Effect of deglycosylation on the structure and hormone-binding activity of the lutropin receptor

Kari P. KEINÄNEN

Biocenter and Department of Anatomy, University of Oulu, Kajaanintie 52 A, SF-90220 Oulu, Finland

Affinity-purified rat ovarian lutropin (LH) receptor is a single 90 kDa polypeptide which binds to immobilized lectins, indicating that the receptor is a glycoprotein [Keinänen, Kellokumpu, Metsikkö & Rajaniemi (1987) *J. Biol. Chem.* **262**, 7920–7926]. In the present study the glycoprotein nature of the rat ovarian LH receptor was investigated in order to determine the contribution of the glycan moiety to receptor's size and hormone-binding properties. Treatment of the ^{125}I -labelled purified LH receptor with neuraminidase and peptide *N*-glycosidase F resulted in a decrease in size of LH receptor from 90 kDa to 79 kDa and 62 kDa respectively, as assessed by SDS/polyacrylamide-gel electrophoresis. Endo- α -*N*-acetylgalactosaminidase treatment did not affect the electrophoretic mobility of the intact or neuraminidase-treated LH receptor. Subjecting the membrane-bound LH receptor to similar enzymic treatments followed by ligand blotting showed that the 79 kDa and 62 kDa forms are capable of specific hormone binding. Furthermore, intact and peptide *N*-glycosidase F-treated membranes bound ^{125}I -labelled human choriogonadotropin with similar affinities. These data suggest that molecular mass of the polypeptide backbone of the LH receptor is 62 kDa. The receptor contains *N*-glycosidically linked oligosaccharide chains with terminal sialic acid residues, with little or no *O*-linked oligosaccharide. *N*-Linked carbohydrate is not required for specific high-affinity hormone binding.

INTRODUCTION

The action of lutropin (LH) is initiated by binding to specific high-affinity LH receptors on the surface of its gonadal target cells [1,2]. In order to gain an understanding of the molecular mechanisms of LH action, purification and characterization of the components of the signal-transduction pathway are necessary. In the first place, mechanisms of receptor function may be elucidated through detailed structural studies of the receptor.

Recently, LH receptor has been purified to near homogeneity in several laboratories [3–6]. The receptor appears on SDS/polyacrylamide-gel electrophoresis as a single 70–90 kDa species. Several indirect studies using different radioactive labelling methods suggest that the receptor has an approximate molecular mass of 90 kDa [7–11]. Contradictory reports supporting a multisubunit structure for the LH receptor have also appeared [12–14]. The receptor's glycoprotein nature is evident from its being retained by immobilized wheat-germ lectin and concanavalin A [4–6] and its being chemically [8] labelled using carbohydrate-specific labelling techniques. The contribution of the oligosaccharide side chains to the function and apparent M_r of the receptor, and the type of glycosidic linkage (*N*-linked or *O*-linked), are, however, not known. To address these questions, we exploited enzymic deglycosylation of affinity-purified and membrane-bound rat ovarian LH receptor. In the present study we describe the effect of enzymic deglycosylation on the hormone-binding properties and electrophoretic mobility of the LH receptor.

EXPERIMENTAL

Materials

Na^{125}I (carrier-free) was from Amersham International (Amersham, Bucks., U.K.). Peptide *N*-glycosidase (PNGase F, glycopeptidase F, glycopeptide *N*-glycosidase, EC 3.2.2.18) and endo- α -*N*-acetylgalactosaminidase were obtained from Boehringer-Mannheim. Neuraminidase (from *Vibrio cholerae*) was from Calbiochem. Molecular-mass markers for SDS/polyacrylamide-gel electrophoresis, *N*-ethylmaleimide and PMSF were products of Sigma. hCG (10900 i.u./mg) was purchased from Diosynth (Oss, The Netherlands). The hormone was radioiodinated by the chloramine-T method [15,16] to a specific radioactivity of 40–60 $\mu\text{Ci}/\mu\text{g}$, determined by the self-displacement method [17]. The bindability of the radioiodinated hCG (determined as the fraction of radioactivity capable of binding to an excess of rat ovarian membranes) was 42–50 %. This percentage was taken into account when calculating the equilibrium binding data. hCG was covalently coupled to Affi-Gel 10 (Bio-Rad) as described previously [5]. Equipment and reagents for electrophoresis were from Bio-Rad Laboratories (Richmond, CA, U.S.A.). All other reagents were of reagent grade and purchased from commercial sources.

