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The human growth hormone receptor of cultured human lymphocytes

Structural characteristics and glycosylation properties

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The structural characteristics and glycoprotein nature of the human growth hormone (hGH) receptor in cultured lymphocytes (IM-9 cell line) were studied with the use of a bifunctional reagent (disuccinimidyl suberate) to couple ^{125}I-hGH covalently to intact cells. After cross-linking, the hormone–receptor complexes were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. A single band of M_r 140000 was identified under reducing conditions. The labelling of this band was blocked by unlabelled hGH but not by insulin, ovine prolactin, bovine or ovine growth hormones. The M_r 140000 band was immunoprecipitated by either anti-hGH antibody or by a monoclonal antibody against rat liver growth hormone receptor. In the absence of reductant two major bands of M_r 270000 and 140000 were found. On two-dimensional gel electrophoresis, with the first dimension in the absence of reductant and the second in its presence, the M_r 270000 complex generated the M_r 140000 band. The nature of the oligosaccharide chains of the receptor was studied by treatment with different glycosidases. The electrophoretic mobility of the M_r 140000 receptor complex was markedly increased after digestion with endoglycosidase F but showed no or little change after digestion with endoglycosidase H. The M_r 140000 band was also sensitive to neuraminidase treatment. In addition the ^{125}I-hGH–receptor complex was adsorbed by immobilized wheat germ agglutinin and to a smaller extent by immobilized concanavalin A, lentil lectin, ricin I and ricin II. In conclusion, taking into account that hGH is a M_r 22000 polypeptide, the binding subunit of the GH receptor in human IM-9 lymphocytes has an M_r of approx. 120000. The native receptor may exist as a homodimer of the binding subunit formed by disulphide bonds. Furthermore, the GH receptor subunit contains asparagine *N*-linked type of oligosaccharide chains. Most, if not all, of these chains are of the complex type and appear to be sialylated whereas no high-mannose type chains are detectable in the mature form of the receptor.

INTRODUCTION

Human growth hormone (hGH) is required for normal linear growth in man. The precise mechanism by which this polypeptide hormone acts is unknown but it is clear, from direct binding studies and from analogy with other polypeptide hormones, that interaction with a cell-surface receptor is the initial step. Since human growth hormone receptors do not react with hormone from lower animal species, the IM-9 cultured human lymphocyte has been used extensively as a model system. It has been shown that: (*a*) IM-9 cells bind hGH with high affinity and specificity [1,2] and these features have been exploited to develop a sensitive and specific radioreceptor assay [3,4]; (*b*) the IM-9 lymphocyte receptor is regulated by hGH [5] (i.e. down-regulated); and (*c*) the hormone–receptor complex is internalized by a receptor-mediated process and the ligand degraded in lysosomes [6,7].

Limited information is available on structural features of the IM-9 hGH receptor. Hughes *et al.* [8] identified an M_r 130000 hormone–receptor complex after cross-linking of ^{125}I-hGH to intact IM-9 lymphocytes, although no further characterization of this complex was attempted. By contrast much more information is available about the structure and post-translational processing of the insulin receptor of IM-9 cells [9–11]. Using the insulin receptor as a model we have now characterized the IM-9 hGH receptor with respect to: (*a*) its structural features as analysed by SDS/polyacrylamide-gel electrophoresis, (*b*) the specificity of the binding subunit, and (*c*) its glycoprotein nature.

Portions of this work have been presented in abstract form in the proceedings of the 67th annual meeting of the Endocrine Society, Baltimore, MD, U.S.A. (abstract no. 274).

METHODS

Materials

Human growth hormone (lot AFP-4793B), hGH antiserum (rabbit, AFP-97720133), bovine GH, ovine GH and ovine prolactin were obtained from the National Pituitary Agency. Na^{125}I was purchased from International Chemical and Nuclear (Irvine, CA, U.S.A.). Porcine ^{125}I-insulin and endoglycosidase F

Abbreviations used: hGH, human growth hormone; GH, growth hormone; PMSF, phenylmethanesulphonyl fluoride.

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(endo-β-*N*-acetylglucosaminidase F, from *Flavobacterium meningosepticum*) were purchased from New England Nuclear (Boston, MA, U.S.A.). Disuccinimidyl suberate and ethyleneglycol bis(succinimidyl succinate) were purchased from Pierce Chemical Company (Rockford, IL, U.S.A.). RPMI 1640 culture medium and fetal bovine serum were from Biofluids (Rockville, MD, U.S.A.) and Flow Laboratories (McLean, VA, U.S.A.), respectively. Endoglycosidase H (endo-β-*N*-acetylglucosaminidase H, from *Streptomyces plicatus*) and wheat germ agglutinin–agarose were purchased from Miles Laboratories (Elkhart, IN, U.S.A.); other lectins coupled to agarose beads were purchased from E.-Y. Laboratories (San Mateo, CA, U.S.A.). Neuraminidase (*Vibrio cholerae*), α_2-macroglobulin and *Staphylococcus aureus* cells (Pansorbin) were purchased from Calbiochem Boehringer (La Jolla, CA, U.S.A.). Monosaccharides, PMSF, leupeptin, pepstatin A and rabbit anti-(mouse IgG) serum were from Sigma (St. Louis, MO, U.S.A.). Monoclonal antibodies against rabbit and rat liver growth hormone receptor were prepared as previously described [12,13]. All reagents for SDS/polyacrylamide-gel electrophoresis were from Bio-Rad (Richmond, CA, U.S.A.). Kodak X-Omat AR5 film was from Eastman Kodak Co. (Rochester, NY, U.S.A.) and Lightning Plus intensifying screens were from DuPont (Wilmington, DE, U.S.A.). All other materials were reagent grade.

