

Time for Molecular Glycobiology

Zhengliang L Wu*

R&D Systems Inc., McKinley Place NE, Minneapolis, USA

Glycobiology, a field of research on glycans, is considered to be distinct from molecular biology, a field of research traditionally focused on protein and nucleic acids. The reason for this distinction is more due to methodological differences. The common practices for molecular biology including molecular cloning and detection methods are not available to glycobiology. However, with the recent technique advances, such as methods for glycosyltransferase assay, and availability of various new enzymatic tools, such as commercially active glycosyltransferases and sulfotransferases, research on glycobiology is going to be more similar to molecular biology and it is reasonable to say that glycobiology is going to enter into a new phase of molecular glycobiology.

Glycobiology is the field of research devoted to the studies of the biological functions of carbohydrates. Molecular biology, by definition, studies the biological functions of all molecules found in a biological system in general. Therefore, glycobiology can be considered as a part of molecular biology. However, traditionally molecular biology only refers to the study of the biological functions of nucleic acids and proteins, because techniques and methods that allow biologists to do functional studies have been available for these molecules. These methods include polymerase chain reaction, recombinant DNA and protein technology, and methods for detection of these molecules such as Southern, Northern and Western blotting. These methods provide the means for obtaining relatively large quantities of pure molecules that are otherwise impossible to obtain and detecting specific molecules among an overwhelmingly abundant unrelated molecules. However, similar methods are not available to study carbohydrates, because carbohydrate synthesis is fundamentally different from those of DNA and proteins, and, carbohydrate detecting has always been challenging.

While DNA and proteins are synthesized with templates, carbohydrates are synthesized by extending scaffold molecules, such as proteins or lipids, with monomeric sugars from activated nucleotide sugar donors by various glycosyltransferases. The synthesized carbohydrates can be further sulfated by various sulfotransferases from the activated sulfate donor 3'-phosphoadenosine 5'-phosphate sulfate (PAPS). Sulfation is most frequently observed on glycosaminoglycans. Although no templates are used, specific carbohydrate structures can be synthesized. These structures are believed to be generated through the availability and specificities of various glycosyltransferases and sulfotransferases in the Golgi apparatus.

Recently, more and more active recombinant glycosyltransferases and sulfotransferases have been made commercially available, which is mainly led by R&D Systems, a biotech company based at Minneapolis, MN. Currently, R&D Systems supplies numerous active glycosyltransferases and sulfotransferases. With these enzymes, it is possible to synthesize or restore specific glycan epitopes on specific targets, such as therapeutic recombinant proteins or therapeutic stem cells. These enzymes also allow us to label and detect specific carbohydrate structures. Some glycosyltransferases can tolerate and therefore allow incorporation of bioorthogonal sugars to native carbohydrates, which becomes a method for detection of these carbohydrates [1,2]. In addition, recombinant sulfotransferases allow incorporation of S-35 directly to any substrate glycans and allows convenient detection of substrate carbohydrates [3]. In addition to the numerous carbohydrate specific synthetic enzymes, more and more

glycosidases have also become commercially available. Companies that supply glycosidases include R&D Systems, Sigma, New England Biolab, Prozyme, QA Bio, EMD Millipore.

In order to utilize these enzymes, one needs to precisely know their enzymatic properties, which requires convenient and quantitative enzymatic assays. This is especially important for glycosyltransferases and sulfotransferases, because these enzymes in general are much more difficult to assay than glycosidases. Recently, a series of phosphatase-coupled enzymatic assays for glycosyltransferases and sulfotransferases have been developed by R&D Systems [4,5]. These assays share the same principle of using specific phosphatases to release inorganic phosphate from the leaving nucleotides of respective reactions. The free phosphate is then detected using malachite-based reagents. These methods are considered to be universal as they are based on detecting the common leaving nucleotides. More importantly, these methods are very quantitative and allow accurate enzyme kinetic and substrate specificity determination, as only one coupling step is involved and the product inhibition caused by the leaving nucleotides is eliminated by hydrolyze the nucleotide.

In the same way that restriction enzymes and DNA/RNA polymerases provided the foundation for molecular biology, glycosidases, glycosyltransferase and sulfotransferases will allow us to establish a solid foundation for research on molecular glycobiology. More specifically, glycosyltransferases and sulfotransferases not only allow us to build up certain glycans or oligosaccharides, but also allow us to detect particular glycans. Glycosidases, on the other hand, allow us to decipher specific glycan structures in combination with other methods such as mass spectrometry and NMR.

References

1. Ramakrishnan B, Boeggeman E, Pasek M, Qasba PK (2011) Bioconjugation using mutant glycosyltransferases for the site-specific labeling of biomolecules with sugars carrying chemical handles. *Methods Mol Biol* 751: 281-296.
2. Prescher JA, Bertozzi CR (2005) Chemistry in living systems. *Nat Chem Biol* 1: 13-21.
3. Wu ZL, Prather B, Ethen CM, Kalyuzhny A, Jiang W (2011) Detection of specific glycosaminoglycans and glycan epitopes by in vitro sulfation using recombinant sulfotransferases. *Glycobiology* 21: 625-633.
4. Wu ZL, Ethen CM, Prather B, Machacek M, Jiang W (2011) Universal phosphatase-coupled glycosyltransferase assay. *Glycobiology* 21: 727-733.
5. Prather B, Ethen CM, Machacek M, Wu ZL (2012) Golgi-resident PAP-specific 3'-phosphatase-coupled sulfotransferase assays. *Anal Biochem* 423: 86-92.

*Corresponding author: Zhengliang L Wu, R&D Systems Inc., 614 McKinley Place NE, Minneapolis, MN 55413, USA, Tel: 612-656-4544; E-mail: zhengliang_wu@yahoo.com

Received July 10, 2012; Accepted July 12, 2012; Published July 14, 2012

Citation: Wu ZL (2012) Time for Molecular Glycobiology. *J Glycobiol* 1:e106. doi:10.4172/2168-958X.1000e106

Copyright: © 2012 Wu ZL. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.