TechNote #TNGL103

Unusual Modifications

These suggestions and data are based on information we believe to be reliable. They are offered in good faith, but without guarantee, as conditions and methods of use of our products are beyond our control. We recommend that the prospective user determine the suitability of our materials and suggestions before adopting them on a commercial scale.

Suggestions for use of our products or the inclusion of descriptive material from patents and the citation of specific patents in this publication should not be understood as recommending the use of our products in violation of any patent or as permission to license to use any patents of ProZyme, Inc.

Dr. Gordon Holt, Oxford GlycoSciences

Glycobiology is undergoing a revolution. Unparalleled numbers of scientists are turning their focus to the study of glycoconjugates in a myriad of biological processes. This surge in interest in turn has brought remarkable results. As can be seen from the reviews provided in this catalog, glycoconjugates are proving to play key roles in many topics at the cutting edge of research today.

One of the unexpected outcomes of this glycobiology revolution is that carbohydrates are now being reported in configurations that were never before described. Perhaps even more surprising, glycoconjugates are also being identified in cell compartments previously thought to be devoid of carbohydrates. The following review briefly lists some of the more novel discoveries in glycobiology.

CARBOHYDRATES IN NEW LINKAGES

Glycosaminoglycans on N-linked Oligosaccharide Cores

For many decades, glycosaminoglycans were thought to be attached to proteins only in O-linkages, i.e. via serine and threonine side chains. However, glycosaminoglycans have now also been detected in N-linkage to asparagine side chains on cell surface glycoproteins. A variety of mammalian cell types bear significant quantities of N-linked chondroitin sulfates, heparins and heparan sulfates(1,2). A 140 kDa glycoprotein modified with both sulfated sialic acid and N-linked heparin/heparan sulfates has also been described(3).

N-Acetylgalactosamine ± (sulfate, sialic acid) on N-linked oligosaccharides

The N-linked oligosaccharides on serum and cell surface glycoproteins have long been known to be terminated in sialic acid, or more rarely with galactose and N-acetylglucosamine. However, lutropin recently was shown to have 4-(SO_4)N-acetylgalactosamine terminals on its N-linked oligosaccharides(4-6). Since this discovery, many other glycoprotein hormones bearing oligosaccharides terminated by N-acetylgalactosamine also have been found(7). Interestingly, several of the N-acetylgalactosamine residues on these hormones are further modified by the addition of SO_4 or sialic acid. The glycosyltransferase and sulfotransferase catalyzing the addition of this modified carbohydrate have also been characterized(8). Sulfated N-acetylgalactosamine does not appear to affect glycoprotein hormone binding to a given hormone receptor, but instead plays a major role in its serum half-life. A hepatic reticuloendothelial cell receptor specific for sulfated N-acetylgalactosamine mediates the rapid clearance of glycoproteins bearing these terminals from the serum(9,10).

O-linked Fucose and Glucose

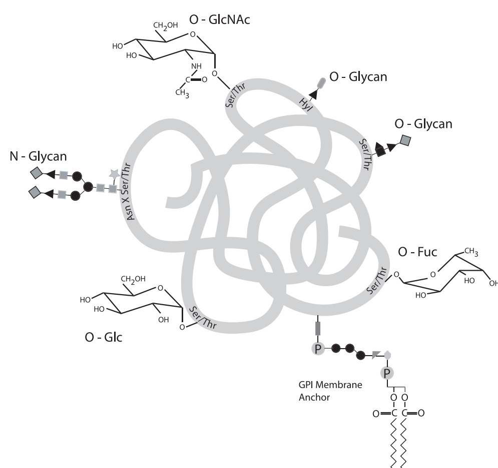
Several authors have reported the discovery of fucose and glucose moieties in O-linkage to glycoproteins that bear certain EGF homology regions, for example epidermal growth factor, tissue plasminogen activator, and coagulation Factors VII, IX and XII(11). O-linked fucose is further processed to a tetrasaccharide chain on Factor IX(12,13). O-linked glucose also is further processed, undergoing the addition of one or two xylose moieties(14). What functions O-linked glucose and fucose oligosaccharides play are unclear, although it has been reported that the removal of O-linked glucose from Factor VII has little effect on the known activities of this glycoprotein(15).

Acid-labile N-acetylgalactosamine on N-linked Oligosaccharides

The presence of mannose-6-PO_4 moieties on hydrolytic enzymes targets the transport of these glycoproteins to lysosomes by a well known receptor-mediated mechanism(16). This targeting signal is created by first transferring N-acetylglucosamine in a phosphodiester linkage to a mannose terminal on a lysosomal enzyme, and then subsequently removing the N-acetylglucosamine to leave phosphorylated mannose. Now, recent studies have hinted at the possibility of an entirely new form of glycoprotein signaling. When Golgi preparations are metabolically labeled with UDP-[6-^{3}H]-N-acetylgalactosamine, a large portion of the radioactive N-acetylgalactosamine is incorporated into acid-labile phosphodiester linkages(17). What functional role these novel phosphodiester-linked N-acetylgalactosmine residues might play is not known. However, it is likely to be different from that of mannose-6-PO_4 addition since this modification only occurs on oligomannose oligosaccharides, while phosphodiester-linked N-acetylgalactosamine is added to complex oligosaccharides(18).