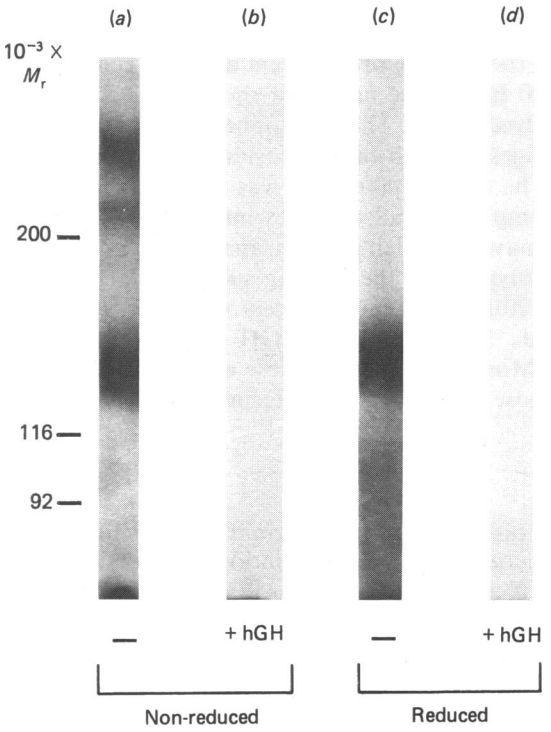

Fig. 1. ^{125}I-hGH cross-linking to IM-9 cells

IM-9 cells were incubated with ^{125}I-hGH for 90 min at 30 °C in the absence (lanes *a* and *c*) or the presence (lanes *b* and *d*) of unlabelled hGH. Cross-linking was carried out with disuccinimidyl suberate, and the samples were incubated in 0.25 M-sucrose containing 5 mM-*N*-ethylmaleimide for 15 min and solubilized in Triton X-100. Solubilized membranes were electrophoresed on a SDS/polyacrylamide (5%) gel under non-reducing (lanes *a* and *b*) or reducing (lanes *c* and *d*) conditions. Autoradiographs were obtained after drying the gels. Gels of this acrylamide concentration (5%), although necessary for separating the high-M_r complexes under non-reducing conditions, yielded quite broad and diffuse bands. A better resolution was achieved at higher acrylamide concentration (7.5%) and was used thereafter throughout this study to analyse the cross-linked receptors under reducing conditions (Figs. 2–7).

Cross-linking of ^{125}I-hGH to IM-9 lymphocytes

^{125}I-hGH (20–40 μCi/μg) was prepared by the chloramine-T method [2]. IM-9 cells were grown in suspension at 37 °C in RPMI 1640 medium supplemented with 10% fetal bovine serum. For cross-linking studies, 2.5×10^7 cells/ml were incubated in 50 mM-Hepes lymphocyte buffer [4] with 0.1% bovine serum albumin, pH 7.4, for 90 min at 30 °C, with ^{125}I-hGH (10–50 ng/ml) in the absence or presence of unlabelled hormone (1 μg/ml). Cells were centrifuged at 500 *g* and the pellet was washed in ice-cold 50 mM-Hepes lymphocyte buffer without albumin and resuspended in the same buffer. Disuccinimidyl suberate, dissolved in dimethyl sulphoxide, was added at a final concentration of 0.1 mM. After 30 min the reaction was stopped by 100 mM-Tris/10 mM-EDTA, pH 7.4. The cells were washed in phosphate-buffered saline and incubated for 15 min in 0.25 M-sucrose containing 5 mM-*N*-ethylmaleimide. They were solubilized in 50 mM-Hepes, pH 7.6, containing 0.15 M-NaCl, 1% Triton X-100, 2 M-PMSF and 5 mM-*N*-ethylmaleimide, and centrifuged at 100000 *g* for 45 min. The supernatant was precipitated with ice-cold 10% (w/v) trichloroacetic acid and the pellet was boiled for 5 min in 2% SDS/10% (v/v) glycerol/0.5 M-2-mercaptoethanol/0.01% Bromophenol Blue/10 mM-sodium phosphate buffer, pH 7.0 (electrophoresis sample buffer).

In other experiments 0.15 mM-ethyleneglycol bis(succinimidyl succinate) was used instead of disuccinimidyl suberate. This cross-linker is cleavable by incubation with 1 M-hydroxylamine in 10 mM-phosphate buffer, pH 8.5, for 5 h.

