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Hypothesis

An antipodean perception of the mode of action of glycoprotein hormones

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Continuance of vertebrate species and maintenance of metabolism have an absolute requirement for the glycoprotein hormones of the anterior pituitary gland. It is now firmly accepted that the *N*-glycans of these and the related placental hormone, chorionic gonadotropin, have essential if undefined roles in their mechanism of action. However, recent investigations by Weisshaar and his colleagues on the oligosaccharides of human luteinizing hormone and chorionic gonadotropin, do not support the generally accepted view of carbohydrates in hormone–receptor interactions and a further concept is proposed that invokes negative charges and changes in structured water.

Glycoprotein hormone; *N*-Glycan; Structured water

The placental glycoprotein hormone, chorionic gonadotropin (CG), is closely related in structure to three hormones that are synthesized and secreted by the anterior pituitary gland in all vertebrates, viz. follicle-stimulating hormone (FSH, follitropin), luteinizing hormone (LH, lutropin) and thyroid stimulating hormone (TSH, thyrotropin). FSH and LH are intrinsic components of the reproductive process, CG like LH stimulates ovary and testis and TSH is an essential regulator of thyroid structure and function.

Each hormone consists of two dissimilar subunits α and β, which are highly cross-linked by disulphide bonds, five in each α and six in each β subunit. While the primary structures of the former and their sites of carbohydrate attachment are virtually the same in all four hormones, the amino acid sequences of the β elements are unique, in part homologous, and their oligosaccharides differ. The oligosaccharides in the pituitary glycoprotein hormones are attached through a proximal *N*-acetylglucosamine residue to the amide nitrogen of asparagine.

Chorionic gonadotropin differs in having a C-terminal extension of 30–35 residues in the β subunit that contains four additional oligosaccharide chains *O*-linked to serine residues in the human hormone. Each subunit is biologically inert, but when associated noncovalently they form the hormonally active heterodimer the specificity of which is conferred by the β component [1].

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The functions of the *N*-glycans in the biological actions of these hormones have been inferred from numerous investigations that mainly employed hCG and it is now widely accepted that while the sugar chains are not required for hormone-receptor binding, their presence is essential for activation of the adenylate cyclase system [2–6]. More recent reports provide evidence for subunit- and site-specific functions of these oligosaccharides; those on the α subunit, primarily at Asn-52, are seemingly more important with respect to hormone assembly, secretion and signal transduction [7–9].

While crystals of hCG suitable for X-ray diffraction have been obtained [10], no data are yet available. However, one model [3] suggests that the *N*-glycans of hCG may interact directly with lectin-like sites in the LH/hCG receptor and/or may influence protein conformation in domains primarily involved in events consequent upon receptor binding. A three-dimensional structure based upon analogies of primary sequence between hCG and chymotrypsin has also been published [11] and in this the *N*-glycans were viewed as a collar that anchored the hormone to laterally opposed lectins.

Because earlier reports on the *N*-glycans of hCG revealed considerable discrepancies, particularly concerning the carbohydrate attachments to the α subunit, the *N*-linked structures were investigated [12] in an attempt to verify their site-specific distribution. The structures of glycopeptides that included specific glycosylation sites were resolved by one- and two-dimensional ^{1}H-NMR spectroscopy. Given that the hormonal activities of hCG and hLH are essentially the same and that they interact with the CG/LH receptor with equal facility, the Auckland group was surprised to find major dif-

ferences in the structure of the peripheral sugar chains when the results were compared with their earlier findings on hLH [13]. Human LHα, unlike hCGα, unexpectedly contained only insignificant amounts of hybrid-type and monoantennary structures.

We view the glycoprotein hormones as consisting of two dissimilar subunits closely associated by hydrophobic interaction with their *N*-linked oligosaccharides in close contact with the protein surface, such that they form an incomplete calyx that permits exposure of hydrophobic domains on both subunits. This perception is based on the findings that the α1-6 bond of diantennary oligosaccharides is flexible [14–18] and that the analytical ultracentrifugation of native and deglycosylated hormones revealed essentially no difference in sedimentation constant [19].