Phosphoester-linked Glucose on Glycogen

Glycogen is well characterized as a highly branched, very high molecular weight polymer of glucose. However, recent reports have shown that glycogen's structure is even more complex than previously suspected. Studies have shown that a cytoplasmic phosphotransferase catalyzes the addition of glucose-1-PO_4 to the C-6 position of a glucose residue in glycogen, resulting in a glucose phosphodiester(19). Subsequent removals of these terminal phosphoester-linked glucose residues may leave glycogen with a significant density of phosphate moieties along its backbone(20).



Carbohydrates are linked to proteins in a number of ways.

CARBOHYDRATES IN NEW PLACES

O-linked N-acetylglucosamine on Cytoplasmic and Nucleoplasmic Proteins

A novel form of protein modification in which single residues of N-acetylglucosamine were shown to be attached in O-linkages to protein was first reported about ten years ago[21]. Since then, O-linked N-acetylglucosamine has been discovered on a wide variety of cytoplasmic and nucleoplasmic proteins[22]. While a function for O-linked N-acetylglucosamine addition remains uncertain, current evidence suggests that this form of protein modification may play a regulatory role analogous to protein phosphorylation[23,24].

Tyrosine Glucosylation Initiates Glycogen Biosynthesis

For decades, glycogen was thought to be composed solely of polymerized glucose; no protein was detected in covalent attachment to this huge glycan. Now, recent studies have demonstrated that glycogen is in fact a highly modified glycoprotein. Glycogen biosynthesis is initiated by the addition of several glucose residues to the hydroxyl side chain on Tyr-194 of glycogenin, a reaction catalyzed by glycogenin itself[25-27]. This oligosaccharide serves as a primer for further elongation by glycogen synthase to form glucose polymers of the much larger sizes classically recognized as glycogen[28]. The tyrosine glucosylation on glycogenin is reversed by another novel mechanism. Glycogen phosphorylase reacts with glucosylated glycogenin and phosphate to produce aglyco-glycogenin and glucose-1-PO_4[29].

Glycoconjugates in the Mitochondria

Several reports have provided evidence to suggest that there are endogenous glycoproteins in the mitochondria[30]. In addition, several enzymes necessary for the synthesis and processing of the N-linked oligosaccharides have also been localized to this organelle[31-33]. No functional role has been proposed for mitochondrial glycoproteins.

Glycosaminoglycans in the Nucleus

Heparins and heparan sulfates on cell surfaces and circulating in the serum have been the subject of tens of thousands of reports over the past fifty years. However, about 8 years ago it was shown that these glycosaminoglycans are also found in the nucleus. Heparan sulfates are transported by an as yet uncharacterized mechanism from the cell surface, through the lipid bilayer, and into the nuclear compartment[34-35]. Unlike its extracellular and cell surface counterparts, nuclear heparan sulfates are not protein-linked and are highly enriched in 2-(SO_4) glucuronic acids moieties[36]. More recently, dermatan sulfates have also been localized to the nucleus of mammalian cells[37]. The function for these nuclear proteoglycans is not known, however several reports have suggested the intriguing possibility that these glycoconjugates are involved in the regulation of cell growth. Nuclear heparan sulfate metabolism correlates with the growth state of hepatoma cells[38]. In addition, nuclear heparan sulfates isolated from confluent cells inhibited the growth rate of log phase cells, while extracts from log phase cells shortened the doubling times of slowly growing cells[39].

REFERENCES

1. Sundblad, G., Holojda, S., Roux, L., Varki, A. & Freeze, H.H. (1988) *J. Biol. Chem.* **263**, 8890-8896
2. Roux, L., Holojda, S., Sundblad, G., Freeze, H.H. & Varki, A. (1988) *J. Biol. Chem.* **263**, 8879-8889
3. Sundblad, G., Kajiji, S., Quaranta, V, Freeze, H.H. & Varki, A. (1988) *J. Biol Chem.* **263**, 8897-8903
4. Parsons, T.F. & Pierce, J. G. (1980) *Proc. Natl. Acad. Sci., U.S.A.* **77**, 7089-7093
5. Green, E. D., van Halbeek, H., Boime, I. & Baenziger, J. U. (1985) *J. Biol. Chem.* **260**, 15623-15630
6. Green, E. D., Boime, I. & Baenziger, J. U. (1986) *J. Biol. Chem.* **261**, 16309-16316
7. Baenziger, J. (1994) *FASEB J.*, **8:13**, 1019-1025
8. Hooper, L.V. & Baenziger, J. U. (1993) *Anal. Biochem.* **212**, 128-133
9. Baenziger, J. U., Kumar, S., Brodbeck, R. M., Smith, P.L. & Beranek, M. C. (1992) *Proc. Natl. Acad. Sci., U.S.A.* **89**, 334-338
10. Fiete, D., Srivastava, V., Hindsgaul, O. & Baenziger, J. U. (1991) *Cell* **67**, 1103-1110.
11. Harris, R.J. & Spellman, M. W. (1993) *Glycobiology* **3**, 219-224
12. Harris, R. J., van Halbeek, H., Glushka, J., Basa, L. J., Ling, V. T., Smith, K. J. & Spellman, M. W. (1993) *Biochemistry* **32**, 6539-6547
13. Nishinura, H., Takao, T., Hase, S., Shimonishi, Y. & Iwanaga, S. (1992) *J. Biol. Chem.* **267**, 17520-17525
14. Nishinura, H., Kawabata, S., Kisiel, W., Hase, S., Ikenaka, T., Takao, T., Shimonishi, Y. & Iwanaga, S. (1989) *J. Biol Chem.* **264**, 20320-20325
15. Bjoern, S., Foster, D. C., Thim, L., Wiberg, F. C., Christensen, M., Komiyama, Y., Pedersen, A. H. & Kisiel, W. (1991) *J. Biol. Chem.* **266**, 11051-11057
16. Dahms, N. M., Lovel, P. and Kornfeld, S. (1989) *J. Biol. Chem.* **264**, 12115-12118
17. Hayes, B. K. & Varki, A. (1993) *J. Biol Chem.* **268**, 16170-16178
18. Hayes, B. K. & Varki, A. (1993) *J. Biol Chem.* **268**, 16155-16169