^{125}I-insulin (100–120 μCi/μg, 5–10 ng/ml) was cross-linked to IM-9 lymphocytes with disuccinimidyl suberate by following a similar protocol to that described for ^{125}I-hGH.

Immunoprecipitation of ^{125}I-hGH–receptor complex either by hGH antibody or by hGH receptor antibody

Aliquots (500 μl) of Triton X-100-solubilized cross-linked ^{125}I-hGH-receptor complex were incubated either with 5 μl of anti-hGH antiserum (1:50) or with control rabbit serum. After overnight incubation at 4 °C, 100 μl of *Staphylococcus aureus* cell suspension (Pansorbin) was added and the incubation was continued for 1 h at 4 °C. The immunoadsorbent was sedimented by centrifugation and washed three times with a solution containing 0.15 M-NaCl, 50 mM-Hepes, 1% Triton X-100 and 0.1% SDS, pH 7.4. After washing, the adsorbed immunocomplex was boiled for 5 min in electrophoresis sample buffer.

For immunoprecipitation with GH receptor monoclonal antibody, 1 ml of solubilized cross-linked ^{125}I-hGH– receptor complex was incubated with either GH receptor monoclonal antibody or control monoclonal antibody at 4 °C overnight; then 50 μl of second antibody [rabbit anti-(mouse IgG)serum] and 25 μl of normal mouse serum (1:50 dilution) were added, and the incu-

bation was continued for 10 h at 4 °C. The samples were centrifuged for 15 min at 8000 *g*, and the pellets were washed three times with 50 mM-Hepes buffer containing 0.1% Triton X-100, and boiled in electrophoresis sample buffer for 5 min.

Endoglycosidase H treatment

Triton X-100 solutions of ^{125}I-hormone–receptor complexes were precipitated with 10% (w/v) ice-cold trichloroacetic acid, washed in 10 mM-sodium phosphate buffer, pH 7.0, and dissolved by boiling for 3 min in 10 mM-sodium phosphate buffer, pH 7.0, containing 1% SDS and 10 mM-dithiothreitol. Following centrifugation, 50 μl aliquots of the supernatant were diluted in 200 μl of 0.3 M-sodium citrate buffer, pH 5.5. Endoglycosidase H was added to 0.5 unit/ml; although this preparation of endoglycosidase H was apparently free of protease activity, 1 mM-PMSF and 10 μM-pepstatin A were added to the samples. Enzymic digestion was performed for 6 h at 37 °C and then terminated by precipitation of the proteins with ice-cold 10% (w/v) trichloroacetic acid. The precipitates were washed in 10 mM-sodium phosphate buffer, pH 7.0, and dissolved in electrophoresis sample buffer.

Endoglycosidase F treatment

Cross-linked cells were solubilized in 1% Nonidet P-40 with 2 mM-PMSF, 1 mM-*N*-ethylmaleimide, 0.5 unit of α_2-macroglobulin/ml and 10 μg of leupeptin/ml. Solubilized samples were precipitated by 10% (w/v) ice-cold trichloroacetic acid. The pellets were washed in 0.1 M-sodium phosphate buffer, pH 6.1, containing 50 mM-EDTA and 1% Nonidet P-40 and boiled for 5 min in the same buffer supplemented with 1% SDS and 1% 2-mercaptoethanol. After centrifugation, 50 μl aliquots of the supernatant were incubated with endoglycosidase F (concentration 70–140 units/ml) with 2 mM-PMSF and 0.5 unit of α_2-macroglobulin/ml for 3 h at 37 °C. The reaction was terminated by precipitation of protein with 10% (w/v) trichloroacetic acid, and then the precipitates were washed in 0.01 M-sodium phosphate buffer, pH 7.0, and dissolved in electrophoresis sample buffer.

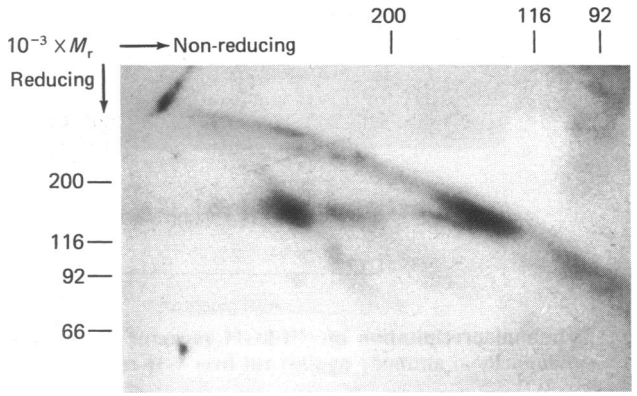

Fig. 2. Two-dimensional gel electrophoresis of ^{125}I-hGH cross-linking to IM-9 cells

Cross-linked ^{125}I-hGH–receptor complex was electrophoresed on a SDS/polyacrylamide (5%) gel under non-reducing conditions. The appropriate lane was cut from the gel, placed on top of a 7.5% polyacrylamide gel, and electrophoresed under reducing conditions. Autoradiography was performed after drying the gels.

