Covalent labelling of the lutropin binding site

Evidence for a single M_r 90 000 sialoglycopolypeptide

M. Kalervo METSIKKÖ

Department of Anatomy, University of Oulu, Kajaanintie 52A, SF-90220 Oulu 22, Finland

(Received 19 September 1983/Accepted 13 January 1984)

Membrane-associated sialoglycopolypeptides of rat ovaries were oxidized with NaIO_4 , reduced with NaB^3H_4 and solubilized with Triton X-100. The solubilized proteins carrying the ^3H label were subjected to affinity chromatography on human choriogonadotropin coupled to agarose. Polyacrylamide-gel electrophoresis in sodium dodecyl sulphate followed by fluorography revealed a single component of apparent M_r 90 000. This component was abolished when ovaries saturated with choriogonadotropin were used as starting material. The above result is identical to that obtained previously by conventional detection methods [Metsikkö & Rajaniemi (1982) *Biochem. J.* **208**, 309–316] and indicates that the ^3H -labelled lutropin/choriogonadotropin sialoglycopolypeptide was observed. The affinity-purified ^3H -labelled protein co-eluted with the choriogonadotropin-binding activity solubilized with Triton X-100 from rat ovarian particles, showed a Stokes' radius of 6.2 nm and sedimented as a single band with a sedimentation coefficient of 5.1 S. The sedimentation coefficient of this ^3H -labelled protein was not significantly altered when boiled in 1% sodium dodecyl sulphate, indicating that non-covalently associated subunits were not present. The ^3H -labelled protein cosedimented with the choriogonadotropin-binding activity solubilized with Triton X-100 from rat ovary. When ^{125}I -choriogonadotropin–receptor complex was covalently crosslinked with glutaraldehyde, an M_r 130 000 component was produced as detected by sodium dodecyl sulphate gel electrophoresis. This component was extracted from the polyacrylamide gel and subjected to sucrose-density-gradient centrifugation in 0.1% Triton X-100. A single band sedimenting at the position of the ^{125}I -choriogonadotropin–receptor complex solubilized from a prelabelled ovary was observed, exhibiting a sedimentation coefficient of 6.5 S. These data suggest that the lutropin-binding site is a single sialoglycopolypeptide of M_r 90 000, which binds one molecule of hormone resulting in an apparent M_r 130 000 complex. The large Stokes' radius (6.2 nm) of the binding site is accounted for by bound detergent.

The lutropin receptor is a cell membrane component which binds with high affinity lutropin and its agonist hormone, human choriogonadotropin. The receptor has been solubilized from gonadal homogenates with nonionic detergents (Dufau *et al.*, 1973, 1974). It behaves as an M_r 194 000 molecule when analysed in a solution containing Triton X-100 (Dufau *et al.*, 1973), while M_r values between 220 000 and 370 000 have been estimated for the choriogonadotropin–receptor complex (Du-

fau *et al.*, 1973; Charreau *et al.*, 1974; Ji *et al.*, 1981; Rebois *et al.*, 1981). When affinity chromatography has been utilized to purify the receptor from rat testis (Dufau *et al.*, 1975) and ovary (Metsikkö & Rajaniemi, 1982) an M_r 90 000 polypeptide was detected by SDS/polyacrylamide-gel electrophoresis. Affinity labelling of the rat testicular receptor yields a compatible result since an M_r 100 000 choriogonadotropin-binding polypeptide has been found by such a technique (Rebois *et al.*, 1981). Furthermore, crosslinking of the rat ovarian ^{125}I -choriogonadotropin–receptor complex with glutaraldehyde produces an M_r 130 000 component

Abbreviations used: SDS, sodium dodecyl sulphate; PBS, 10 mM-sodium phosphate, pH 7.0, containing 140 mM-NaCl.

(Metsikkö & Rajaniemi, 1982). However, porcine granulosa cells have been reported to produce several components when photoaffinity labelled with ^{125}I -choriogonadotropin and subsequently analysed by SDS/polyacrylamide-gel electrophoresis and autoradiography (Ji & Ji, 1981; Ji *et al.*, 1981). No data are available concerning the possible subunit structure of the M_r 194000 lutropin receptor solubilized from gonadal tissues.

In this study a sensitive ^3H -radiolabelling approach was developed for the rat ovarian lutropin receptor. Analysis of physical parameters of the ^3H -labelled receptor protein in the non-denatured and denatured state suggests that about half of its M_r in a medium containing Triton X-100 is accounted for by bound detergent. In addition, evidence is presented that the ^{125}I -choriogonadotropin-receptor complex solubilized with Triton X-100 possesses an M_r of about 130000, although its Stokes' radius suggests a higher value.

