COMMENTARY

Use of a mutant OGA for detecting O-GlcNAc modified proteins

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In the previous issue of *Biochemical Journal* Mariappa et al. [(2015) Biochem J. **470,** 255–262] demonstrate a new method for visualizing O-linked *N*-acetylglucosamine (O-GlcNAc) modified proteins by making use of a catalytically dead version of the enzyme that normally removes this modification. They show their approach has broader specificity than current antibody-based techniques and higher specificity than lectin and chemical biology-based labelling approaches. This commentary discusses methods for O-GlcNAc detection and the significance of this work for characterizing this common, but currently poorly understood regulatory modification.

Key words: glycosylation, mass spectrometry, OGA, O-linked *N*-acetylglucosamine (O-GlcNAc).

INTRODUCTION

Cells employ a large selection of post-translational modifications (PTMs) to dynamically alter the activity of proteins. One of the most common is the addition of a single *N*-acetylglucosamine (GlcNAc) residue on to serine and threonine residues of nuclear and cytoplasmic proteins. O-GlcNAcylation is a widespread modification, being found in all multicellular organisms. It contrasts with other types of glycosylation, in that it is not added in the endoplasmic reticulum (ER)/Golgi secretory pathway and it is a highly dynamic regulatory modification. Despite the discovery of O-GlcNAcylation over 30 years ago [1], it is only in the last five to ten years that the modification has started to attract a lot of attention, with its links to diabetes, neurodegenerative disorders and cancer now under intensive investigation [2]. Probably, the main reason for the slow uptake in the study of this PTM has been a lack of effective tools for its analysis.

A few O-linked *N*-acetylglucosamine (O-GlcNAc) antibodies are commercially available, but these were each raised against specific targets, such as the CTD110.6 antibody that targets GlcNAc modification sites in the C-terminal domain of RNA polymerase II and RL2, which was raised against modified nuclear pore proteins. Whereas these each detect a range of O-GlcNAc modification sites, they only detect a subset of all O-GlcNAc PTMs present.

Another set of strategies employed for O-GlcNAc detection have made use of galactosyltransferases specific to terminal GlcNAc residues to add modified sugar residues. These added residues could contain a radioactive tag for detection [3] or a chemical tag that can be exploited for derivatization. Ketone groups have been incorporated that allow reaction with amino-oxy groups or an azide group can be used to attach a tag through azide-alkyne cycloaddition. These reactions allow incorporation of a fluorescent tag for visualization [4,5] or a biotin tag for enrichment [6,7]. In an innovative spin on this approach, derivatization with a large polyethylene glycol mass tag allows determination of O-GlcNAc stoichiometry on individual proteins by molecular mass shifts on a 1D polyacrylamide gel [8]. Metabolic incorporation directly of an azide-containing GlcNAc has also been demonstrated [9,10], although this only labels newly modified proteins.

A third strategy employed has been to enrich for the modification using lectins, which are sugar-binding proteins. The most commonly used lectin for this approach has been wheat germ agglutinin (WGA), which has primary affinity for terminal GlcNAc residues and sialic acid moieties. However, its affinity for a single GlcNAc residue is low, so bind-and-elute strategies are not successful; rather weak affinity chromatography has been employed to enrich for modified peptides [11,12].

Both lectin and chemical derivatization strategies suffer from lack of specificity between O-GlcNAc and certain other types of glycosylation. Galactosyltransferases will label all terminal GlcNAc residues, some of which can be found in complex N-linked glycans. WGA lectin chromatography enriches an even broader distribution of complex glycopeptides in addition to O-GlcNAc, to the extent that it has been used for global glycopeptide analysis [13,14]. In particular, the discovery of single N-linked GlcNAc residues on sites where one would expect extended glycan structures [15] has uncovered a modification that neither enzyme nor lectin approaches are able to differentiate from O-GlcNAc. The only way to reliably determine that a modification is indeed an O-GlcNAc modification is through the use of MS.

Fragmentation of modified peptides using MS can usually differentiate between N- and O-linked GlcNAc modifications, as the glycosidic link in N-linked modifications is stable, whereas the O-linked glycosidic link is labile under the most common type of fragmentation, collision-induced dissociation (CID) [16]. This means that the observation of glycosylated fragment ions is indicative of N-linked modification. Also, simply identifying the modified peptide sequence is a strong clue, due to the well-defined N-linked glycosylation motif N-X-S/T (where X is anything other than a proline); if this motif is found in the peptide then

Abbreviations: CID, collision-induced dissociation; O-GlcNAc, O-linked *N*-acetylglucosamine; PTM, post-translational modification; WGA, wheat germ agglutinin.

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it is probably (although not always) an N-linked glycosylation. Determination of O-GlcNAc sites is generally not possible using CID due to the modifications lability under fragmentation, but by using alternative fragmentation approaches such as electron transfer dissociation, a number of studies have discovered a large number of O-GlcNAc modification sites [7,12,15,17].

O-GlcNAcylation is often contrasted with phosphorylation [2], as they can modify the same residues, so in certain instances must have competing regulatory effects. However, one significant difference between O-GlcNAcylation and phosphorylation is the modification machinery; whereas there are over 500 kinases in the cell and many phosphatases, there are only single enzymes responsible for the addition (OGT) [18] and removal (OGA) [19] of O-GlcNAc. These two proteins can be thought of as holoenzymes, where they are the catalytic subunit, but other binding proteins provide target specificity [20].

In an article in the previous issue of *Biochemical Journal,* Mariappa et al. [21] have exploited a mutant version of a bacterial OGA to develop a new method for O-GlcNAc detection. Their mutant protein binds substrate, but does not have catalytic activity, so does not release its bait. Their approach was to express a GST-tagged version of this mutant OGA protein and use antibodies against GST as readout for O-GlcNAcylated proteins. They show that their far-Western approach visualizes more modified proteins on a 1D polyacrylamide gel than an anti-O-GlcNAc antibody and that signal is decreased upon pre-treatment with wild-type OGA, confirming signal specifically corresponds to the level of O-GlcNAcylation. They then show that addition of PNGase F (Peptide-N-Glycosidase F), which removes complex N-linked glycans, did not affect the protein banding pattern. This indicates that their readout has no cross-reactivity against complex N-glycosylation, unlike other strategies. Finally, they used their method to visualize changes in the O-GlcNAcylated protein profile during *Drosophila* embryo development, where in general there was an accumulation of modified proteins.

Their approach shows a lot of promise for surveying protein O-GlcNAc patterns, but obviously it is important to be able to determine what the modified proteins are. Hence, if their strategy could be adapted to an immunoprecipitation approach then it would be much more powerful, as enriched samples could then be analysed by MS and proteins and modification sites identified. As previously mentioned, there have been a few significant successes recently in global O-GlcNAc protein and site identification, because lower specificity purification steps can be compensated for by the high discriminative power of the MS data. Nevertheless, having a purer sample loaded on to the mass spectrometer should allow a significant improvement in the depth of O-GlcNAc modification site characterization, which will hopefully assist in uncovering the many biological roles this regulatory modification performs.

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