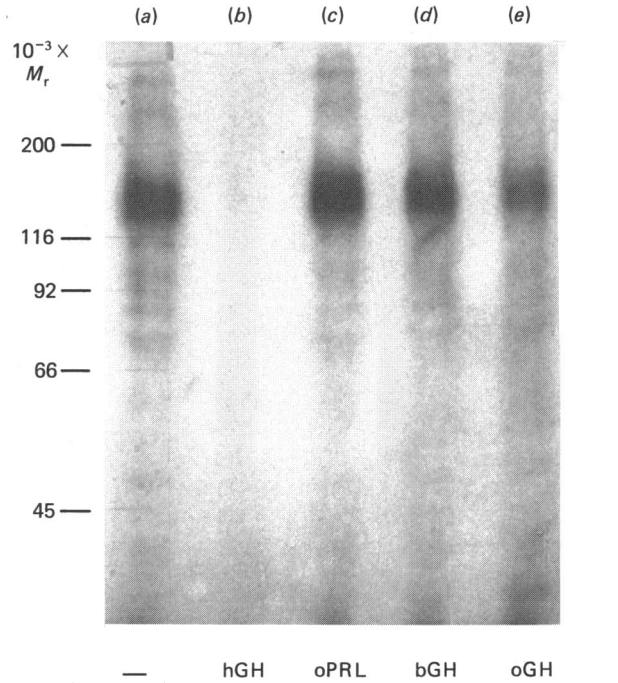

Fig. 3. Effects of growth hormones and prolactin on ^{125}I-hGH cross-linking to IM-9 cells

IM-9 cells were incubated with ^{125}I-hGH for 90 min at 30 °C in the absence (lane *a*) or the presence of human GH (1 μg/ml) (lane *b*), ovine prolactin (10 μg/ml) (lane *c*), bovine GH (10 μg/ml) (lane *d*) or ovine GH (10 μg/ml) (lane *e*). Cross-linking was carried out with disuccinimidyl suberate, and samples were solubilized and analysed by SDS/polyacrylamide (7.5%) gel electrophoresis under reducing conditions and autoradiography.

Lectin chromatography

Cross-linked ^{125}I-hormone–receptor complexes solubilized in Triton X-100 were chromatographed on lectin columns as previously described for the insulin receptor [14]. Briefly, the solubilized samples were applied to 2 ml of lectin–agarose; after extensive washing with 0.15 M-NaCl/50 mM-Hepes/0.1% Triton X-100, pH 7.6, bound material was eluted by addition to the buffer of the appropriate inhibitory sugars (0.3 M). Wheat germ columns were eluted with *N*-acetyl-D-glucosamine, concanavalin A and lentil columns with α-methyl-D-mannopyranoside, ricin I and II with β-methyl-D-galactopyranoside, soybean columns with *N*-acetyl-D-galactosamine, peanut columns with α-methyl-D-galactopyranoside, and *Ulex europaeus* I columns with L-fucose.

Gel electrophoresis and autoradiography

Samples were analysed on SDS/polyacrylamide-gel electrophoresis according to Laemmli [15]. The gels were stained for 5 min in 0.1% Coomassie Brilliant Blue dye dissolved in 50% methanol/7% acetic acid. The gels were destained in 10% methanol/7% acetic acid and dried. Autoradiography was carried out using Kodak X-Omat film with intensifying screens.

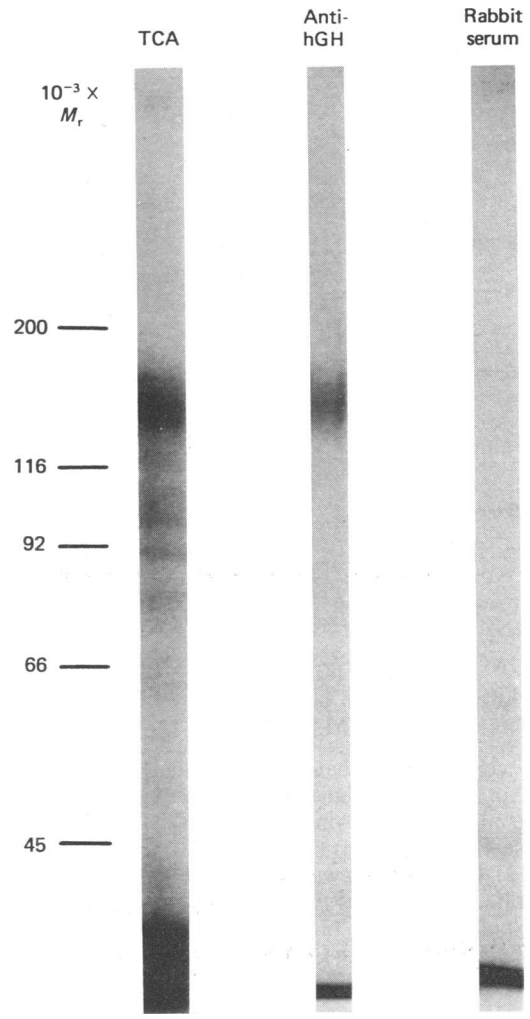

Fig. 4. Immunoprecipitation of ^{125}I-hGH–receptor complex by hGH antibody

IM-9 cells were cross-linked to ^{125}I-hGH with disuccinimidyl suberate, solubilized and immunoprecipitated with hGH antiserum or normal rabbit serum. The immunoprecipitates were analysed by SDS/polyacrylamide (7.5%) gel electrophoresis and autoradiography.