Experimental

Materials

Female 25-day-old rats (Wistar) were induced pseudopregnant by treatment with pregnant mare serum gonadotropin (Diosynth, Oss, Holland) and choriogonadotropin (Diosynth) as described by Parlow (1961). The biological potency of choriogonadotropin was 10 100 i.u./mg and it was labelled with Na^{125}I (Amersham International, Amersham, Bucks., U.K.) by a modification (Markkanen *et al.*, 1980) of the chloramine-T method (Hunter & Greenwood, 1962). Specific radioactivity of the labelled hormone was 20 Ci/g. NaB^3H_4 (5.7 Ci/mmol) was obtained from Amersham International and was dissolved in 0.01 M-NaOH and stored at -70°C (Gahmberg *et al.*, 1970). Affi-Gel 10 was purchased from Bio-Rad Chemicals, Richmond, CA, U.S.A., and Sephacryl S-400 from Pharmacia Fine Chemicals, Uppsala, Sweden. All other reagents were of analytical grade.

Labelling of ovarian particles with periodate/ NaB^3H_4

The labelling procedure was a modification of that of Gahmberg & Andersson (1977). Pseudopregnant rat ovarian tissue (300 mg) was homogenized in 1 ml of PBS with a glass homogenizer (100 rev./min for 2 min) and centrifuged at 600g for 10 min at 4°C . The supernatant was then centrifuged at 20000g for 30 min at 4°C and the pellet suspended in 1 ml of PBS. Sodium periodate (0.5 M) was added to a final concentration of 2 mM, and the sample was incubated for 10 min at 4°C in the dark. Excess periodate was removed by centrifugation at 20000g for 30 min. The pellet was washed once with 1 ml of PBS and centrifuged as above. The

final pellet was suspended in 1 ml of 50 mM-phosphate buffer, pH 8.3, containing 0.1 M-NaCl, whereafter 5 mCi (5 μl) of NaB^3H_4 solution was added. The suspension was kept at 20°C for 1 h, and centrifuged at 20000g for 30 min at 4°C . The pellet was extracted with Triton X-100.

Labelling of the ovarian lutropin receptor with ^{125}I -choriogonadotropin

^{125}I -Choriogonadotropin (5×10^6 c.p.m., 100 μl) was injected into the tail vein of a pseudopregnant rat, and the animal was killed 2 h later. The uptake of ^{125}I radioactivity into the ovaries was 20–30% of that injected. The ovaries were homogenized in PBS and centrifuged at 20000g for 30 min at 4°C and the pellet was extracted with Triton X-100.

Extraction of ovarian particles with Triton X-100

The ovarian pellet obtained after treatment with NaB^3H_4 or from the ovaries labelled with ^{125}I -choriogonadotropin was suspended in PBS containing 1% (w/v) Triton X-100 and 10 mM-iodoacetamide (2–3 mg of particle protein/ml) and stirred on ice for 30 min. The suspension was diluted to 0.5% Triton X-100 with PBS containing 10 mM-iodoacetamide and centrifuged at 100000g for 2 h at 4°C .

Affinity chromatography

The affinity gel was prepared by coupling human choriogonadotropin to Affi-Gel 10 as described earlier (Metsikkö & Rajaniemi, 1982). The Triton X-100 extract of the periodate/ NaB^3H_4 -labelled ovarian particles (4 ml, 2.7 mg of protein) was agitated with choriogonadotropin-Affi-Gel 10 (100 μl) overnight at 4°C . The gel beads were collected by centrifugation at 500g for 5 min and packed into a column (0.5 cm $\times$ 1 cm). The column was washed as described earlier (Metsikkö & Rajaniemi, 1982) and then eluted with 100 μl of 100 mM-sodium acetate, pH 4.0, containing 0.1% Triton X-100. The eluate was neutralized immediately.

Crosslinking of the ^{125}I -choriogonadotropin-receptor complex

The ^{125}I -choriogonadotropin-receptor complex solubilized with Triton X-100 was crosslinked with glutaraldehyde as previously described (Metsikkö & Rajaniemi, 1982), with some modifications. Glutaraldehyde (100 mM) was added to a final concentration of 5 mM and the complex was incubated with the crosslinker for 1 h at 20°C , whereafter 100 mM-Tris/HCl, pH 7.3, was added to a final concentration of 10 mM.

