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ISOLATION AND CHARACTERISATION OF N-GLYCANS OF OVINE AND HUMAN LUTEINIZING HORMONES

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry, University of Auckland, 1991
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ABSTRACT

Gonadotrophic hormones are heterodimeric glycoproteins and their N-glycans attached to specific amino acid residues are currently thought to play important roles in hormonal biosynthesis, secretion and function. The studies reported in this thesis aimed at isolation and characterisation of structural properties of the N-glycans on ovine and human luteinizing hormones.

Initially, chromatographic methods were developed using reverse-phase HPLC for the analytical separation of the three human pituitary glycoprotein hormones and their subunits. Separation of intact oLH and its subunits was also effected by a single HPLC step.

A preparative procedure was developed for the efficient purification of hLH and hTSH from crude human pituitary extracts using hydrophobic chromatography which gave highly purified hormones in good yields and with high biological activities. This method did not significantly influence the hormones' extensive charge heterogeneity and it offered potential advantages in the characterisation of their carbohydrate structures.

A preparative scheme was developed for the isolation of the N-linked oligosaccharides from each glycosylation site of o- and hLH. Charge heterogeneity of oligosaccharides, which were released by hydrazinolysis from subunits and glycopeptides, was characterised by anion-exchange HPLC.

$^1$H-NMR analysis showed that the structures of all three N-glycans on hLH were highly heterogeneous but mainly diantennary complex-type, with site-specific patterns of terminal sialylation and sulphation as well as core-fucosylation.
Sulphated/sialylated and/or disialylated oligosaccharides were the major components at each site. A set of new mono- and disialylated oligosaccharides with the terminal sequence NeuAcα2-6GalNAcβ1-4GlcNAcβ1-2Manα1-3 was identified. This finding suggested unique site-specific terminal sialylation of oligosaccharides at Asn 78 (hLHα) by an unknown α2-6 sialyltransferase(s) in the human pituitary gonadotroph cell.

Each glycosylation site in oLH had a distinct set of oligosaccharides ranging from mainly monosulphated hybrid-type at the two sites of oLHα to predominantly disulphated diantennary complex-type on oLHβ. Core-fucosylation also differed at each site. These results suggested that processing of the oligosaccharides of the α- and β-subunits by α-mannosidase II and α1-6 fucosyltransferase was differently regulated by protein structure in oLH.

Whereas hCG, hLH and oLH share similar biological activities, no apparent relationship between their N-glycan structures was found, which suggested that specific branching and peripheral structures of N-glycans on LH and hCG may not be essential for biological function, although the N-glycan nearer the N-terminus of the α-subunit of hCG has been implicated in hormonal activity.
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ABBREVIATIONS

Asn  asparagine
BSA  bovine serum albumin
cAMP adenosine 3',5'-cyclic monophosphate
CM   carboxymethyl
cpm  counts per minute
DEAE diethylaminoethyl
EDTA ethylenediaminetetraacetic acid
Fuc  L-fucose
Gal  D-galactose
GalNAc N-acetylgalactosamine (2-acetamido-2-deoxy-D-galactose)
Glc  D-glucose
GlcNAc N-acetylgalcosamine (2-acetamido-2-deoxy-D-glucose)
GlcNAc-ol N-acetylglucosaminitol
GTP  guanosine 5'-triphosphate
HF   hydrogen fluoride
1H-NMR proton nuclear magnetic resonance
HPLC high-performance liquid chromatography
i.p. intraperitoneal
IU   international units
kDa  kilodalton
Man  D-mannose
NeuAc N-acetylneuraminic acid (sialic acid)
NIADDK National Institutes of Arthritis, Diabetes and Digestive and Kidney Diseases
NIH  National Institutes of Health, Bethesda, Maryland, U.S.A.
pI   isoelectric point
RIA  radioimmunoassay
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<tr>
<td>RP</td>
<td>reverse-phase</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecylsulphate</td>
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<tr>
<td>TPCK</td>
<td>L-1-tosylamido-2-phenylethyl chloromethyl ketone</td>
</tr>
<tr>
<td>Tris</td>
<td>tris (hydroxymethyl) aminomethane</td>
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