Animals

Female 27-day-old Sprague-Dawley rats were rendered pseudo-pregnant by sequential treatments with pregnant-mare serum gonadotropin and hCG [18].

Abbreviations used: LH, lutropin; PNGase F, peptide *N*-glycosidase F; PMSF, phenylmethanesulphonyl fluoride; hCG, human choriogonadotropin; PBS, phosphate-buffered saline (10 mM-sodium phosphate, pH 7.4, containing 140 mM-NaCl); iodoreceptor, ^{125}I -labelled LH receptor; BSA, bovine serum albumin.

Animals were killed on day 7 after the hCG injection, and the ovaries were collected, freed from extraneous tissue, immersed in liquid N₂, and stored frozen at -80 °C until use.

Preparation of ovarian membranes

Ovaries were homogenized in PBS/5 mM-EDTA/5 mM-*N*-ethylmaleimide/0.2 mM PMSF (buffer A). The homogenate was centrifuged for 10 min at 160 *g*, and the resulting supernatant was further centrifuged for 30 min at 27 000 *g*. The crude membrane pellet was suspended in appropriate buffer as described below.

Purification of LH receptor

The LH receptor was solubilized in buffer A containing 1% (v/v) Triton X-100 and 20% (v/v) glycerol, and affinity-purified on hCG-Affigel 10 as described previously [6]. The receptor purified by one cycle of affinity chromatography was dialysed against 0.1 M-sodium phosphate, pH 7.4, and radioiodinated by using the chloramine-T method [15] as described previously [6]. ¹²⁵I-labelled LH receptor (iodoreceptor) was resolved by SDS/polyacrylamide-gel electrophoresis on a 7.5% acrylamide slab gel, and the *M_r*-85 000–95 000 band was excised after staining with Coomassie Brilliant Blue. Iodoreceptor was eluted electrophoretically by using a Bio-Rad model 422 Electro-Eluter. Elution buffer was 50 mM-(NH₄)₂CO₃/0.1% SDS. Electroeluted iodoreceptor was evaporated to dryness with Savant Speed-Vac concentrator and used for the deglycosylation experiments. Electroeluted receptor migrated as a single 90 kDa band on SDS/PAGE. During prolonged storage, however, faint bands corresponding to 59 kDa and 34.5 kDa became gradually apparent.

Enzymic deglycosylation of iodoreceptor

The iodoreceptor was incubated with neuraminidase (final concn. 50 munits/ml), endo- α -*N*-galactosaminidase (final concn. 25 munits/ml) or both in 20 mM-sodium phosphate, pH 7.0, containing 0.5% Triton X-100 and 0.5 mM-PMSF for 3 h at 30 °C. Enzymes were omitted from a control tube which was subjected to similar incubation. In some experiments, 50 mM-sodium acetate, pH 5.5, containing 0.1% Triton X-100 and 0.5 mM-PMSF, was used for neuraminidase treatment.

PNGase F treatment was performed in 50 mM-sodium phosphate, pH 7.4, containing 50 mM-EDTA, 1% 2-mercaptoethanol, 0.1% SDS, 1% Triton X-100 and 0.2 mM-PMSF. A portion (1 μ l, 0.2 unit) of PNGase F was added to 50 μ l of receptor solution and the mixture was incubated for 1–18 h at 30 °C.

Reaction products were analysed by SDS/polyacrylamide-gel electrophoresis after precipitation with trichloroacetic acid (final concn. 10%). Soybean trypsin inhibitor (10 μ g) was added as a carrier. The pellet was washed once with ethanol/diethyl ether (1:1, v/v) and solubilized by boiling in SDS sample buffer. Over 90% of the radioactivity was precipitated, and no differences in the trichloroacetic acid-precipitability were observed between the enzyme-treated and untreated samples.

