Review

Distribution of *O*-GlcNAc Transferase in the Rat Pancreas

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Received May 2, 2000; accepted May 18, 2000

O-GlcNAc transferase (OGT) catalyzes the attachment of *N*-acetylglucosamine monosaccharides to the hydroxyl group of serine or threonine residues of intracellular proteins and may play an important role in the hexosamine pathway. We examined the localization of OGT protein in the rat pancreas. Immunofluorescence staining with antibody raised against OGT stained both the exocrine acinar cells and endocrine islet cells. The acinar cell nucleus and the zymogen granule region were intensely stained. In the islets

of Langerhans, especially in the A cells, intense staining with anti-OGT antibody was observed. Immuno-electron microscopy showed the OGT to be restricted to the euchromatin of the nucleus and around the secretory granules of exocrine acinar cells and endocrine islet cells. These results suggest that OGT is involved in the regulation of transcription and of granular secretion. Thus, *O*-GlcNAcylated protein(s) may be an important component of the glucose-sensing mechanism in the pancreas.

Key words: *O*-GlcNAc transferase, Rat pancreas, Diabetes, Streptozotocin, Immunohistochemistry

I. Introduction

Classically, glycosylation has been thought to occur on proteins that are excreted or localized to the cell surface. However, in 1984 Torres and Hart found that many nuclear and cytoplasmic proteins are modified by a single *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) linked to a Ser/Thr residue [7, 17, 20]. The *O*-GlcNAc modification (termed *O*-GlcNAcylation) is a dynamic regulatory posttranslational modification that exhibits properties more like those of phosphorylation than those of typical *O*- and *N*-linked glycosylation. The *O*-GlcNAc modification has been found on a variety of eukaryotic nuclear and cytoplasmic proteins such as RNA polymerase II, transcriptional regulatory proteins, cytoskeletal proteins,

kinases, phosphatases, oncogene proteins, steroid receptors, viral proteins, and nuclear pore proteins. All of these *O*-GlcNAc-modified proteins are also known to be phosphoproteins. The localization of *O*-GlcNAc at Ser/Thr is near or identical to the phosphorylation sites. In some cases the two modifications appear to have a reciprocal relationship (Fig. 1). One proposed function of *O*-GlcNAcylation is capping of the potential phosphorylation sites [6, 7, 17]. *O*-GlcNAcylation has been implicated in the regulation of a number of cellular functions such as the DNA-binding activity of the p53 tumor suppressor, protein synthesis, neurofilament assembly, transcription, and oncogenic activity. In this review, we focus on the localization of *O*-GlcNAc transferase in the pancreas.

II. *O*-GlcNAc Transferase

A UDP-*N*-acetylglucosamine: peptide *N*-acetylglucosaminyl-transferase (OGT; EC 2-4-1), specific for the attachment of *O*-GlcNAc to proteins, has been purified and characterized. The rat liver *O*-GlcNAc transferase (OGT) is a hetero-trimer composed of two catalytic 110-

Selected by "The 40th Memorial Scientific Meeting of the Japan Society of Histochemistry and Cytochemistry" held in Kyoto, Japan on Dec. 6–7, 1999.

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Fig. 1. *O*-GlcNAcylation and *O*-phosphorylation of protein. A model representing the multiple pathways involved in regulating the post-translational state of *O*-GlcNAcylated phosphoproteins. Analogous to Ser/Thr protein kinases and phosphatases, which attach and remove phosphates, are the *O*-GlcNAc transferase and *O*-GlcNAcase, which likewise attach and remove *O*-GlcNAc.

kDa subunits and one 78-kDa (regulatory) subunit. The 110-kDa subunit is present in all tissues examined. OGT contains phosphorylated tyrosine, and the enzyme is itself modified by *O*-GlcNAc. OGT contains several tandem repeats of the tetratricopeptide repeat (TPR) motif in the amino-terminal half. This suggests that OGT may interact with other proteins via these TPR domains to form a multimeric complex. The catalytic activity exists in the carboxyl-terminal half. Unlike other glycosyltransferases previously described, this enzyme is localized in both the nucleus and the cytosol [11]. The gene encoding the 110-kDa subunit has been cloned from rat liver, *C. elegans*, and human erythrocytes [11, 12]. The gene encoding OGT shares no sequence homology with any other cloned UDP-GlcNAc transferase. However, a gene highly related to OGT has been found in all higher eukaryotes examined. OGT is expressed in all tissues examined as far. Northern blot analysis of OGT mRNA in human demonstrated that OGT expression is highest in the pancreas, at least 12-fold higher than in any other tissue examined [12]. Previously we showed by *in situ* hybridization histochemistry that the OGT gene in the rat pancreas is expressed in both the exocrine acinar cells and the islets of Langerhans [1].