Assay of receptor activity

The receptors solubilized with Triton X-100

were determined as described by Dufau *et al.* (1973). Aliquots of sample (200 μ l) were incubated with 50 μ l of 125 I-choriogonadotropin (70000 c.p.m.) overnight at 4°C and the 125 I-choriogonadotropin-receptor complex was precipitated with poly(ethylene glycol). Non-specific binding was determined in the presence of 1 μ g of choriogonadotropin, and it was usually 3% of total counts.

SDS/polyacrylamide-gel electrophoresis

Polyacrylamide-gel electrophoresis in the presence of SDS was performed according to Laemmli (1970) using 5% or 7% stacker gels (1 cm) and 7% or 9% separating gels (10 cm), correspondingly. Standard proteins, detected by staining with Coomassie Brilliant Blue (Fairbanks *et al.*, 1971), were used for calibration of the gels: myosin (M_r 200000), phosphorylase *b* (M_r 94000), bovine serum albumin (M_r 66000) and ovalbumin (M_r 45000). Samples were dialysed into the Laemmli sample buffer containing 1% (w/v) SDS and boiled for 1 min before electrophoresis. 125 I radioactive samples were analysed by cylindrical gels which were cut into 1 mm slices and the slices were counted for radioactivity in a gamma counter (LKB Wallac). 3 H radioactive samples were analysed by slab gels which were treated for fluorography (Bonner & Laskey, 1974). Fluorography of the dried slabs was performed at -70°C using Kodak XR-2 film. Exposure time was 5–10 days.

Density gradient centrifugation

Centrifugation in linear 5–20% (w/v) sucrose gradients made in PBS containing 0.1% Triton X-100 was performed according to Martin & Ames (1961) in a Beckman L5-50B ultracentrifuge employing an SW-40 rotor. The standard proteins used were human choriogonadotropin (2.9S), bovine serum albumin (4.3S) and bovine fibrinogen (7.9S) which were detected by determining protein in the fractions. Sample size was 200 μ l. After centrifuging for 20 h at 40000 rev./min at 4°C, fractions (200 μ l) were collected. 125 I-radioactive fractions were counted with a gamma counter, and 3 H radioactive fractions were counted in a toluene-based cocktail in a liquid-scintillation counter (LKB Wallac 1215 Rackbeta II).

Centrifugation in isopycnic gradients of CsCl was performed employing the SW-40 rotor. Samples of 50 μ l were layered on 5.5 ml of 26% (w/w) CsCl solution made in 10 mM-phosphate buffer, pH 7.4, containing 0.1% Triton X-100, and centrifuged for 40 h at 40000 rev./min at 4°C. Phosphate buffer was layered on the samples to fill the tubes. Fractions of 150 μ l were collected and their densities determined by measurement of refraction index at 25°C (Bruner & Vinograd, 1965).

Gel filtrations

Samples (250 μ l) were applied to a column (0.9 cm $\times$ 60 cm) of Sephacryl S-400 equilibrated with PBS containing 0.1% Triton X-100 and 10 mM-iodoacetamide. Flow rate was 2.5 ml/h and fractions (0.53 ml) were collected. Proteins of known Stokes' radius were used as standards: equine cytochrome *c* (1.0 nm), bovine serum albumin (3.5 nm), bovine gamma globulin (5.6 nm), and bovine fibrinogen (10.7 nm).

Protein determination

Protein was determined by a modification (Dulley & Grieve, 1975) of the method of Lowry *et al.* (1951).

Identification of 3 H-labelled 5-acetamido-3,5-di-deoxy-L-arabino-2-heptulosonic acid

Periodate/ NaB^3H_4 -labelled sample was treated with 0.1 M- H_2SO_4 at 80°C for 1 h, and neutralized with BaCl_2 . The hydrolysis product was analysed by paper chromatography on Whatman no. 3MM paper with *n*-butyl acetate/acetic acid/water (3:2:1, by vol.) as described by Liao *et al.* (1973).

Results

Labelling of the ovarian particles with periodate/ NaB^3H_4

Rat ovarian particles containing 4.5 mg of protein were oxidized with 2 mM-periodate and reduced with 5 mCi of NaB^3H_4 . This treatment did not significantly affect the maximal hormone binding capacity or the affinity of binding. The incorporation of radioactivity into the particles was 1.27×10^5 d.p.m./ μ g of protein. The ovarian particles were solubilized with Triton X-100 when 84% of the radioactivity incorporated was obtained in the supernatant fraction (Table 1). About 75% of the radioactivity in this supernatant was precipitable with 10% (w/v) trichloroacetic acid. The polypeptide profile of the labelled proteins is shown in Fig. 1 (lane 1).