Deglycosylation of membrane-bound receptor

For glycosidase treatments, crude ovarian membranes (20–30 μ g of protein) were suspended in 200 μ l of 0.1 M-sodium phosphate, pH 7.0, containing 0.2 mM-PMSF. Next, 10 μ l of PNGase F (2 units), neuraminidase

(10 munits) or buffer (control) was added, and the mixtures were incubated at 30 °C for the indicated period of time. After deglycosylation, membranes were assayed for ¹²⁵I-hCG binding as described below, or centrifuged, washed once with 10 mM-sodium phosphate, containing 140 mM-NaCl, and solubilized in 1% Triton X-100 containing 20% (v/v) glycerol, and subjected to ligand blotting [6,19].

Hormone-binding assays

Binding of ¹²⁵I-hCG to membrane-bound receptor was performed essentially as described previously [6]. Briefly, the membranes were suspended in buffer A containing 0.1% BSA. Membranes were incubated for 16 h at 20 °C in a final volume of 250 μ l with increasing amounts of ¹²⁵I-hCG [(0.03–1) $\times$ 10⁶ c.p.m.]. After incubation, the membranes were collected by centrifugation, washed once with buffer A, and counted for ¹²⁵I radioactivity. Results were corrected for non-specific binding obtained in the presence of a large excess of unlabelled hCG (1 μ g/250 μ l). In some cases, the hormone-binding activity was assayed after solubilization of the enzymically treated membranes. The soluble ¹²⁵I-hCG-binding activity was measured as described previously [6,20]. The two methods yielded similar results in terms of binding affinity, but the total binding in the soluble fraction was consistently 10–20% higher than in the membranes. The binding data were transformed into a Scatchard [21] plot to yield the equilibrium dissociation constant (*K_d*) and maximal binding capacity (*B_{max}*).

SDS/polyacrylamide-gel electrophoresis

Electrophoresis was performed by using the discontinuous buffer system of Laemmli [22], and homogeneous 1 mm-thick slab gels containing 7.5% acrylamide monomer in the separating gel and 4% acrylamide in the stacking gel. Sample buffer was 62.5 mM-Tris/HCl (pH 6.8)/2% (w/v) SDS/10% (v/v) glycerol/0.001% Bromophenol Blue/1% (w/v) 2-mercaptoethanol. Mercaptoethanol was omitted from samples subjected to ligand blotting. Following electrophoresis (200 V, 45 min, Bio-Rad Mini-Protean apparatus), the gels were stained with Coomassie Brilliant Blue or silver nitrate [23]. For autoradiography, gels stained with Coomassie Brilliant Blue were destained, dried, and exposed on Kodak XAR-5 film overnight at -80 °C. The apparent *M_r* of the labelled band was determined from a standard curve based on the mobility of molecular-mass markers co-electrophoresed with the samples.

Ligand blotting

Solubilized receptor (2–5 μ g of protein) was mixed with 2 vol. of SDS buffer (without reductant) and subjected to SDS/polyacrylamide-gel electrophoresis on 1.5 mm-thick slab gels (7.5% acrylamide). Resolved proteins were blotted on to nitrocellulose (100 V, 1 h; Bio-Rad Mini Trans-Blot apparatus) and processed further as described previously [6,19].

Miscellaneous

Protein was assayed by the Lowry [24] method, with BSA as a standard. The results described in the present study are representative of experiments that were performed at least three times with essentially identical results.