RESULTS

Structural characteristics of IM-9 hGH receptor

When IM-9 cells cross-linked to ^{125}I-hGH with disuccinimidyl suberate were treated with *N*-ethylmaleimide, solubilized, and electrophoresed in the absence of reductant, three bands were predominantly seen. The major band had M_r 140000, a second moderately radioactive band had M_r 270000, and a minor band migrated at M_r 230000 (Fig. 1). An excess of unlabelled hGH blocked the formation of all three components. In contrast, electrophoretic analysis in the presence of reductant revealed only a band of M_r 140000; the labelling of this band was also blocked by an excess of unlabelled hGH. To determine whether the high-M_r complex contained the M_r 140000 species, we employed two-dimensional gel electrophoresis. In the first dimension, the hGH–receptor complex was analysed without reductant; the appropriate lane of the gel was then placed on a second gel and analysed with reductant. The M_r 270000 band generated the M_r 140000 band, and the minor M_r 230000 band also contained the M_r 140000 band and a lesser amount of lower M_r components (Fig. 2). Thus, the M_r 270000 complex appears to represent the disulphide-linked homodimer of the M_r 140000 component. On the other hand, the M_r 230000 band represents probably proteinase cleavage of the M_r 270000 component.

Since it is known that hGH molecules may undergo disulphide interchange, we next wished to determine whether the disulphide link (i.e. the band cleaved by the reductant) was between ligand or receptor molecules. Using a cleavable cross-linker [ethyleneglycol bis(succinimidyl succinate)] we found that hGH monomer was liberated from both the M_r 270000 and 140000 bands, indicating that the disulphide bond exists between receptor molecules and not ligand molecules (results not shown).

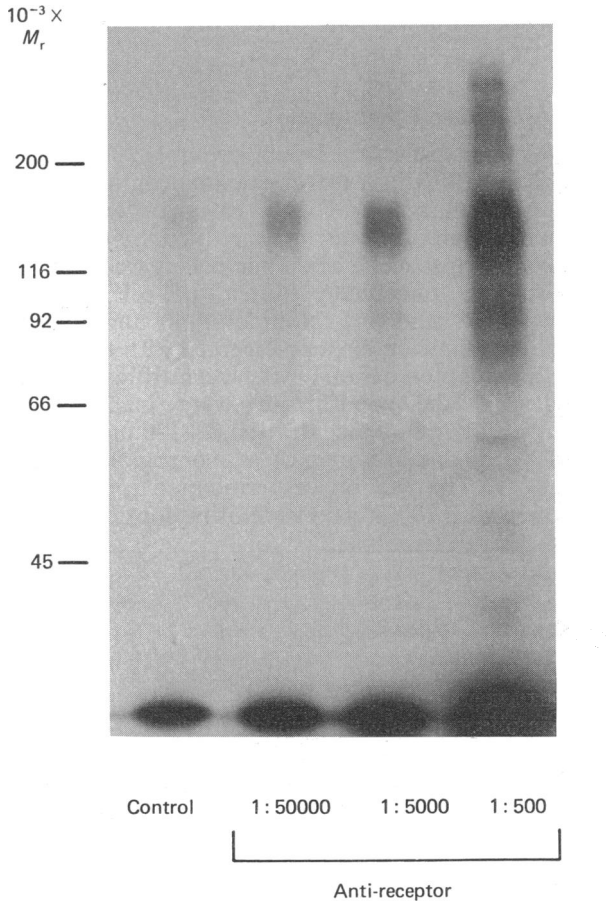

Fig. 5. Immunoprecipitation of ^{125}I-hGH–receptor complex by monoclonal antibody against rat liver GH receptor

IM-9 cells were incubated with ^{125}I-hGH for 90 min at 30 °C and cross-linking was carried out with disuccinimidyl suberate. The cross-linked samples were solubilized and incubated with monoclonal antibody against purified GH receptor from rat liver membranes overnight at 4 °C. The immunocomplexes were precipitated with a second antibody and analysed by SDS/polyacrylamide (7.5%) gel electrophoresis under reducing conditions and autoradiography.