Isolation and identification of the 3 H-labelled lutropin binding site

The Triton X-100 extract of the ovarian particles labelled with the NaB^3H_4 was subjected to affinity chromatography on choriogonadotropin-Affi-Gel 10. About 0.04% of the radioactivity in the Triton X-100 extract was recovered in the acetate buffer eluate of the affinity gel (Table 1). A single component with an apparent M_r 90000 was observed when this eluate was analysed by SDS/polyacrylamide-gel electrophoresis and fluorography (Fig. 1, lane 2). Ovaries saturated with 100 i.u. of choriogonadotropin by an intravenous injection 2 h before killing were used as control tissue for the

Table 1. Labelling of rat ovarian sialoglycopolypeptides with ^3H

Pseudopregnant rat ovarian particles (4.5 mg of protein) were labelled by the periodate/ NaB^3H_4 method, and then solubilized with Triton X-100. The Triton X-100 extract was incubated with choriogonadotropin-Affi-Gel 10 which was thereafter washed, and eluted with sodium acetate buffer, pH 4.0, containing 0.1% Triton X-100.

Preparation	Total ^3H incorporated (d.p.m.)	Specific radioactivity (d.p.m./ μg of protein)
Ovarian particles	5.7×10^8	1.27×10^5
Triton X-100 extract	4.8×10^8	1.78×10^5
pH 4 eluate	2.0×10^5	—

NaB^3H_4 labelling procedure and subsequent affinity chromatography. The M_r 90000 component was then absent (Fig. 1, lane 4). Boiling of the ^3H -labelled receptor protein for 1 min in a solution containing 1% (w/v) SDS and 1% (v/v) 2-mercaptoethanol caused appearance of two faint, more rapidly moving, components but the parent M_r 90000 band was not abolished (Fig. 1, lane 3).

All the label was released from the ^3H -labelled component by 0.1 M- H_2SO_4 at 80°C for 60 min. The released label comigrated with the ^3H -labelled sialic acid derivative 5-acetamido-3,5-dideoxy-L-arabino-2-heptulosonic acid when analysed by paper chromatography.

Analysis of physical properties of the ^3H -labelled receptor protein

The ^3H -labelled receptor glycoprotein purified by affinity chromatography sedimented as a single band when analysed by sucrose density gradient centrifugation in a solution containing 0.1% Triton X-100. The partial specific volume of the ^3H -labelled receptor protein was $0.812 \pm 0.005 \text{ cm}^3/\text{g}$ (s.d., four determinations) as determined by isopycnic CsCl density gradient centrifugation. A sedimentation coefficient of $5.1 \pm 0.3 \text{ S}$ (s.d., five determinations) was estimated for the ^3H -labelled species by means of standard proteins after correction for a partial specific volume of $0.812 \text{ cm}^3/\text{g}$, using the tables of McEwen (1967) (Fig. 2). Boiling in 1% SDS for 1 min did not alter the partial specific volume of the ^3H -labelled receptor glycoprotein. After boiling in SDS, an aliquot of the ^3H -labelled receptor protein was diluted to 1:10 with PBS containing 0.1% Triton X-100 and subjected to sucrose-density-gradient centrifugation. A single band was observed with a sedimentation coefficient of $5.0 \pm 0.3 \text{ S}$ (s.d., five determinations) when corrected for the influence of the high partial

Fig. 1. Electrophoresis and fluorography of the ^3H -labelled ovarian sialoglycopolypeptides isolated by choriogonadotropin-Affi-Gel 10

Pseudopregnant rat ovarian particles with receptors unoccupied or occupied with choriogonadotropin were labelled with the periodate/ NaB^3H_4 method and extracted with Triton X-100. The extract was subjected to affinity chromatography on choriogonadotropin immobilized to Affi-Gel 10. The radioactivity which detached from the affinity gel with sodium acetate buffer, pH 4.0, was analysed together with the Triton X-100 extract by electrophoresis in SDS using a 9% polyacrylamide slab gel, followed by fluorography. Lane 1, Triton X-100 extract; lane 2, radioactivity detached from the affinity gel at pH 4.0; lane 3, radioactivity detached at pH 4.0 and reduced with β -mercaptoethanol; lane 4, radioactivity detached from the affinity gel which was incubated with the Triton X-100 extract obtained from ^3H -labelled ovarian particles saturated with choriogonadotropin *in vivo*. About 50000 c.p.m. were applied to lane 1, and 5000 c.p.m. were applied to each of lanes 2-4.

specific volume of the labelled protein. Boiling in SDS thus did not significantly alter the sedimentation properties of the ^3H -labelled receptor protein.

