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O-GlcNAc transfer: size matters

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Abstract

O-GlcNAc transferase is an essential protein catalyzing the *O*-GlcNAc modification of hundreds of intracellular proteins in higher eukaryotes. The structure of human *O*-GlcNAc transferase represents a leap in our understanding of the catalytic mechanism and recognition of protein substrates.

Post-translational modification of nucleocytoplasmic proteins by the addition of a single *O*-linked sugar, β -*N*-acetylglucosamine (*O*-GlcNAc), is a dynamic, abundant and essential process in higher eukaryotes¹. Hundreds of *O*-GlcNAc proteins have been identified, and dysregulation of cellular *O*-GlcNAc levels is thought to be associated with Alzheimer's disease, cancer and diabetes. Recent reports suggest wide-ranging regulatory effects of *O*-GlcNAcylation, reminiscent of regulatory protein phosphorylation. However, in contrast to the >500 protein kinases needed to establish complex cellular phosphorylation-mediated signal transduction networks, only a single enzyme, *O*-GlcNAc transferase (OGT), is responsible for the addition of all cellular *O*-GlcNAc. How this single enzyme is able to recognize and *O*-GlcNAcylate hundreds of individual protein substrates and regulate cellular processes has baffled the *O*-GlcNAc field for the past two decades. In a recent *Nature* report², Lazarus and colleagues present crystal structures of human OGT that represent a major advance in our understanding of OGT substrate recognition and the mechanism of glycosyl transfer.

The structures reveal two distinct regions that are found throughout the GT41 glycosyltransferase family (Fig. 1a). The N terminus consists of a set of tetratricopeptide repeats (TPRs), variable in number and known to be important for recognition of protein substrates³. The C-terminal catalytic domain contains three small subdomains, two of which form the classical glycosyltransferase type-B fold, whereas the third is found exclusively in metazoa and reveals a previously unobserved fold (Fig. 1a). Previous studies have reported the structure of the first 11.5 human OGT TPRs⁴ and the structures of a bacterial OGT homolog from *Xanthomonas campestris* in complex with UDP and UDP-GlcNAc analogs^{5–7}. Although these earlier structures defined the location of the active site and showed that the TPR repeats form a superspiral that seamlessly connects into the active site groove, the questions of mechanism and peptide substrate recognition remained. The work by the Walker group² provides a highly sought-after ternary complex with UDP (a reaction product) and a substrate peptide derived from the casein kinase II (CKII)—a bona fide OGT substrate (Fig. 1b). These ligands define the OGT active site, including the possible identity of the key catalytic base, and a mode of nucleotide binding that is in agreement with the bacterial OGT structures. The combination of the structural data of this ternary complex

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with the structure of a bacterial OGT in complex with a UDP-C-GlcNAc derivative⁵ approximately defines the sugar-binding pocket (Fig. 1b). This pocket is completely covered by the peptide, indicating a reaction mechanism whereby UDP-GlcNAc must bind first, followed by binding of the acceptor protein.

The binding mode of the CKII peptide, fully defined by 1.95-Å diffraction data, gives two crucial new insights into how OGT may differentially recognize protein substrates. First, the peptide extends from the catalytic core to the concave surface of the TPR helix, bridging these two previously identified sites of recognition (Fig. 1a,b). Precisely how the TPR helix recognizes large protein substrates remains unclear—superposition of the p53 DNA binding domain (that possesses a characterized and ordered *O*-GlcNAc site on Ser149 (ref. 8)) onto the CKII peptide results in severe clashes with the TPR helix (Fig. 1a). Together with molecular dynamics simulations conducted by Lazarus *et al.*, this suggests that the TPR helix may well be more flexible than can be gleaned from static crystal structures. Second, the peptide complex hints at how OGT might recognize *O*-GlcNAc sites. Some of the subsites (in particular -3, -1 and +2, Fig. 1c) are shallow pockets that can only accommodate small side chains. All of the side chains lining these pockets are conserved from *Caenorhabditis elegans* to human. The calculated volumes of these pockets correlate well with the degenerate *O*-GlcNAc site sequence pattern presented by Lazarus *et al.*² and others⁹ (Fig. 1c). Thus, the depth of these pockets may help OGT to select from many potential acceptor serines or threonines in the human proteome.

Current attempts to probe the functional role of *O*-GlcNAc *in vivo* rely on the use of exquisitely potent and selective inhibitors of *O*-GlcNAcase, the enzyme that removes *O*-GlcNAc^{10,11}. In addition to the new insights into mechanism and specificity, the OGT structures reported by Lazarus *et al.* will advance the rational development of potent OGT inhibitors as invaluable cell biological probes to study *O*-GlcNAc. Finally, the Lazarus *et al.* work now enables future studies to effectively target the catalytic mechanism, dissect the relative contributions of the catalytic core and TPRs to substrate binding and define the function of the enigmatic middle domain.

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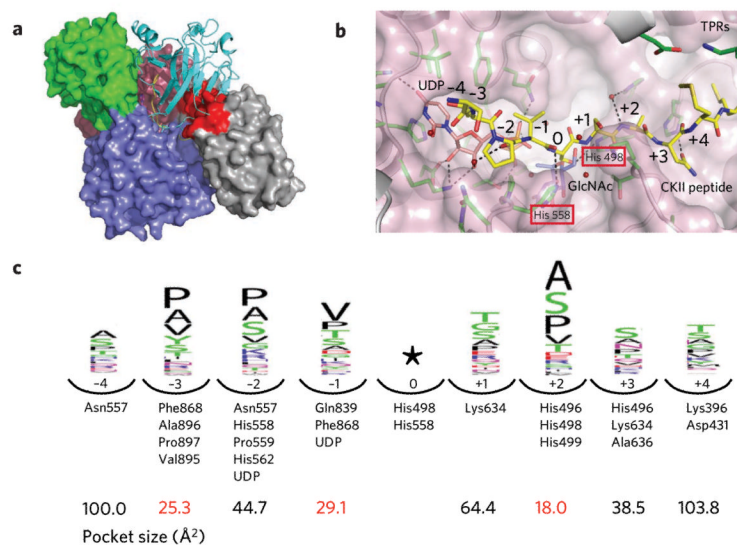


Figure 1. Insights into the human OGT structure. **(a)** The structure reported by Lazarus *et al.*² (pink and blue, GT-B fold; green, middle domain; gray, TPRs; yellow, CKII peptide). The OGT substrate p53 (PDB ID: 1TUP) was superposed into the active site by matching the substrate backbones for -2 to +2 subsites and is shown in cyan. The steric clashes between the OGT TPRs and p53 are highlighted in red. **(b)** Structure of the Lazarus *et al.*² hOGT ternary complex (protein surface in pink) with CKII peptide (sticks with yellow carbons) and UDP (sticks with pink carbons). The position of the sugar as obtained by superposition with the *X. campestris* OGT UDP-C-GlcNAc complex⁵ (blue transparent sticks) is also shown. The -4 to +4 subsites of peptide binding are labeled, and the two candidates for the catalytic base are labeled in red. **(c)** Schematic of human OGT substrate peptide subsite pockets. The sequence preference (recalculated from Lazarus *et al.* Supplementary Table 4 (ref. 2)) is placed in context of a schematic of the subsites formed by the human OGT residues listed below the pockets. Using the Lazarus *et al.* coordinates, the size of each of the subsites was approximated by the solvent accessibility of a valine residue (standard rotamer) placed at each of the positions on the CKII peptide—the smallest subsites (-3, -1 and +2) are highlighted with red boxes.