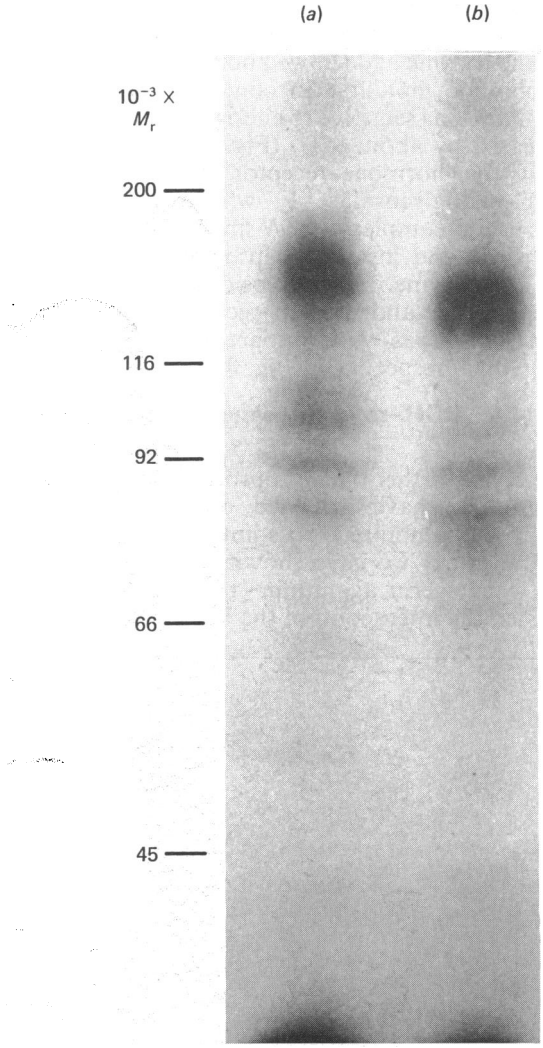

Fig. 6. Neuraminidase treatment of ^{125}I-hGH–receptor complex

IM-9 cells cross-linked with ^{125}I-hGH were treated with 0.025 unit of neuraminidase/ml in Dulbecco's phosphate-buffered saline for 10 min at 37 °C and washed. The complex was solubilized and analysed by SDS/polyacrylamide (7.5%) gel electrophoresis and autoradiography. Lane *a*, no treatment; lane *b*, neuraminidase treatment.

Specificity of the M_r 140000 hGH receptor subunit

To study the binding specificity of the hGH-receptor monomer, IM-9 cells were incubated with ^{125}I-hGH in the presence of hGH, ovine prolactin, bovine growth hormone and ovine growth hormone. hGH blocked the formation of the M_r 140000 band in a dose-dependent fashion. By densitometry the amount of the M_r 140000 band was reduced from 100 to 63, 33, and 0% of control values in the presence of 0, 20, 100 and 1000 ng of hGH/ml, respectively (results not shown). Ovine GH and bovine GH (10 μg/ml) slightly inhibited the production of the M_r 140000 band, but 10 μg of ovine prolactin/ml had no effect (Fig. 3). Further, insulin did not inhibit the production of the hGH–receptor complex and hGH did not affect the cross-linking of insulin to its receptor (results not shown).

Immunoprecipitation of hGH–receptor complex

When the ^{125}I-hGH–receptor complex was incubated with anti-hGH serum at 1:5000 dilution, the M_r 140000 band was immunoprecipitated with a high degree of specificity (Fig. 4). Since monoclonal antibodies have recently been prepared against the rabbit liver membrane GH receptor [12], we wished to determine whether these monoclonal antibodies were able to immunoprecipitate the human ^{125}I-hGH–receptor complex. Eight antibodies were tested and none demonstrated inhibition of hormone binding or capacity to immunoprecipitate the receptor. However, a ninth (263) raised against rat liver GH receptors was able to react with the human IM-9 receptor. ^{125}I-hGH cross-linked to IM-9 cells was incubated with monoclonal antibody (MAb 263) and precipitated by using a second antibody. The M_r 140000 receptor complex was immunoprecipitated by this monoclonal antibody at 1:500–1:50000 dilution, but not by control antibody (Fig. 5).

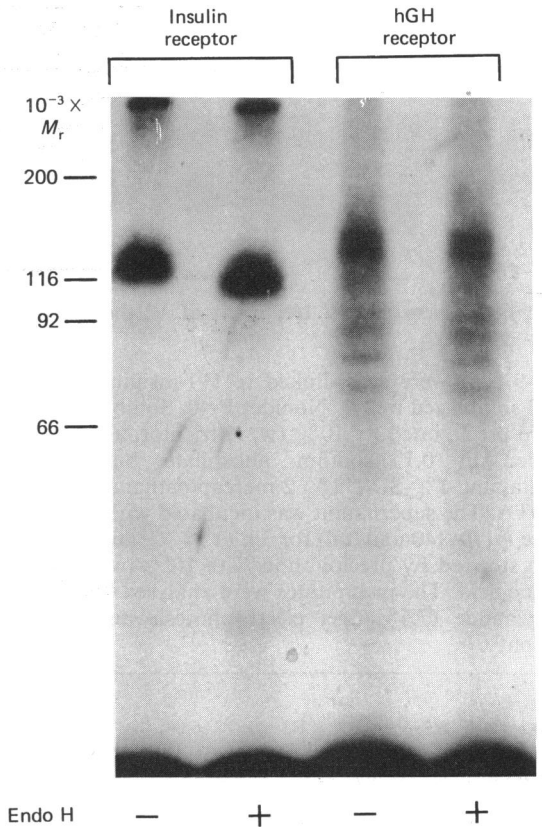

Fig. 7. Endoglycosidase H treatment of ^{125}I-hormone–receptor complex

IM-9 cells were cross-linked to ^{125}I-insulin or ^{125}I-hGH and solubilized in 1% Triton X-100. Solubilized samples were precipitated with 10% (w/v) trichloroacetic acid and boiled in 10 mM-sodium phosphate buffer, pH 7.0, containing 1% SDS and 10 mM-dithiothreitol. The supernatant was diluted (1:5) in 0.3 M-sodium citrate buffer, pH 5.5, and incubated with endoglycosidase H (0.5 unit/ml) for 6 h at 37 °C. The reaction was stopped by precipitation with 10% (w/v) trichloroacetic acid and the precipitates were dissolved in sample electrophoresis buffer and analysed by SDS/polyacrylamide (7.5%) gel electrophoresis and autoradiography.