Fig. 1. Purification of LH receptor

LH receptor was purified from rat ovaries by affinity chromatography on hCG-Affigel 10. The receptor was eluted in 0.1 M-sodium acetate, pH 4.0, containing 20% glycerol and 0.1% Triton X-100 and analysed by SDS/7.5%-polyacrylamide-gel electrophoresis under reducing (lanes a-c) or non-reducing (d-e) conditions. Polypeptides were revealed by silver staining (lanes a and b) or autoradiography (lane c). The receptor was identified by binding of ^{125}I -hCG to a protein blot after non-reducing electrophoresis (ligand blotting). Lanes: a, molecular-mass markers; b, pH 4 eluate; lane c, autoradiogram of iodoreceptor; lane d, ligand blot; lane e, ligand blot in the presence of unlabelled hCG (1 $\mu\text{g/ml}$). Molecular-mass markers were myosin heavy chain (205 kDa), β -galactosidase (116 kDa), phosphorylase b (94 kDa), BSA (66 kDa) and ovalbumin (45 kDa).

RESULTS

Deglycosylation of purified receptor

Previous studies from this laboratory have shown that affinity-purified rat ovarian LH receptor consists of a single 90 kDa polypeptide which bound to immobilized wheat-germ lectin and concanavalin A, suggesting the presence of *N*-linked carbohydrate [5,6]. One purpose of the present study was to determine the contribution of the receptor's oligosaccharide chains to its apparent M_r . To this end, LH receptor was first purified from pseudo-pregnant-rat ovaries by one-step affinity chromatography [6]. Fig. 1 shows a SDS/polyacrylamide-gel electrophoretic analysis of a typical receptor preparation (with a specific hormone-binding activity of 3080 pmol/mg of protein). Revelation of the polypeptides by silver staining (Fig. 1b) or autoradiography after chemical iodination (Fig. 1c) revealed the presence of a major 90 kDa polypeptide, corresponding to the LH receptor, and minor bands in the 40–60 kDa range. Transfer of resolved receptor preparation to nitrocellulose, followed by incubation with ^{125}I -hCG (ligand blotting) and autoradiography, demonstrates binding of ^{125}I -hCG to the 90 kDa species (Fig. 1d). This binding is specific, as it is

Fig. 2. Effect of neuraminidase and endo- α -N-acetylgalactosaminidase on the electrophoretic mobility of the iodoreceptor

Iodoreceptor was incubated alone (control) or with neuraminidase (50 munits/ml), endo- α -N-acetylgalactosaminidase (25 units/ml) or both for 3 h at 30 $^{\circ}\text{C}$, and the reaction products were analysed by SDS/polyacrylamide-gel electrophoresis after precipitation by 10% (w/v) trichloroacetic acid. An autoradiogram of the dried gel is shown. Lanes: a, control (untreated receptor); b, neuraminidase treatment; c, neuraminidase plus endo- α -N-acetylgalactosaminidase treatment; d, endo- α -N-acetylgalactosaminidase treatment. The sizes (in kDa) of molecular-mass markers are indicated on the left.

displaced by an excess of unlabelled hCG (Fig. 1e). These data are in agreement with our previous results [6] and support the notion that a single 90 kDa polypeptide represents ovarian receptor for LH and hCG.

For enzymic deglycosylation experiments, radio-iodinated LH receptor was chosen for the substrate as autoradiography provides a convenient and unequivocal method of identification of the deglycosylated receptor species. Because of the presence of minor 30–60 kDa bands, which might complicate interpretation of the results, the 90 kDa iodoreceptor was first electroeluted from the SDS-containing gel. Contribution of sialic acid residues and serine/threonine-linked oligosaccharides to the apparent molecular mass of LH receptor was investigated using neuraminidase and endo- α -N-acetylgalactosaminidase (Fig. 2). A marked increase in the electrophoretic mobility of the iodoreceptor from approx. 90 kDa to approx. 79 kDa was observed after neuraminidase treatment (Fig. 2b). This indicates that LH receptor contains terminally sialylated oligosaccharide side chains. Endo- α -N-acetylgalactosaminidase is capable of liberating the disaccharide Gal(β 1 $\rightarrow$ β 3)GalNAc from *O*-glycans of glycoproteins, provided the disaccharide is not substituted by sialic acid or other sugars. Endo- α -N-acetylgalactosaminidase treatment did not change the electrophoretic mobility of the intact (Fig. 2d) or neuraminidase-treated (Fig. 2c) receptor, suggesting that *O*-linked oligosaccharide chains are absent. Longer incubations (up to 18 h) with neuraminidase and endo- α -N-acetylgalactosaminidase did not change the electrophoretic pattern (results not shown).

