

DIFFERENCES IN THE CARBOHYDRATE PORTION OF THE α SUBUNIT OF PORCINE LUTROPIN (LH), FOLLITROPIN (FSH) AND THYROTROPIN (TSH)

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Received 17 September 1975

1. Introduction

Hypophyseal and placental glycoprotein hormones are closely related in term of chemical structure. Indeed, LH*, FSH, TSH and CG are made of two non-covalently-linked subunits, α and β . Primary structures studies [2–9] have shown that, within the same species, the amino acid sequences of the α subunits of LH, FSH, TSH and CG are identical if one excepts some shortening of the polypeptide chain at the amino-terminal end. The β subunit of these hormones is responsible for the hormonal specificity of the $\alpha\beta$ complex [10] as the amino acid sequences of the β chains are different.

Being involved in structural studies of LH, TSH and FSH, we have examined the characteristics of the α chains of these hormones within a single species. We observed that the carbohydrate moieties of the α subunits are significantly different in LH, FSH and TSH. This finding suggests that biosynthesis of the α subunit of LH, TSH and FSH occurs in different cells of the pituitary gland.

2. Materials and methods

Procedures used for the purification of LH and TSH, from frozen pituitary glands, have already been

described [11,12]. Purification of FSH will be detailed elsewhere (manuscript in preparation). Final preparations of the porcine hormones had the following biological potencies: LH, 1 U/mg, when assayed by the ovarian ascorbic acid depletion test [13] with NIH-LH-S17 as standard; TSH, 34 IU/mg as determined by bioassay in mice according to McKenzie [14] using the International Thyrotropin Standard as reference; FSH, 75 times more potent than the NIH-FSH-S9 standard when tested by Steelman and Polhey's assay [15].

Dissociation of the three hormones was performed in 8 M urea at acidic pH. Separation and purification of the subunits were made by ion exchange chromatography on SP-Sephadex for LH [11] or on DEAE-Sephadex for TSH [12] and FSH (manuscript in preparation). After reduction and S-carboxymethylation of the glycoproteins, the tryptic glycopeptides of TSH α and FSH α were obtained and purified by a procedure already described for LH α [7]. This procedure involved gel filtration on Sephadex G-50 followed by ion exchange chromatographies on QAE-Sephadex. Their purity was assessed by amino-terminal amino acid analysis [16], high voltage paper electrophoresis, paper chromatography and amino acid analysis in the conditions already described [17]. Neutral sugar analyses were performed by gas liquid chromatography of the alditol acetates [18] with the modification already described [19]. The determination of glucosamine and galactosamine was made using an Amino Acid Analyzer Beckman 121 using the same hydrolysate than that used for neutral sugars [18]. Sialic acid content was determined by the thiobarbituric assay of Warren [20].

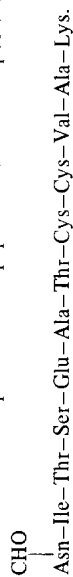
*Abbreviations: LH: lutropin [1] (formerly: luteinizing hormone of interstitial-cell-stimulating hormone) FSH: follitropin [1] (formerly: follicle-stimulating hormone). TSH: thyrotropin. CG: choriogonadotropin [1] (formerly: chorionic gonadotropin).

Table 1
Carbohydrate composition of the α subunits of LH, TSH and FSH of porcine origin.
Residues/molecule^a

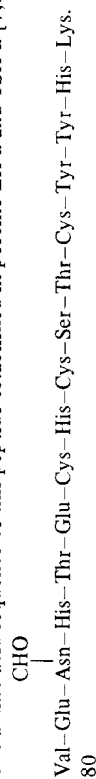
	<i>LH</i> α subunit		<i>TSH</i> α subunit		<i>FSH</i> α subunit	
	α subunit	Tryptic glycopeptides	α subunit	Tryptic glycopeptides	α subunit	Tryptic glycopeptides
		Internal ^b	carboxy-terminal ^c	Internal ^b	carboxy-terminal ^c	Internal ^b
Fucose	0.9	0.1	0.6	0.9	0.6	0.7
Mannose	6.8	2.6	2.7	5.7	2.8	1.8
Galactose	1.0	0.4	0.4	1.1	0.7	1.2
<i>N</i> -acetyl glucosamine	4.2	2.1	2.1	6.4	3.4	2.0
<i>N</i> -acetyl galactosamine	1.7	1.0	0.8	2.8	1.7	0.0
Sialic acid	1.1	n.d	n.d	0.7	n.d	0.3
					1.4	0.1