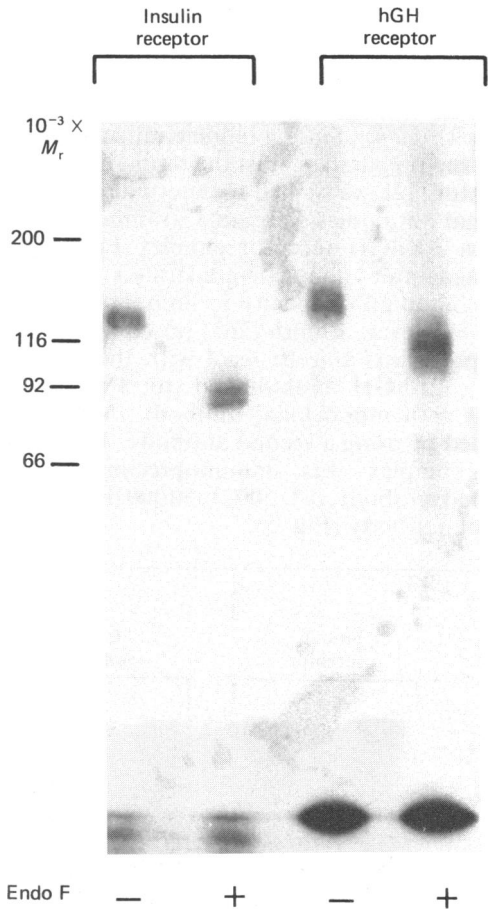

Fig. 8. Endoglycosidase F treatment of ^{125}I-hormone–receptor complex

IM-9 cells were cross-linked to ^{125}I-insulin or ^{125}I-hGH and solubilized in 1% Nonidet P-40. Solubilized samples were precipitated by 10% (w/v) trichloroacetic acid and boiled in 0.1 M-sodium phosphate buffer, pH 6.1, containing 1%-SDS, 1% 2-mercaptoethanol and 50 mM-EDTA. The supernatant was incubated with endoglycosidase F (70–140 unit/ml) for 3 h at 37 °C, and the reaction was stopped by precipitation with 10% (w/v) trichloroacetic acid. The precipitates were analysed by SDS/polyacrylamide (7.5%) gel electrophoresis and autoradiography.

Digestion of hGH–receptor complex with glycosidic enzymes

To investigate the nature of the carbohydrate side chains of the hGH receptor, the effect of three separate enzyme digestions was studied. First the ^{125}I-hGH–receptor complex was incubated with neuraminidase and its electrophoretic mobility was determined. Following neuraminidase digestion the M_r 140000 receptor complex migrated with M_r 125000 (Fig. 6). This suggests that the receptor contains terminal sialic acid residues which are accessible to the enzyme.

In order to determine the presence of *N*-linked high-mannose and/or complex-type oligosaccharide chains, cross-linked ^{125}I-hGH–receptor complex was treated with either endoglycosidase H or endoglycosidase F. For the purpose of comparison cross-linked ^{125}I-insulin–receptor complex was also treated with these enzymes. When solubilized hGH–receptor complex was treated with endoglycosidase H, the complex was resistant to the enzyme and showed no or little change in the electrophoretic mobility. In contrast, the insulin–receptor complex was sensitive to endoglycosidase H and its M_r decreased by about 9000 (Fig. 7). On the other hand, when the hormone–receptor complexes were treated with endoglycosidase F, which cleaves both high-mannose and complex-type *N*-linked oligosaccharides, both hGH– and insulin–receptor complexes were sensitive to the enzyme. Endoglycosidase F altered the mobility of hGH– and insulin–receptor complexes, resulting in complexes with apparent M_r values of 112000 and 90000 respectively (Fig. 8).