PNGase F is a recently characterized and commercially

Fig. 3. Effect of PNGase F on the electrophoretic mobility of the iodoreceptor

Denatured iodoreceptor was incubated either alone (4 h) or with PNGase F (4 units/ml) for 1–4 h at 30 °C, and the reaction products were analysed by SDS/polyacrylamide-gel electrophoresis after precipitation with 10% (w/v) trichloroacetic acid. An autoradiogram of a representative gel is shown. Lanes: a, intact receptor; b–d, PNGase F-treated receptors: b, 1 h treatment; c, 2 h treatment; d, 4 h treatment. The sizes (in kDa) of molecular-mass markers are indicated on the left.

available enzyme which removes *N*-glycosidically linked oligosaccharides from glycoproteins, producing essentially deglycosylated proteins in terms of *N*-linked carbohydrate [25,26]. Fig. 3 shows the effect of PNGase F on LH receptor. A decrease in the molecular mass of the iodoreceptor from 90 kDa to approx. 62 kDa was noted after PNGase F digestion (Figs. 3c–3d). Longer incubations (up to 18 h) or complete denaturation of the receptor sample by boiling for 1 min in the presence of 0.1% SDS and 1% 2-mercaptoethanol before addition of the enzyme did not produce any species of smaller M_r , indicating that deglycosylation had proceeded to completion (results not shown). Conversion of the iodoreceptor from a 90 kDa species into a 62 kDa species after treatment with PNGase F is most likely due to the removal of sugar moieties from the receptor and not due to proteolysis. This is supported by several facts. According to the manufacturer, the enzyme preparation used contained no proteinase activity. Incubation was performed in the presence of proteinase inhibitors EDTA and PMSF. Furthermore, no changes in the trichloroacetic acid-precipitability of the radioactive label were observed between the control and treated samples. Lastly, the 90 kDa receptor, but not the 62 kDa species, bound to, and was eluted specifically from, immobilized wheat-germ lectin (results not shown). The decrease in the molecular size of the LH receptor by 28 kDa after PNGase F treatment indicates the presence of a substantial amount of *N*-linked carbohydrate.

Effect of deglycosylation on hormone-binding properties of LH receptor

The experiments described above demonstrate the presence of asparagine-linked carbohydrate in the LH receptor. Another purpose of the present study was to

Fig. 4. Identification of LH receptor by ligand blotting after neuraminidase treatment of the membranes

Membranes were incubated with neuraminidase (final concn. 0.1 unit/ml) for 0–2 h at 30 °C. After digestion, membranes were collected by centrifugation, washed and solubilized in 1% Triton X-100/20% glycerol. Solubilized receptor was subjected to SDS/polyacrylamide-gel electrophoresis under non-reducing conditions, electroblotted on to nitrocellulose, and incubated with 125 I-hCG for 16 h at room temperature. An autoradiogram of a dried blot is shown. Lanes: a, control (no neuraminidase), zero time; b, neuraminidase, 30 min; c, neuraminidase, 1 h; d, neuraminidase, 2 h. The sizes (in kDa) of molecular-mass markers are indicated on the left.

investigate the effect of enzymic deglycosylation on the hormone-binding characteristics of the LH receptor. For these experiments, membrane-bound receptor was used because of the possibility of completely removing the enzyme from the receptor preparation before hormone-binding assay. This is important, because hCG is a glycoprotein hormone, and partial or complete deglycosylation of the hormone influences the hormone–receptor interaction [27]. Furthermore, after solubilizing the membranes, the molecular size of the deglycosylated receptor species would be revealed by ligand blotting [6,19], provided the deglycosylated receptor was able to bind hormone.

