COMMENTARY

Glycosylation mutations of serine/threonine-linked oligosaccharides in low-density lipoprotein receptor: indispensable roles of O-glycosylation

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Introduction

The low-density lipoprotein (LDL) receptor is a surface glycoprotein that mediates the cellular uptake of LDL, a cholesterol-carrying plasma protein (Goldstein et al. 1985). After receptor-mediated endocytosis of LDL, the receptor recycles to the cell surface from the acid compartment, the endosome, and LDL is then transported and degraded in lysosomes where the cholesterol ester core is hydrolysed and from which the unesterified cholesterol is released. The cholesterol molecules from LDL regulate de novo cholesterol biosynthesis and LDL receptor expression. Mutations in the receptor gene for the LDL receptor impair LDL uptake into cells and cause familial hypercholesterolemia (FH) because of the lack of normal regulation of cholesterol metabolism (Goldstein et al. 1985).

The mature LDL receptor consists of 839 amino acids and five domains (Yamamoto et al. 1984; Russell et al. 1984). The first domain (Fig. 1 (1)) of the LDL receptor consists of the NH₂-terminal 292 amino acids with a seven cysteine-rich repeat sequence; the second domain (2) of 400 amino acids is 35% homologous to a portion of the extracellular domain for EGF; the third domain (3) of 58 amino acids contains 18 serine/threonine residues where O-linked oligosaccharide chains are clustered; the fourth domain (4) of 22 hydrophobic amino acids spans the plasma membrane; the fifth domain (5) of 50 amino acid cytoplasmic tail is a COOH-terminal segment (Fig. 1). The mature LDL receptor contains both N-linked and O-linked oligosaccharides (Fig. 2). Among the five major domains of LDL receptor the third domain contains many serine/threonine (Ser/Thr) residues that are the clustered sites for the O-glycosylation. In addition to the clustered Ser/Thr-linked oligosaccharides, the O-glycosylation site is supposed to be located within the first or/and second domains of the receptor (Cummina et al. 1983; Davis et al. 1986). The LDL receptor also contains two asparagine-linked oligosaccharides (Cummings et al. 1983) (Fig. 1). The LDL receptor is synthesized in rough endoplasmic reticulum (ER) as a precursor form of 120 000 Mr, and processed into a mature form of 160 000 Mr. During processing in the ER–Golgi complex, high-mannose N-linked sugar chains of the precursor are converted into the complex N-linked sugar chains in the mature form (see Fig. 2). The human LDL receptor precursor carries Ser/Thr-linked core oligosaccharides with only one monosaccharide, N-acetylgalactosamine (GalNAc) (Cummings et al. 1983), and the initial step for O-glycosylation attachment to GalNAc appears to occur in the rough ER or the transition zone of the ER in some cell lines (Pathak et al. 1988). In this report, we discuss whether O-glycosylation is indispensable for the normal expression and function of LDL receptors.

Construction of truncated LDL receptor lacking the O-glycosylation clustered domain

In addition to the third domain, isolated Ser/Thr-linked oligosaccharides are located within the first two domains of the LDL receptor. Davis et al. (1986) have constructed an LDL receptor cDNA deletion mutant (pLDL-R2Δ) that is without the region of the cDNA encoding the polypeptide region for these clustered O-linked domains of the third domain. The truncated LDL receptor appearing in the plasma membrane of pLDL-R2Δ cDNA-transfected CHO cells shows similar activity to that expressed in wild-type LDL receptor cDNA (pLDL-R2)-transfected CHO cells (Davis et al. 1986) (see Table 1). The O-glycosylation of Ser/Thr-linked oligosaccharides in the third domain thus does not appear to be required for the receptor activity. This study by Davis et al. (1986), however, does not demonstrate whether O-glycosylation in the first and/or second domain of the receptor is required for receptor activity.

LDL receptors in an Idl-D mutant of CHO

Krieger and his colleagues have isolated an Idl-D mutant from CHO, which has a reversible defect in O-glycosylation (Kingsley et al. 1986; Krieger et al. 1985). Idl-D is deficient in UDP-Gal/UDP-GalNAc 4-epimerase and cannot synthesize UDP-galactose(Gal)/UDP-GalNAc in the absence of exogenous Gal/GalNAc. Under normal culture conditions in the absence of GalNAc the mutant Idl-D cells cannot add GalNAc to the Ser/Thr-linked oligosaccharides. However, this O-glycosylation defect is rapidly corrected when GalNAc is added to the culture medium. One can thus examine the role of O-glycosylation of various glycoproteins by manipulation of the Idl-D