The *N*-linked glycans of hCG and hLH are identical only in respect of the pentasaccharide core that links the proximal *N*-acetylglucosamine residue to an asparagine residue in the polypeptide backbone. Human LH was found to contian predominantly diantennary, *N*-acetyllactosamine-type structures at all three glycosylation sites, with only insignificant amounts of hybrid-type and monoantennary chains. In contrast, the *N*-glycans of hCG comprised monosialylated monoantennary, disialylated diantennary and monosialylated hybrid-type structures. Such major differences in the peripheral sugars of hCG and hLH cast doubt on the validity of theories that involve recognition between hormonal *N*-glycans and lectins in the receptor, especially in the case of hCG and hLH which share the same physiological actions and the same receptor.

We are particularly impressed by the presence of chainterminating negatively charged groups which occur in hCG [12], hFSH [20], hLH [13], oLH [21] and hTSH [22] and by the observation that the water in small hydrophobic cavities has strong intermolecular hydrogen bonds which make it viscous and unreactive. It excludes hydrophobic solutes and highly hydrated ions (e.g. Na^+, H^+, Ca^{2+}, Mg^{2+}), but selectively accumulates K^+ and univalent anions, the larger the anion the greater its selective uptake. Water decreases its local density in this manner because molecules close to a hydrophobic surface are in a state of high enthalpy, being unable to satisfy their hydrogen-bonding potential; they decrease their local chemical potential by collectively moving apart, doing work of expansion. When, however, such water selectively accumulates K^+ and anion, the osmolality inside the cavity exceeds that of the external solution. Internal water now has a lower chemical potential than external water, so that more water enters the cavity down its chemical potential gradient, and to complete its equilibration, water in the cavity collectively compacts, becoming fluid, reactive and with diametrically opposite solvent properties [23–25].

Applying these principles to the hormone-receptor interaction, $-SO_3^-$ or $-CO_2^-$ attached to a hormone molecule, together with their counter-cations, readily enter the predominantly hydrophobic cavity. The resulting increase in solute concentration decreases the activity of water, more water diffuses in, the cavity opens more widely and water–water hydrogen bonds decrease in strength. The hormonal sugar moiety makes specific interactions with the surface of the receptor cavity.

While strongly-bonded low-density water was a barrier to movement of Ca^{2+} through the channel which has been proposed in the hFSH receptor, [26,27], weakly-bonded water induced by accumulation of anionic groups, K^+ and water, attracts Ca^{2+} into the cavity, from which it diffuses through its transmembrane channel to elicit the biological response. At the same time the switch in water structure inside the cavity, reverses selective accumulation of oxyanions and counter-cations. The hormone diffuses out, closing the Ca^{2+} channel behind it, as water reverts to its initial low-density state.

It has been observed [2,19] that if the charged groups are removed from the hormone it binds more strongly to the receptor and evokes a markedly reduced biological response. Without the anionic groups and counter cations to weaken water–water hydrogen bonds inside the receptor cavity, the sugar moiety can bind to surface groups, but the channel is still closed to Ca^{2+}. Moreover, without the oscillatory behaviour of water induced by charged groups the hormone remains bound.

The LH/CG receptor, like its complementary hormones is glycosylated and it has been shown in tissue and cell lines derived from the rat that enzymatic deglycosylation does not abolish hormone-binding ability [28,29].

In conclusion, we have two glycoprotein hormones with virtually identical α subunits, β subunits with highly homologous primary sequences and which in heterodimeric form bind to the same receptor with equal avidity to elicit the same biological response. The main structural differences lie in their peripheral oligosaccharide chains whose terminal sugars possess charged groups. The proposed model provides another view of how these particular glycoproteins might interact with their receptor to evoke their biological responses and may be applicable to other glycoprotein hormones including erythropoietin.

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