Fig. 4 illustrates a ligand blot prepared after neuraminidase digestion of the membranes. Conversion of the intact 90 kDa receptor (lane a) to a 79 kDa species (lanes c and d) via an 85 kDa intermediate (lane b) is observed. Binding of 125 I-hCG to the 90 kDa and 79 kDa species was specific, as it was displaced by an excess of unlabelled hCG (results not shown). Longer incubations failed to produce any further alteration in the mobility of the receptor, but resulted gradually in loss of hormone binding.

Incubation of the ovarian membranes with PNGase F induced conversion of the receptor from a 90 kDa to a 62 kDa species (Fig. 5). This compares well with the size change observed after complete deglycosylation of the iodoreceptor, indicating that the oligosaccharides of the native membrane-bound receptors are accessible to

Fig. 5. Identification of LH receptor by ligand blotting after PNGase F treatment of the membranes

Membranes were incubated alone or with PNGase F (10 units/ml) for 2 h at 30 °C. After digestion, membranes were collected by centrifugation, washed and solubilized in 1% Triton X-100/20% glycerol. Solubilized receptor was subjected to SDS/polyacrylamide-gel electrophoresis under non-reducing conditions, electroblotted on to nitrocellulose and incubated with ^{125}I -hCG for 16 h at room temperature. An autoradiogram of a dried blot is shown. Lanes: a, c, d, control membranes (untreated); b and e, PNGase F-treated membranes. An excess of unlabelled hCG was present during the hormone-incubation step of lanes d and e. The sizes (in kDa) of molecular-mass markers are indicated on the left.

enzymic removal. Furthermore, the very existence of the 62 kDa band in the ligand blot provides direct evidence for the hormone-binding ability of the polypeptide moiety of the LH receptor.

To further evaluate the role of carbohydrate in the hormone-receptor interaction, quantitative binding assays were performed. Fig. 6 illustrates Scatchard [21] plots of binding of ^{125}I -hCG to intact and PNGase F-treated membrane-bound LH receptor. It is clear that, after PNGase F treatment, the binding of ^{125}I -hCG to the membranes is essentially unaltered, the K_d values being $1.27(\pm 0.31) \times 10^{-10}$ M for the PNGase F-treated and $1.45(\pm 0.31) \times 10^{-10}$ M for the control (intact) membranes respectively (means $\pm$ S.D., $n = 4$). Maximal binding capacity of the deglycosylated membranes calculated from the equilibrium binding data as well as the signal produced by the deglycosylated membrane-bound LH receptor was often, but not always weaker (by 10–20%) than those obtained when intact receptor was analysed. This issue was not pursued further, but it could reflect greater sensitivity of the deglycosylated membrane receptor was often, but not always, weaker (by 10–20%) complete deglycosylation of the membrane-bound LH receptor with PNGase F does not have significant effects upon the hormone-binding characteristics of the receptor.

DISCUSSION

Membrane-bound receptors for hormones and growth factors are generally glycosylated. The function of the glycan moiety has been studied mainly by using inhibitors of *N*-linked glycosylation and oligosaccharide

Fig. 6. ^{125}I -hCG binding to intact and deglycosylated membrane-bound LH receptor

Membranes were treated with PNGase F as described in the legend to Fig. 5. Washed membranes were incubated with increasing amounts of ^{125}I -hCG for 16 h at 30 °C. Membranes were collected by centrifugation, washed, and counted for radioactivity. Non-specific binding was determined in the presence of an excess of unlabelled hCG (4 $\mu\text{g}/\text{ml}$) and subtracted from the total binding to obtain the specific binding. A Scatchard [21] plot of the binding data is shown. ○, Control membranes; ●, PNGase F-treated membranes. Equilibrium dissociation constants (K_d) were derived from the plot: K_d (control) = 1.27×10^{-10} M; K_d (treated) = 1.51×10^{-10} M.

processing, and recently also by using specific enzymes capable of removing all or most of the glycoprotein glycans, but no generalizations on the role of carbohydrate in membrane receptor function can be drawn. At least partial glycosylation is required for processing and acquirement of hormone-binding activity of insulin receptor [28] and epidermal-growth-factor receptor [29,30]. On the other hand, inhibition of *N*-glycosylation by tunicamycin does not affect expression or function of β -adrenergic receptors in mouse lymphoma cells [31]. In accordance with this, partial or near-complete enzymic deglycosylation of the β -adrenergic receptor does not affect its agonist-binding properties [32,33].