^a The values have been calculated on the basis of mol. wts 15 500, 3300 and 4000 for the α subunit, the internal tryptic glycopeptide and the carboxy-terminal glycopeptide respectively. A 10% correction has been applied for moisture. Sugar analyses were performed in duplicate, if one excepts the tryptic glycopeptides of FSH α whose composition results from a single analysis. n.d: not determined.

^b The amino acid sequence of this peptide established in porcine LH α and TSH α [7,8] is the following:



^c The amino acid sequence of this peptide established in porcine LH α and TSH α [7,8] is the following:



3. Results

Carbohydrate compositions of the α subunits of LH, TSH and FSH from porcine origin are given in table 1 together with the sugar compositions of the tryptic glycopeptides isolated from these α subunits. The three α subunits differ significantly in their neutral hexose composition. Indeed, the ratio mannose:galactose is 6.8 for LH α , 5.2 for TSH α and 1.7 for FSH α . On the basis of their total neutral sugar content, the three subunits studied are classified in the following order: FSH α , LH α , TSH α . The reverse order is obtained when these subunits are classified according to their osamine content. The FSH α molecule is devoid of galactosamine.

A more precise location of the differences between the polysaccharide moieties of the three α subunits is obtained from the study of their glycopeptides (table 1). No significant difference is observed between the two glycopeptides of the LH α subunit, while the two tryptic glycopeptides of TSH α as well as FSH α differ in their hexose content. The neutral sugar distribution between both polysaccharide chains of the α subunit differs thus according to the hormone considered. The different compositions in neutral and amino sugars between the α subunits of the three hormones noted above can now be located in their glycopeptides: one can note in particular the high galactose content of the carboxyterminal FSH α glycopeptides and the high percentage of osamines in both peptides of TSH α .

4. Discussion

These analytical data represent the first detailed report of carbohydrate composition of the α subunits of three glycoprotein hormones, LH, TSH and FSH, within a single species. The sugar analyses were performed by the same analytical method in the same laboratory. This situation allows unambiguous conclusions concerning the non-identity of the sugar portion of the α subunits of three porcine hormones. Comparative study of LH and TSH α subunits from bovine origin suggested similar conclusion, bovine LH α being richer in mannose but poorer in osamines than bovine TSH α [21]. However, bovine FSH is not yet characterized. Our results indicate that, in spite of the

identity of their amino acid sequence [2–4, 7–9] and their possible interchange in 'in vitro' reassociation experiment with the corresponding or non-corresponding β subunits [10,22], the α subunits differ in their covalent structure at the level of the polysaccharide prosthetic groups. Biosynthesis of glycoprotein implies that sugars are added one by one to the protein by a series of specific enzyme reactions. The unique sequence of sugars in the heterosaccharide portion of a glycoprotein is the result of the specificity of the glycosyl transferases found in the cells producing that particular glycoprotein [23]. Major differences in the composition of the carbohydrate moiety of glycoproteins such as those observed among the α subunits of FSH, LH and TSH indicate thus that they are synthesized in different cell types.

The structural differences between the carbohydrate side chains of the α subunits should perhaps be responsible for the different biological potencies recorded between hybrid and natural recombined $\alpha\beta$ hormonal dimers [10,22].

Acknowledgement

This work has been partly supported by the Fonds de la Recherche Scientifique et Médicale Belge. Mrs Poncelet and Dockier provided their helpful technical assistance.

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