The ^3H -labelled and affinity-purified receptor glycoprotein cosedimented with the choriogonadotropin binding activity solubilized from pseudopregnant rat ovaries by Triton X-100, but a sedi-

Fig. 2. Radioactivity profiles from sucrose-density-gradient centrifugation of the ^3H -labelled receptor protein before and after boiling in SDS

The ^3H -labelled receptor protein isolated by affinity chromatography was subjected to centrifugation in a 5–20% sucrose density gradient in a medium containing 0.1% Triton X-100 (●). An aliquot of the ^3H -labelled protein was boiled for 1 min in a medium containing 1% SDS before centrifugation (○). By the use of standard proteins, a sedimentation coefficient of $4.8 \pm 0.3\text{S}$ was obtained for the ^3H -labelled protein that was not boiled in SDS, and 4.7 ± 0.3 for that boiled in SDS, assuming the receptor has a partial specific volume of a 'typical' protein ($0.73\text{cm}^3/\text{g}$). When corrected for the true partial specific volume of $0.812\text{cm}^3/\text{g}$, values of 5.1S and 5.0S were obtained, correspondingly. Inset: ^3H -R, the ^3H -labelled receptor protein; hCG, human choriogonadotropin.

mentation coefficient of $5.6 \pm 0.3\text{S}$ (s.d., five determinations) was now observed (Fig. 3). This value differs significantly from that observed for the ^3H -labelled receptor alone ($P \leq 0.05$ as calculated by Student's *t* test). Similarly, the ^3H -labelled species coeluted with the choriogonadotropin binding activity when analysed by gel filtration. A Stokes' radius of 6.2 nm (mean of three determinations) was estimated for the ^3H -labelled species (Fig. 4).

Hormone-binding activity of the ^3H -labelled receptor protein

The specific uptake of the ^3H -labelled receptor by choriogonadotropin-Affi-Gel 10 was deter-

Fig. 3. Demonstration of cosedimentation on sucrose-density gradient centrifugation of the ^3H -labelled receptor protein and the choriogonadotropin binding activity solubilized with Triton X-100 from pseudopregnant rat ovaries

The ^3H -labelled receptor protein (10000 c.p.m.) was mixed with Triton X-100 extract (200 μl , 1.5 mg of protein) of pseudopregnant rat ovaries, and applied onto a 5–20% sucrose gradient made in PBS containing 0.1% Triton X-100 and 10 mM-iodoacetamide. After centrifuging for 20 h at 40000 rev./min, fractions (200 μl) were collected and divided into two equal portions, which were assayed for ^3H radioactivity (●) or specific ^{125}I -choriogonadotropin-binding capacity (○). The ^3H -labelled receptor protein cosedimented with the choriogonadotropin binding activity, exhibiting a sedimentation coefficient of 5.6S after correction for the partial specific volume of $0.812\text{cm}^3/\text{g}$, as was observed in five experiments.

mined. The ^3H -labelled species recovered by affinity chromatography was incubated overnight at 4°C with 50 μl of choriogonadotropin-Affi-Gel 10 in a solution (100 μl) containing PBS and 0.1% Triton X-100. Nonspecific binding was determined in the presence of 500 μg of choriogonadotropin, and it was about 1% of total radioactivity. The specific uptake by the gel ranged between 5 and 11% (means of three determinations), depending on the purified receptor batch.

Properties of the ^{125}I -choriogonadotropin-receptor complex crosslinked with glutaraldehyde

The ^{125}I -choriogonadotropin-receptor complex solubilized from pseudopregnant rat ovaries with Triton X-100 was subjected to density gradient

Fig. 4. Demonstration of co-elution on a column of Sephacryl S-400 of the ^3H -labelled receptor protein and the hormone-binding activity solubilized from pseudopregnant rat ovaries with Triton X-100

The ^3H -labelled receptor protein was simultaneously applied with the Triton X-100 extract of pseudopregnant rat ovaries to a column of Sephacryl S-400 (0.9 cm $\times$ 60 cm). Fractions (0.53 ml) were collected and divided into two equal portions which were counted for ^3H radioactivity ($\bullet$) or assayed for specific ^{125}I -choriogonadotropin-binding capacity ($\circ$). The two activities coeluted at $K_{av} = 0.49$, this value corresponding to a Stokes radius of 6.2 nm (inset). V_0 , void volume; V_t , total volume. The arrows mark the positions of standard proteins: 1, fibrinogen; 2, gamma globulin; 3, albumin; 4, cytochrome c.