In the present study, enzymic deglycosylation of rat ovarian LH receptor was used to determine the size of the deglycosylated receptor and to yield information on the function of the carbohydrate moiety of the receptor. There is some variation in the molecular size reported for the affinity-purified LH receptor by different laboratories; e.g. porcine luteal LH receptor migrated as a 60–77 kDa species depending on the electrophoretic conditions [3,34], whereas the size of purified rat ovarian LH receptor was reported to be 72 kDa [4] or 90 kDa [6]. It seems possible that, besides differences in methodology

and electrophoretic conditions, some of the variation may be caused by different extents of glycosylation.

Conversion of purified and membrane-bound LH receptors from a 90 kDa to a 62 kDa form after PNGase F treatment indicates that approx. 28 kDa of the molecular mass of LH receptor is contributed by asparagine-linked oligosaccharide chains. The fact that many of these chains are sialylated (i.e. of hybrid or complex type) is suggested by a marked increase in the electrophoretic mobility of the receptor induced by neuraminidase. On the other hand, unaltered electrophoretic mobility after endo- α -N-acetylgalactosaminidase suggests that the receptor does not contain serine- or threonine-linked oligosaccharides. Thus, on the basis of combined evidence from enzymic deglycosylation studies, it is most likely that the 62 kDa receptor species represents the completely deglycosylated LH receptor, and thus gives a measure of the size of the polypeptide backbone of the receptor. (Very recently, similar findings to those obtained in the present study on the contribution of carbohydrate to the apparent M_r of the LH receptor were reported by Kusuda & Dufau [35].)

From quantitative binding studies and ligand blotting experiments it is clear that specific hormone-binding activity is not lost after deglycosylation of LH receptor. Radiolabelled ^{125}I -hCG bound to PNGase F-treated LH receptor with unchanged affinity. This indicates that the glycan moiety of LH receptor is not necessary for specific high-affinity hormone binding. Furthermore, results of ligand blotting showed that the deglycosylated forms are also capable of refolding to active (in terms of hormone binding) conformation after SDS/polyacrylamide-gel electrophoresis. It was noteworthy that the signal produced by the deglycosylated LH receptors in ligand blotting was often weaker than that obtained from intact receptor, despite similar binding to membrane-bound receptor (see, e.g., Fig. 4). This is suggestive of greater sensitivity of the deglycosylated receptor to proteolysis or irreversible denaturation during the sample preparation. It is also possible that the weaker signal in ligand blotting is due to impaired folding (renaturation) of the LH receptor to its active hormone-binding conformation after denaturing conditions of the electrophoresis. It should be emphasized, however, that weaker signal produced by deglycosylated receptor was not a consistent finding.

In conclusion, the results of the present study show that deglycosylated LH receptor is a 62 kDa polypeptide with essentially unaltered hormone-binding properties. Thus the specific function, if any, of the oligosaccharide side chains of the LH receptor is clearly not involved in the ligand-binding process. Further studies are required to assess whether the deglycosylated receptor is capable of activating adenylate cyclase and possibly other effectors.

I thank Dr. Hannu Rajaniemi for support and critical reading of the manuscript. The skilful technical assistance of Ms. Paula Soininen is gratefully acknowledged. This study was supported by Medica Research Foundation, the Cultural Foundation of Finland and The Academy of Finland.