III. Light Microscopic Localization of OGT and *O*-GlcNAc in Rat Pancreas

By use of a polyclonal antibody that reacts with both 110-kDa and 78-kDa subunits of OGT, the distribution of OGT in the pancreas was examined by immunofluorescence microscopy (Figs. 2, 3) [1]. To compare the localization of OGT with that of *O*-GlcNAc residues, we

employed monoclonal anti-*O*-GlcNAc antibodies (HGAC85 and RL2). In the exocrine acinar cells, staining signals for OGT were observed in the nuclei and in the zymogen granule region (Fig. 2a, b). Multiple staining showed that glucagon-, or pancreatic polypeptide-secreting cells were also intensely positive for OGT and *O*-GlcNAc (Fig. 2c-f). In the insulin- or somatostatin-secreting cells, staining for OGT was also observed, but was weaker than that in glucagon-positive cells. The OGT staining pattern in the islets was almost the same as that of *O*-GlcNAc. The results obtained with another monoclonal antibody (RL2) against *O*-GlcNAc were the same as those found with HGAC 85.

IV. Electron Microscopic Localization of OGT in Rat Pancreas

Detailed subcellular location of OGT was determined at the electron microscopic level (Fig. 4) [1]. In the acinar cells colloidal gold particles that bound to OGT were observed mainly in the euchromatin of the nucleus (Fig. 4a), and there were many colloidal gold particles around the zymogen granules in these cells. In the A cells colloidal gold particles for OGT were observed around the granules (Fig. 4b). In the B cells the same distribution pattern was observed, but the number of colloidal gold particles for OGT was less than that in the A cells.

A number of *O*-GlcNAcylated proteins have been identified in the nucleus, including p62, which is a nuclear pore protein, RNA polymerase II, and numerous transcription factors. *O*-GlcNAcylation of these and other nuclear proteins suggests that OGT activity may play a role in regulating transcription and nuclear import. OGT is abundant in the cytoplasm of acinar and A cells and less abundant in the cytoplasm of B cells. The localization of OGT suggests that OGT is also involved in the process of secretion of these granules. By catalyzing the *O*-GlcNAcylation of cytoskeletal proteins such as microtubule-associated proteins, which are involved in the secretion of granules and are heavily *O*-GlcNAcylated [2, 3], OGT may regulate granular secretion. Many cytoskeletal and membrane proteins, such as band 4.1, vinculin, cytokeratins, synapsins, ankyrins, and talins, are known to be *O*-GlcNAcylated [2, 4].

V. OGT and Diabetes

The hexosamine biosynthesis pathway plays an important role in the induction of insulin resistance [13, 14]. In this pathway, glutamine:fructose-6-phosphate amidotransferase (GFAT) plays an integral role in the development of insulin resistance. OGT utilizes cytoplasmic UDP-GlcNAc, which is synthesized in the hexosamine biosynthetic pathway. Animals with STZ-induced diabetes and hyperglycemia show greatly increased levels of hexosamine metabolites including UDP-GlcNAc [13]. This increase in hexosamine biosyn-

Fig. 2. Distribution of reactivity with immunofluorescent anti-OGT in the normal rat pancreas. **a, b:** Localization of OGT in the acinar cells. Pancreas was fixed with 4% formaldehyde. Semithin frozen sections were reacted with anti-OGT antibody, and then with Cy3-donkey anti-rabbit IgG. Nuclei were stained with DAPI. **a:** Distribution of OGT (red). Nuclei and the zymogen granule region (asterisk) are stained intensely. Nucleoli are not stained. **b:** Double exposure image of OGT and DAPI. **c, d, e, f:** Localization of OGT in the Langerhans islet (the same optical field). The sections were reacted with anti-OGT antibody, and then with Cy3-donkey anti-rabbit IgG. The sections were next incubated with anti-glucagon antibody, and then with Cy5-donkey anti-mouse IgG. The sections were finally incubated with anti-pancreatic polypeptide antibody, and then with FITC-donkey anti-guinea pig IgG. Nuclei were stained with DAPI (blue). **c:** Distribution of OGT. **d:** Distribution of glucagon reactivity (blue). **e:** Double-exposure image for pancreatic polypeptide (green) and nucleus (blue). **f:** Triple-exposure images for OGT, pancreatic polypeptide, and nucleus. White arrows in (**c, d, e, f**) indicate both glucagon- and pancreatic polypeptide-positive cells. Yellow arrow indicates the glucagon-positive cell. Bars = 10 μ m (**a, c**).