Key words: glycosylation, low density lipoprotein, oligosaccharides.
Fig. 1. Structure of LDL receptor and a model for its biosynthesis in CHO and Mon'-31 cells. Five domains from the NH₂-terminal domain are presented (see text). Precursor forms of LDL receptor with high-mannose N-linked sugar chains are converted into the mature forms with different O-glycosylation in CHO and Mon'-31 cells (Seguchi et al. 1991). (○) N-acetylgalactosamine (GalNAc); (■) galactose (Gal); (□) mannose (Man); (○) N-acetylglucosamine (GlcNAc); (●) sialic acid.

mutant. By using ldl-D mutant cells, Kozarsky et al. (1988a) have shown that newly synthesized LDL receptor is rapidly degraded and the steady-state surface expression is very low in the absence of O-glycosylation (Table 1). In that report, the authors demonstrate that the lack of O-glycosylation of the Ser/Thr residues in all three domains (first, second and third) of LDL receptor prevents the normal surface expression of LDL receptors. The ldl-D mutant has also been used to study the effects of O-glycosylation of the β-subunit of human chorionic gonadotropin on the synthesis and secretion of the human chorionic gonadotropin α/β heterodimer (Matzuk et al. 1987), the gp55 subunit of the human interleukin-2 receptor (Kozarsky et al. 1988a), apolipoprotein E (apo E) (Zanni et al. 1989), the gp120/41 envelope protein of human immunodeficiency virus (Kozarsky et al. 1989), decay-accelerating factor (Reddy et al. 1989) and other glycoproteins. As summarized in Table 2, O-glycosylation of Ser/Thr-linked oligosaccharides is required for expression and intracellular sorting of some glycoproteins, but not for others. This somatic cell mutant is a very powerful tool for examining the effects of O-glycosylation.

Fig. 2. Structure of N- and O-linked oligosaccharide chains. In the rough endoplasmic reticulum and the Golgi complex, the initial high-mannose type of N-linked oligosaccharide can be added to proteins and then converted to the complex type. O-linked oligosaccharide can be attached to Ser/Thr and then converted to the mature form from the precursor form.

LDL receptors in int mutants of hamster cells

We have isolated, by independent selection, two different somatic cell mutants that show altered expression of LDL receptor (Kuwano and Ono, 1989a,b). A compactin-resistant mutant, MF-2, was isolated from the Chinese hamster V79 cell line and showed very high cholesterol synthesis in the presence of LDL (Masuda et al. 1982). Compactin-resistant mutants showed less binding and internalization of LDL, and the mature LDL receptor was apparently 5000 M₉ smaller than that of the parental V79 cells, but the molecular size of the precursor form in MF-2 cells is similar to that in the parental V79 cells. Treatment of the mature forms of LDL receptor with O-glycanase can diminish the difference in the molecular sizes of MF-2 and V79 cells, which suggests altered O-glycosylation in the compactin-resistant mutant (Yoshida et al. 1987). On the other hand, a monensin-resistant (Mon'-31) mutant derived from CHO (Ono et al. 1984) also showed a decreased response to LDL as well as aberrant cholesterol metabolism (Tomita et al. 1987). The Mon'-31 mutant was found to be defective in internalization of ricin (Ono et al. 1984) and insulin (Sato et al. 1984; Seguchi et al. 1989). The mature LDL receptor produced in Mon'-31 was also, seemingly, 5000 M₉ smaller than that of the parental CHO
cells, and the mutation in Mon'-31 specifically alters O-linked sugar chains, not N-linked sugar chains (Yoshimura et al. 1987). This has raised the possibility that these two mutants might fall into the same complementation group. To analyse the possible relatedness of these alleles, cell-cell hybridization was done. Hybrids between MF-2 and Mon'-31 still produced LDL receptor molecules with properties intermediate between those of the parental CHO and Monr-31 lines. However, further characterization of the receptor from Mon r-31 cells, however, showed that it contains about one-third fewer Ser/Thr-linked oligosaccharides than the parental CHO receptor from parental CHO cells and/or the receptor from Mon r-31 cells. A plasmid pLDL-R2A containing a cDNA coding for a truncated human LDL receptor from parental CHO cells was transfected into Mon r-31 cells and its glycosylation was analysed. The mature truncated human receptor has an Mr of 120,000, and lacks and clustered Ser/Thr-linked oligosaccharides while retaining a small number of isolated Ser/Thr-linked oligosaccharides (Davis et al. 1986). The mature form of the human receptor in CHO has an apparent Mr of 120,000 while that in Mon r-31 has a Mr of 110,000 (Seguchi et al. 1991). Treatment of the mature human receptor with sialidase causes a decrease in the apparent relative molecular mass from 120,000 to 105,000 in CHO, and from 110,000 to 100,000. However, further reduction in the apparent relative molecular mass is found only for the human receptor expressed in CHO cells when treated with both sialidase and O-glycanase (Seguchi et al. 1991): O-glycanase can cleave Ser/Thr-linked oligosaccharides that have an unmodified core structure of Gal-GalNAc. The apparent relative molecular mass of the sialidase-treated human receptor in Mon r-31 cells is equivalent to that of the receptor in CHO cells that is treated with both sialidase and O-glycanase. This also suggests that Mon r-31 cells produce a human receptor lacking O-linked oligosaccharides. These results demonstrate that the LDL receptor produced by the Mon r-31 cells contains Ser/Thr-linked oligosaccharides in the clustered domain (domain 3), but is missing the Ser/Thr-linked oligosaccharides in the unclustered domains (domains 1 and 2) of the receptor (see Fig. 2). Ligand blotting assays with LDL and its antibody show much less binding of LDL to the truncated human receptors in Mon r-31 cells than those expressed in CHO cells (Seguchi, T., unpublished data). The O-glycosylation at the first or/and second domain may be critical for LDL receptor function.