centrifugation. A single peak with a sedimentation coefficient of $6.5 \pm 0.3\text{S}$ (s.d., five determinations) was observed (Fig. 5a). The peak fractions of the gradient tubes (Fig. 5a) were incubated with 5 mM-glutaraldehyde to crosslink the ^{125}I -labelled choriogonadotropin to its receptor. Electrophoresis of this crosslinked complex in SDS revealed a single ^{125}I -labelled component which showed an apparent M_r of 130 000, in addition to the hormone and its α -subunit bearing the ^{125}I -label (Markkanen *et al.*, 1980) (Fig. 5b). It was estimated from the peak areas in Fig. 5(b) that about 30% crosslinking of ^{125}I radioactivity to the receptor protein occurred. The M_r 130 000 component was extracted from the corresponding gel slices and analysed by sucrose-density-gradient

centrifugation. A single component was observed, showing a sedimentation coefficient of 6.5S (mean of three determinations) (Fig. 5c).

The crosslinked ^{125}I -choriogonadotropin-receptor complex eluted as a single peak on gel filtration on a column of Sephacryl S-400, showing a retardation coefficient of 0.46 (mean of three determinations). This value was identical with that of the native ^{125}I -choriogonadotropin-receptor complex on this column, corresponding to a Stokes' radius of 7.0 nm. Boiling of the crosslinked complex in 1% (w/v) SDS did not alter its elution position, as was observed in three experiments.

Discussion

In the present study rat ovarian particulate sialoglycoproteins were oxidized with periodate and reduced with NaB^3H_4 . After solubilization with Triton X-100 a single ^3H -labelled component was isolated by affinity chromatography on choriogonadotropin immobilized to agarose, as was shown by SDS/polyacrylamide-gel electrophoresis. This component showed an apparent M_r 90 000 when analysed on 9% gels but this value must be regarded as provisional, since electrophoresis in SDS does not show real M_r values for glycopolypeptides (Leach *et al.*, 1980). Occupation of tissue receptor sites with choriogonadotropin abolished the M_r 90 000 component, indicating that a specific choriogonadotropin binding polypeptide was detected (Fig. 1). This observation is consistent with a previous report from this laboratory when an M_r 90 000 Coomassie Blue-staining polypeptide was purified from rat ovaries together with some other components, perhaps peripheral proteins (Metsikkö & Rajaniemi, 1982). Furthermore, Rebois *et al.* (1981) have shown by an affinity labelling approach that ^{125}I -choriogonadotropin can be incorporated into an M_r 100 000 polypeptide in rat testis. Recently, a choriogonadotropin receptor polypeptide with an apparent M_r 79 000 was endogenously labelled in Leydig cells using [^{35}S]-methionine (Aubry *et al.*, 1982). Apparently all these observations represent the same polypeptide. In this study it was shown that the rat ovarian choriogonadotropin-binding component is a sialoglycopolypeptide.

When the ^3H -labelled receptor sialoglycopolypeptide was boiled in SDS in the presence of a reductant, faint bands migrating more rapidly on SDS gel electrophoresis appeared in addition to the M_r 90 000 component (Fig. 1, lane 3). Probably proteolytic cleavage had occurred in the polypeptide chain and the cleavage products were dissociated upon reduction.

Boiling in SDS had no marked influence on the sedimentation coefficient of the ^3H -labelled recep-

Fig. 5. Relationship between the native ^{125}I -choriogonadotropin-receptor complex sedimenting at 6.5S and the glutaraldehyde-crosslinked complex showing an apparent M_r 130000

Pseudopregnant rat ovaries labelled with ^{125}I -choriogonadotropin *in vivo* were extracted with Triton X-100 and the extract subjected to centrifugation on a 5–20% sucrose gradient, whereafter fractions (200 μl) were collected and assayed for ^{125}I radioactivity, as shown in (a). The peak fraction from the gradient tube was incubated for 1 h with 5 mM-glutaraldehyde. The solution was dialysed against the Laemmli (1970) sample buffer diluted 1:3 and then evaporated to one-third of the volume, and subjected to electrophoresis in SDS on a 7% polyacrylamide gel. The gel was sliced and the slices were counted for radioactivity. A single peak migrating with an apparent M_r 130000 was observed, in addition to the hormone and its α -subunit migrating with the dye (b). The radioactivity migrating in the M_r 130000 region was extracted with PBS containing 0.1% Triton X-100 and the extract was analysed by sucrose-density-gradient centrifugation. A single band showing an identical sedimentation coefficient to the native complex was observed (c). The peak in (c) represents the middle of the peak in (a) and is sharper than this peak, suggesting heterogeneity of the native ^{125}I -choriogonadotropin-receptor complex. The arrows mark the position of standard proteins with their M_r ($\times 10^{-3}$).

tor protein (Fig. 2), nor did it alter the partial specific volume of the labelled species. These findings suggest that the ^3H -labelled protein exhibiting the sedimentation coefficient of 5.1S and the M_r 90000 polypeptide observed by SDS gel electrophoresis are identical. It appears that during the centrifugation through the sucrose gradient the SDS bound to the polypeptide chain was exchanged for Triton X-100, as has been shown for the erythrocyte membrane proteins by the use of [^{35}S]SDS (Bhakdi, 1980).