REFERENCES

- Catt, K. J., Ketelslegers, J.-M. & Dufau, M. L. (1976) in *Methods in Receptor Research* (Blecher, M., ed.), vol. 1, pp. 175–250, Marcel Dekker, New York
- Ascoli, M. (ed.) (1985) *Luteinizing Hormone Action and Receptors*, CRC Press, Boca Raton, FL
- Wimalasena, J., Moore, P., Wiebe, J. P., Abel, J., Jr. & Chen, T. T. (1985) *J. Biol. Chem.* **260**, 10689–10697
- Kusuda, S. & Dufau, M. L. (1986) *J. Biol. Chem.* **261**, 16161–16168
- Metsikkö, M. K. & Rajaniemi, H. J. (1982) *Biochem. J.* **208**, 309–316
- Keinänen, K. P., Kellokumpu, S., Metsikkö, M. K. & Rajaniemi, H. J. (1987) *J. Biol. Chem.* **262**, 7920–7926
- Rebois, R. V., Omedeo-Sale, F., Bardy, R. O. & Fishman, P. H. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2086–2089
- Metsikkö, M. K. (1984) *Biochem. J.* **219**, 583–591
- Rapoport, B., Hazum, E. & Zor, U. (1984) *J. Biol. Chem.* **259**, 4267–4271
- Ascoli, M. & Segaloff, D. L. (1986) *J. Biol. Chem.* **261**, 3807–3815
- Kim, I.-C., Ascoli, M. & Segaloff, D. L. (1987) *J. Biol. Chem.* **262**, 470–477
- Ji, L., Bock, J. H. & Ji, T. H. (1985) *J. Biol. Chem.* **260**, 12815–12821
- Dattatreyaumurthy, B., Rathnam, P. & Saxena, B. B. (1983) *J. Biol. Chem.* **258**, 3140–3157
- Bruch, R. C., Thotakura, N. R. & Bahl, O. P. (1986) *J. Biol. Chem.* **261**, 9450–9460
- Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496
- Markkanen, S., Töllikkö, K., Jääkeläinen, K. & Rajaniemi, H. (1980) *Horm. Res.* **12**, 32–45
- Ketelslegers, J.-M., Knott, G. D. & Catt, K. J. (1975) *Biochemistry* **14**, 3075–3083
- Parlow, A. F. (1961) in *Human Pituitary Gonadotropins* (Albert, A., ed.), pp. 300–310, Charles C. Thomas, Springfield, IL
- Keinänen, K. P., Kellokumpu, S. & Rajaniemi, H. J. (1987) *Mol. Cell. Endocrinol.* **49**, 33–38
- Dufau, M. L., Charreau, E. H. & Catt, K. J. (1973) *J. Biol. Chem.* **248**, 6973–6982
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Merril, C. R., Goldman, D. & Van Keuren, M. L. (1984) *Methods Enzymol.* **104**, 441–447
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Plummer, T. H., Jr., Elder, J. H., Alexander, S., Phelan, A. W. & Tarentino, A. L. (1984) *J. Biol. Chem.* **259**, 10700–10704
- Tarentino, A. L., Gomez, C. M. & Plummer, T. H., Jr. (1985) *Biochemistry* **24**, 4665–4671
- Sairam, M. R. (1985) in *The Receptors* (Conn, P. M., ed.), vol. 2, pp. 307–340, Academic Press, New York
- Ronnett, G. V. & Lane, M. D. (1981) *J. Biol. Chem.* **256**, 4704–4707
- Söderquist, A. M. & Carpenter, G. (1984) *J. Biol. Chem.* **259**, 12586–12594
- Slieker, L. J., Martensen, T. M. & Lane, M. D. (1986) *J. Biol. Chem.* **261**, 15233–15241
- George, S. T., Ruoho, A. E. & Malbon, C. G. (1986) *J. Biol. Chem.* **261**, 16559–16564
- Stiles, G. L. (1985) *Arch. Biochem. Biophys.* **237**, 65–71
- Cervantes-Olivier, P., Durieu-Trautman, O., Delavier-Klutchko, C. & Strosberg, A. D. (1985) *Biochemistry* **24**, 3765–3770
- Wimalasena, J., Abel, J. A., Jr., Wiebe, J. P. & Chen, T. T. (1986) *J. Biol. Chem.* **261**, 10689–10697
- Kusuda, S. & Dufau, M. L. (1988) *J. Biol. Chem.* **263**, 3046–3049