, 128 Academia Road, Section 2, Nankang, Taipei 115, Taiwan. Tel.: 1 (858) 784 2487; fax: 1 (858) 784 2409.
E-mail addresses: wong@scripps.edu or chwong@gate.sinica.edu (C.-H. Wong).

http://dx.doi.org/10.1016/j.mam.2016.05.003
0098-2997/

1. Introduction

Oligosaccharides are often displayed on the cell surface through conjugation to lipids and proteins, and are involved in various intercellular communications and carbohydrate-mediated recognition processes. Since the biosynthesis of glycans depends on the expression of genome-encoded enzymes, the composition of cell-surface carbohydrates is specific to the cell type and alters as the cell undergoes developmental and functional changes. However, elucidation of the biological functions of glycans can be a complex task, and requires effective methods and tools for the study. Over the last 25 years, an impressive progress has been attained in our understanding the role of carbohydrates in biology (National Research Council Committee, 2012). Much of this achievement is due to the improvements in the methods of oligosaccharides synthesis, which can deliver substantial amounts of pure material required for the functional study. Another element that helped expand our knowledge of glycosylation events was the emergence of new tools for the study of glycosylation, such as methods for specific gene expression and sequencing, structural analysis and glycoproteomics, the design of glycosylation probes and glycan arrays. These advances made possible identification of novel glycan structures involved in various carbohydrate-mediated biological recognitions, which led to the development of carbohydrate-based medicines.

2. Glycan synthesis and automation

One of the major challenges in oligosaccharide synthesis is selective glycosidic bond formation. The stereoselectivity of chemical glycosylation depends on the structural features of the glycosyl donors and protecting groups (PGs). Although many glycosylation procedures have been developed and can provide a high degree of stereocontrol, their practicality remains a major challenge. Recent efforts have been directed toward the development of simple and efficient methods for glycan assembly, such as one-pot procedure for the construction of complex oligosaccharides (Raghavan and Kahne, 1993), including variations of this concept using the PG-controlled reactivity of glycosyl donors (Douglas et al., 1998; Huang et al., 2004), and the orthogonal one-pot strategy (Yamada et al., 1994) through highly specific activation of leaving groups (Yasomanee and Demchenko, 2013).

2.1. Programmable one-pot synthesis

Although the one-pot strategy has proven to be quite efficient for the synthesis of complex oligosaccharides, it is limited to the laboratories with the expertise in carbohydrate chemistry. To transform the highly specialized carbohydrate syntheses into a routine operation, a programmable strategy of oligosaccharide assembly has been developed by the Wong group (Zhang et al., 1999). This method utilizes the designed software for the selection of thioglycoside building block donors based on their relative reactivity values (RRVs). The RRVs are determined from competition experiments against peracetylated thiomannoside (RRV=1) and reflect the effect of protecting groups on the reactivity of anomeric center. The current database contains more than 400 building blocks and has been successfully applied to the synthesis of various oligosaccharides, including colon cancer antigen Le^y, sLe^x, fucosyl GM_1, and embryonic stem cell surface carbohydrates Lc_4 and $IV^2Fuc\text{-}Lc_4$ (Hsu et al., 2011). Fig. 1a illustrates synthesis of the Globo H antigen, which was performed in a highly efficient manner (Burkhart et al., 2001).

2.2. Automated solid-phase synthesis

Development of automated oligosaccharide synthesizer has been highly anticipated for quite some time. The most advanced prototype developed by the Seeberger group utilizes the reengineered peptide synthesizer with several adjustments to accommodate for glycosylation conditions, including temperature control of the reaction vessel and the use of inert gas atmosphere to handle sensitive reagents (Plante et al., 2001). As illustrated in the preparation of Globo H, the reducing end sugar is coupled to the solid support and is used as an initial glycosyl acceptor. The cycles of coupling – selective removal of temporary PG and glycosylation of solid-bound acceptor – are repeated until the oligosaccharide of the desired length is assembled (Fig. 1b) (Werz et al., 2007). In general, each coupling step proceeds with high efficiency (80–90%) and can be monitored by UV trace of the Fmoc group. The release from solid support via Grubbs' metathesis gives pentenyl glycoside donor. More recently, a new bifunctional linker was introduced, thus giving access to amine functionalized glycans suitable for the immediate application, such as conjugation to the carrier protein for vaccine design or attachment to activated surfaces for the glycan array fabrication (Krock et al., 2012). The main drawbacks of the method are the need for selective deprotection after each coupling step and the use of a large excess of building blocks. These drawbacks could be partially addressed by the solution phase automated synthesis using flow reactors (Geyer et al., 2009), fluorous tags (Jaipuri and Pohl, 2008; Zhang et al., 2009) or using reagent free donor activation as in the case of electrochemical synthesis of oligoglucosamines (Nokami et al., 2013, 2015). Nevertheless, the solid-phase automated synthesis was successfully applied to the synthesis of several notable targets including Le^y, Le^x antigens, short glycopeptides, glycosaminoglycan oligosaccharides, and GPI glycolipids (Seeberger, 2015). The idea of automated synthesis with enzymes immobilized on solid supports, coined artificial Golgi, was originally demonstrated by the Nishimura group (Nishimura, 2005), and inspired further studies toward enzymatic synthesis of oligosaccharides in the digital microfluidic devices (Martin et al.,