The ^3H -labelled protein isolated by affinity chromatography coeluted with the choriogonadotropin binding activity solubilized from rat ovarian particles when analysed on a column of Sephacryl

S-400 (Fig. 4), and cosedimented with the hormone-binding activity (Fig. 3). However, a sedimentation coefficient of 5.6S instead of 5.1S was observed for the ^3H -labelled receptor protein when mixed with the ovarian Triton X-100 extract. It is possible that the high amount of protein in the Triton X-100 extract disturbed the sucrose gradient, but the change may also indicate adherence of an additional component to the ^3H -labelled species. Dimerization of the M_r 90000 component could not have been responsible for the change observed.

The ^{125}I -choriogonadotropin-receptor complex sedimenting at 6.5S was crosslinked with glutaraldehyde in this study. The crosslinked complex migrated as a single M_r 130000 component, in

addition to the hormone and its α -subunit carrying the ^{125}I -label, when analysed by SDS gel electrophoresis (Fig. 5b). No larger components were observed, which argues against the idea of a dimeric receptor or an additional, non-hormone-binding component. Consistently, it was demonstrated that the M_r 130 000 complex sedimented identically to the native ^{125}I -choriogonadotropin-receptor complex obtained in soluble form from rat ovary by using Triton X-100 and exhibiting a sedimentation coefficient of 6.5S (Fig. 5). Accordingly, the ^{125}I -choriogonadotropin-receptor complex is not analogous with the ternary complex detected for the β -adrenergic receptor between agonist, the receptor, and a GTP-binding protein (Limbird & Lefkowitz, 1978; Limbird *et al.*, 1980).

The above M_r 130 000 for the ^{125}I -choriogonadotropin-receptor complex is much smaller than has been calculated from physical parameters by the use of the method of Siegel & Monty (1966) when a value 230 000 was derived (Dufau *et al.*, 1973). The Stokes' radii determined for the unoccupied receptor (6.2 nm) and the hormone-receptor complex (7.0 nm) in this study are in relation to those obtained by others (Dufau *et al.*, 1973, 1974; Charreau *et al.*, 1974) but do not correspond to the M_r values obtained by SDS gel electrophoresis, namely 90 000 and 130 000. Although the sedimentation coefficient of the ^3H -labelled receptor protein increased when mixed with the ovarian Triton X-100 extract, it was still smaller than that observed for the ^{125}I -choriogonadotropin-receptor complex exhibiting the M_r 130 000. The unduly large Stokes' radii are probably due to bound detergent. With non-denaturing detergents the amount of detergent bound to a membrane protein is often that of one micelle (Tanford & Reynolds, 1976; Helenius *et al.*, 1979), the M_r of which for Triton X-100 is about 90 000 (Kushner & Hubbard, 1954). It has been shown for the insulin receptor by sedimentation analysis in H_2O and $^2\text{H}_2\text{O}$ that about 90 000 of the M_r 440 000 receptor is accounted for by bound Triton X-100 (Siegel *et al.*, 1981). On the other hand, the glycoprotein nature of the receptor and its hormone complex may be responsible for the discrepancy between the Stokes' radii and the M_r values obtained by SDS gel electrophoresis.

Only 5–11% of the ^3H -labelled receptors were able to rebind to choriogonadotropin-Affi-Gel 10. Possibly denaturation of the receptors occurred at pH 4.0, but it may be that an additional factor is required for hormone binding, as suggested by the enlargement of receptor size when mixed with the ovarian Triton X-100 extract (Fig. 3). The yields of active receptor have been in the range of 10% for many cell-surface receptors in spite of better protein yields (Jacobs *et al.*, 1977; Kanellopoulos *et al.*, 1979; Waters & Friesen, 1979; Liscia &

Vonderhaar, 1982) although the β -adrenergic receptor has been recently purified with a 30% yield (Shorr *et al.*, 1982).