Many studies have suggested that O-glycosylation may be a post-translational process, but the O-glycosylation pathways are variable (Carraway and Hull, 1989). It is unclear why the lack of Ser/Thr-linked oligosaccharides at selected sites on the LDL receptor is produced by an int mutation in Mon r-31 cells. If the int mutation causes abnormal folding of LDL receptor during processing in rough ER–Golgi apparatus, certain polypeptide regions are not fully accessible to the GalNAc transferase that transfers GalNAc from UDP-GalNAc to Ser/Thr residues. The first domain (the ligand binding domain) of the receptor is known to contain numerous disulfide bonds (Goldstein et al. 1985). int mutation causes an O-glycosylation defect in human LDL receptors that are expressed in CHO ldl-D mutant

<table>
<thead>
<tr>
<th>Protein</th>
<th>Effect of O-glycosylation</th>
<th>References</th>
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<tbody>
<tr>
<td>Decay-accelerating factor</td>
<td>No effect</td>
<td>Matzuk et al. (1987)</td>
</tr>
<tr>
<td>Decay-accelerating factor</td>
<td>No effect</td>
<td>Zanini et al. (1988)</td>
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<td>Decay-accelerating factor</td>
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<tr>
<td>Major envelope glycoprotein antigen of Epstein-Barr virus</td>
<td>No effect</td>
<td>Reddy et al. (1989)</td>
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<td>gp55 subunit of the human interleukin-2 receptor</td>
<td>No effect</td>
<td>Reddy et al. (1989)</td>
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<tr>
<td>gp120/41 envelope protein of human immunodeficiency virus</td>
<td>No effect</td>
<td>Cited by Krieger et al. (1989)</td>
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Table 1. Role of O-glycosylation of LDL receptors

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<thead>
<tr>
<th>Mutant</th>
<th>Loss of O-glycosylation</th>
<th>Effect of O-glycosylation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ldl-D</td>
<td>All domains (first, second and clustered O-linked domains)</td>
<td>Stability: protection from proteolysis</td>
<td>Kingsley et al. (1986), Rozansky et al. (1988)</td>
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<td>No effect</td>
<td>Davis et al. (1986)</td>
</tr>
<tr>
<td>Mon r-31</td>
<td>Selected first or/and second domain</td>
<td>Reduced binding activity</td>
<td>Yoshiida et al. (1987), Seguchi et al. (1991)</td>
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Table 2. Role of O-glycosylation of various glycoproteins in a CHO ldl-D mutant
pLDL-R2 or pLDL-R2Δ DNA-transfected hamster Mon'-31 cells (Yoshimura et al. 1987; Seguchi et al. 1991). Primary structures of LDL receptors themselves appear to be identical in CHO and Mon'-31 cells (Yoshimura et al. 1987). The Golgi apparatus is the major subcellular location of the enzymes that are responsible for further O-glycosylation during processing of LDL receptors and other glycoproteins. The topology of O-glycosylation enzymes is variable in the Golgi complex. For example, GalNAc transferases (Roth, 1984) and sialyl-transferases (Roth et al. 1986; Trinchera and Ghidon, 1986; Shite et al. 1990) are located in different compartments of the Golgi complex. If the topology of O-glycosylation in ER–Golgi is altered in Mon'-31 cells, selected parts of LDL receptors may not be O-glycosylated during transport in the ER–Golgi complex. Further experiments are required to determine the precise mutation site(s) for the int mutation.

Conclusion
Three independent studies demonstrate roles for O-glycosylation in the expression and activity of LDL receptors (Table 1). Of approximately 18 Ser/Thr-linked oligosaccharides, about two-thirds are present in a clustered region near the transmembrane domain and the remaining one-third at undefined locations, possibly first and second domains, N-terminal to the clustered domain. O-glycosylation of the Ser/Thr residues at the N-terminal proximal domains may be indispensable for the LDL receptor.

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References

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