Fig. 3. The localization of OGT in rat pancreas. Schematic drawing. Red color denotes the localization of the OGT. OGT is localized mainly in the nuclei of both exocrine and islets cells. It also exists in the cytoplasm, being especially abundant in the cytoplasm of exocrine cells, A cells, and PP cells of islets.

thesis may stimulate OGT activity, inducing *O*-GlcNAc modification (*O*-GlcNAcylation) of proteins that are involved in the glucose transport system or in granule secretion. Recent kinetic analyses have confirmed that

OGT is exquisitely regulated by the UDP-GlcNAc concentration across a broad range, from low micromolar to millimolar levels [10]. Thus we hypothesize that abnormal *O*-GlcNAcylation of transcription factors, perhaps due to elevated UDP-GlcNAc levels, is a major mechanism behind the pleiotropic effects of glucose toxicity and insulin resistance.

Streptozotocin, is a glucosamine analogue that specifically destroys islet B cells [21]. In the STZ diabetic pancreas, necrosis of B cells occurs and severe degranulation is observed in the B cells. However, all B cells do not become necrotic. Limited proliferation derived from pre-existing precursor B cells occurs about 4 days after STZ administration to rats. The B cells or B cell lines that express glucose transporter 2 (GLUT 2) show enhanced transport of STZ [16]. Meanwhile, kidney, liver, and small intestine, all of which also express GLUT2 [18, 19], are less susceptible to STZ. STZ has been demonstrated to induce DNA strand breaks or free radicals [8, 22]. However, the mechanism of STZ-induced B cell toxicity is not clearly understood. Since STZ is a weak inhibitor of *O*-GlcNAcase ($IC_{50} \sim 2.5$ mM, Parker and Hart, unpublished), the enzyme responsible for *O*-GlcNAc removal, it has been proposed that inhibition of *O*-GlcNAcase induces the accumulation of *O*-GlcNAcylated proteins, thus contributing to selective B cell apoptosis by STZ [5, 15]. We think that OGT plays a role in the

Fig. 4. Ultrastructural localization of OGT by immunogold labeling of an ultrathin cryosection with colloidal gold-conjugated secondary antibodies. Ultrathin frozen sections were incubated with anti-OGT antibody, and then with colloidal gold (6 or 12 nm)-conjugated goat anti-rabbit IgG. **a:** Enlargement of a nucleus in an acinar cell. Colloidal gold particles (12 nm) representing OGT are localized mainly in the euchromatin. **b:** Enlargement of an A cell. The alpha granules have a dense spherical core and a less dense outer region bounded by a membrane. There are many colloidal gold particles (6 nm) in the cytosol around the alpha granules (200–250 nm in diameter). Bars: (a) = 1 μ m, (b) = 200 nm.

modulation of O-GlcNAc levels in the B cells of the STZ-diabetic pancreas. Recently, it was shown that STZ and glucose increase the O-GlcNAcylation of B cell proteins, especially 135-kD protein [9]. Thus, the O-GlcNAcylation of proteins in the B cells may play a role in the development of STZ- and glucose-induced diabetes.

VI. Perspectives

Many proteins involved in granule secretion have now been characterized. The membrane of the granules and the adjacent plasma membrane contain proteins that are involved in membrane fusion and allow exocytosis, such as alpha SNAP, beta SNAP, SNAP 25, syntaxin, synaptobrevin, etc. The regulation of O-GlcNAcylation and phosphorylation of these proteins in the process of exocytosis needs to be examined. Further studies will be required to determine the role of OGT and O-GlcNAc in the nucleus and the cytoplasm of pancreatic cells by using the cDNA and inhibitors of OGT.

VII. Acknowledgments

We thank Ms. B. J. Hart for peptide synthesis, as well as Dr. R. Marchase, Dr. J. E. Kudlow and all the members of the Hart Lab for helpful discussions. This work was supported in part by the Juvenile Diabetes Foundation and Fifty 50 Foods Inc., by NIH Grant HD13563 awarded to G.W.H., and by a gift from Oxford Glycosystems, Inc. We also wish to express our appreciation to Mr. M. Fukuda, Ms. S. Matsubara, Ms. M. Kanai, and Ms. S. Shibata (Laboratory for Electron Microscopy and Department of Anatomy, Kyorin University School of Medicine) for their technical assistance. This work was supported in part by Grants-in-Aid for scientific research from the Ministry of Education, Science, Sports, and Culture of Japan and the Promotion and Mutual Aid Corporation for Private Schools of Japan.

VIII. References

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