In conclusion, evidence was presented that the lutropin binding site is a single sialoglycopolypeptide exhibiting an apparent M_r 90 000. Although the possible requirement of an additional component for hormone binding could not be excluded, it was shown that the choriogonadotropin-receptor complex is composed solely of the M_r 90 000 polypeptide and the hormone, exhibiting an apparent M_r 130 000.

I thank Dr. Hannu Rajaniemi for valuable suggestions and discussions. I am grateful to Sirpa Jämsä for expert technical assistance. This work was supported by The Medical Research Council of the Academy of Finland and by The Aaltonen Foundation.

References

- Aubry, M., Collu, R., Ducharme, J. R. & Crine, P. (1982) *Endocrinology* **111**, 2129–2131
- Bhakdi, S. (1980) *J. Biochem. Biophys. Methods* **2**, 79–90
- Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88
- Bruner, R. & Vinograd, J. (1965) *Biochim. Biophys. Acta* **108**, 18–29
- Charreau, E. H., Dufau, M. L. & Catt, K. J. (1974) *J. Biol. Chem.* **249**, 4189–4196
- Dufau, M. L., Charreau, E. H. & Catt, K. J. (1973) *J. Biol. Chem.* **248**, 6973–6982
- Dufau, M. L., Charreau, E. H., Ryan, D. & Catt, K. J. (1974) *FEBS Lett.* **39**, 149–153
- Dufau, M. L., Ryan, D. W., Baukal, A. J. & Catt, K. J. (1975) *J. Biol. Chem.* **250**, 4822–4824
- Dulley, J. R. & Grieve, P. A. (1975) *Anal. Biochem.* **64**, 136–141
- Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617
- Gahmberg, C. G. & Andersson, L. C. (1977) *J. Biol. Chem.* **252**, 5888–5894
- Gahmberg, C. G., Häyry, P. & Andersson, L. C. (1970) *J. Cell Biol.* **68**, 642–653
- Helenius, A., McCaslin, D. R., Fries, E. & Tanford, C. (1979) *Methods Enzymol.* **56G**, 734–749
- Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496
- Jacobs, S., Shechter, Y., Bissell, K. & Cuatrecasas, P. (1977) *Biochem. Biophys. Res. Commun.* **77**, 981–988
- Ji, I. & Ji, T. H. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5465–5469
- Ji, I., Yoo, B. Y., Kaltenbach, C. & Ji, T. H. (1981) *J. Biol. Chem.* **256**, 10853–10858
- Kanellopoulos, J., Rossi, G. & Metzger, H. (1979) *J. Biol. Chem.* **254**, 7691–7697
- Kushner, L. M. & Hubbard, W. D. (1954) *J. Phys. Chem.* **58**, 1163–1167
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Leach, B. S., Collawn, J. F., Jr. & Fish, W. W. (1980) *Biochemistry* **19**, 5734–5741

- Liao, T.-H., Gallop, P. M. & Blumenfeld, O. O. (1973) *J. Biol. Chem.* **248**, 8247–8253
- Limbird, L. E. & Lefkowitz, R. J. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 228–232
- Limbird, L. E., Gill, D. M. & Lefkowitz, R. J. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 775–779
- Liscia, D. S. & Vonderhaar, B. K. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5930–5934
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Markkanen, S., Töllikkö, K., Jääskeläinen, K. & Rajaniemi, H. (1980) *Horm. Res.* **12**, 32–45
- Martin, R. G. & Ames, B. N. (1961) *J. Biol. Chem.* **236**, 1372–1379
- McEwen, C. R. (1967) *Anal. Biochem.* **20**, 114–149
- Metsikkö, K. & Rajaniemi, H. (1982) *Biochem. J.* **208**, 309–316
- Parlow, A. F. (1961) in *Human Pituitary Gonadotropins* (Albert, A., ed.), pp. 300–310, C. C. Thomas, Springfield, IL
- Rebois, R. V., Omedeo-Sale, F., Brady, R. O. & Fishman, P. H. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2086–2089
- Shorr, R. G. L., Heald, S. L., Jeffs, P. W., Lavin, T. N., Strohsacker, W. M., Lefkowitz, R. J. & Caron, M. G. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2778–2782
- Siegel, L. M. & Monty, K. J. (1966) *Biochim. Biophys. Acta* **112**, 346–362
- Siegel, T. W., Ganguly, S., Jacobs, S., Rosen, O. M. & Rubin, C. S. (1981) *J. Biol. Chem.* **256**, 9266–9273
- Tanford, C. & Reynolds, J. A. (1976) *Biochim. Biophys. Acta* **457**, 133–170
- Waters, M. J. & Friesen, H. G. (1979) *J. Biol. Chem.* **254**, 6815–6825