19. Marchase, R. B., Bounelis, P., Brumley, L.M., Dey, N., Browne, B., Auger, D., Fritz, T. A., Kulesza, P. & Bedwell, D. M. (1993) *J. Biol Chem.* **268**, 8341-8349
20. Lomako, J., Lomako, W. M., Whelan, W. J. & Marchase, R. B. (1993) *FEBS Lett.* **329**, 263-267
21. Torres, C. R. & Hart, G. W. (1984) *J. Biol Chem.* **259**, 3308-3317
22. Haltiwanger, R. S., Kelly, W. G., Roquemore, E. P., Blomberg, M.A., Dong, L. Y., Kreppel, L., Chou, T. Y. & Hart, G. W. (1992) *Biochem. Soc. Trans.* **20**, 264-269
23. Hart, G. W., Haltiwanger, R.S., Holt, G.D. & Kelly, W. G. (1989) *Annu. Rev. Biochem.* **58**, 841-874
24. Holt. G.D., Haltiwanger, R.S., Torres, C.-R., & Hart, G.W. (1987) *J. Biol Chem.* **262**, 14847-14850
25. Lomako, J., Lomako, W. M. & Whelan, W. J. (1992) *Carbohydr. Res.* **227**, 331-338
26. Smythe, C. and Cohen, P. (1991) *Eur. J. Biochem.* **200**, 625-631
27. Cao, Y., Mahrenholz, A. M., Depaoli-Roach, A. A. & Roach, P. J. (1993) *J. Biol Chem.* **268**, 14687-14693
28. Tolmasky, D. S., Salmoral, E. M., Blumenfeld, M. L. & Krisman, C. R. (1993) *Cell Mol. Biol.* **39**, 301-308
29. Cao, Y., Skurat, A. V., DePaoli-Roach, A. A. & Roach, P. J. (1993) *J. Biol Chem.* **268**, 21717-21721
30. Levrat, C., Ardail, D. & Louisot, P. (1990) *Int. J. Biochem.* **22**, 287-293
31. Gasnier, F., Lerme, F., Rousson, R., Roussouly, P., Vaganay, E., Louisot P. & Gateau-Roesch, O. (1991) *Clin. Chim. Acta* **199**, 69-82
32. Gasnier, F., Rousson, R., Lerme, F., Vaganay, E., Louisot, P. & Gateau-Roesch, O. (1992) *Eur. J. Biochem.* **206**, 853-858
33. Ardail, D., Lerme, F. & Louisot, P. (1990) *Biochim. Biophys. Acta* **1024**, 131-138
34. Ishihara, M., Fedarko, N. S. & Conrad, H. E. (1987) *J. Biol Chem.* **262**, 4708-4716
35. Ishihara, M., Fedarko, N. S., & Conrad, H. E. (1986) *J. Biol Chem.* **261**, 13575-13580
36. Fedarko, N. S. & Conrad, H. E. (1986) *J. Cell Biol.* **102**, 587-599
37. Hiscock, D. R. R., Yanagashita, M. & Hascall, V. C. (1994) *J. Biol Chem.* **269:6**, 4539-4546
38. Ishihara, M. & Conrad, H.E. (1989) *J. Cell Physiol.* **138**,467-76
39. Fedarko, N.S., Ishihara, M. & Conrad, H.E. (1989) *J. Cell Physiol.* **139**, 287-294

1933 Davis Street, Suite 207
San Leandro, CA 94577-1258

TOLL FREE (800) 457-9444
PHONE (510) 638-6900
FAX (510) 638-6919

E-MAIL info@prozyme.com
WEB www.prozyme.com

ProZyme®, Glyko® and FACE® are registered trademarks of ProZyme, Inc., San Leandro, CA USA