Adsorption of the hGH–receptor complex to immobilized lectins

To characterize further the glycoprotein pattern of the hGH receptor we have extended our study of the adsorption of the solubilized cross-linked hGH receptor to lectins. Previously we have shown that the complex adsorbs to wheat germ agglutinin [16]. In the present study we tested the interaction of the ^{125}I-hGH–receptor

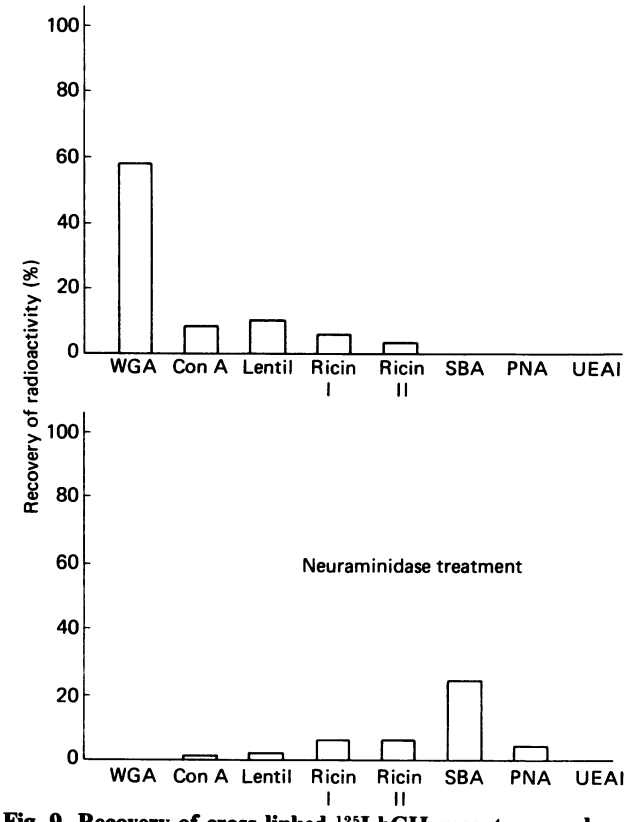

Fig. 9. Recovery of cross-linked ^{125}I-hGH–receptor complex on immobilized lectin columns

IM-9 cells were cross-linked to ^{125}I-hGH, solubilized and applied on lectin columns. The columns were washed and eluted with the appropriate monosaccharides. The radioactivity in the eluate was plotted as a percentage of the total applied. Upper panel, recoveries in the absence of prior neuraminidase treatment; lower panel, recoveries after neuraminidase treatment of the cells. Key: WGA, wheat germ agglutinin; Con A, concanavalin A; SBA, soybean agglutinin; PNA, peanut agglutinin; UEAI, *Ulex europaeus* agglutinin I.

complex with a panel of eight different immobilized lectins (Fig. 9). We confirmed the adsorption of the complex by wheat germ agglutinin and found only a very moderate interaction with concanavalin A, lentil, ricin I and ricin II lectins. Prior neuraminidase treatment of the receptor complex abolished the interaction with wheat germ agglutinin but had little effect on the reactivity with other lectins, except for soybean and peanut agglutinins which showed now a small degree of adsorption of the receptor complex.

DISCUSSION

In this work we have investigated the structure of the hGH receptor in human IM-9 lymphocytes with the use of bifunctional reagents to couple ^{125}I-hGH covalently to intact cells and subsequent analysis of the complexes on SDS/polyacrylamide-gel electrophoresis. A single band of apparent M_r 140000 was identified under reducing conditions. In the absence of reductant two major bands of M_r 270000 and M_r 140000 were found. Thus, our data indicate that the M_r 140000 band represents the binding subunit of the hGH receptor in IM-9 lymphocytes and that this subunit can dimerize via disulphide bonds. It is possible that the native receptor in the membrane may exist in both monomeric and dimeric forms. Taking into account that hGH is a single polypeptide chain of M_r 22000, it can be estimated that the true M_r of the hGH receptor is probably closer to 120000. Obviously these values should be considered with caution given the anomalous behavior of glycoproteins on SDS/polyacrylamide gels. As is frequently observed with glycoproteins, the GH receptor migrated on SDS/acrylamide gels as a broad band. Occasionally (Figs. 3, 7 and 8) a doublet pattern can be observed, which suggests that the heterogeneity of this band may be due, in great part, to two major species.

Our results on the structure of the hGH receptor of IM-9 lymphocytes are in good agreement with those reported by other authors in rat adipocytes [17,18] and in rat hepatocytes [19]. These authors have also found the hGH receptor to exist in monomeric and dimeric forms with similar M_r values to those reported here. On the other hand, our results differ somewhat from those of Hughes *et al.* [8] who found only an M_r 130000 monomeric form of the hGH receptor in IM-9 lymphocytes, both in the presence and the absence of reductant. The failure to detect the M_r 270000 complex is probably due to the fact that these authors used only 9% acrylamide gels, which make the resolution of such a high-M_r band very difficult.

In contrast with the similarities of the structural features of the hGH receptor in human IM-9 lymphocytes, rat hepatocytes and rat adipocytes, the hGH receptor of rabbit liver membranes appears to be strikingly different. Although estimates of the native receptor range from M_r 200000 [20] to 300000 [21], several authors have reported that the monomeric form of GH receptor in this tissue has M_r 60000–80000 [8,21–23]. It cannot be determined at present whether this is truly a species difference or a difference in methodology, although the latter is unlikely since Hughes *et al.* [8] found a similar M_r for the IM-9 receptor as reported here. Actually, a species difference would not be surprising given the well-documented differences in binding specificities for different somatotropic and lactogenic peptides that the GH receptor of these tissues exhibit [2]. In fact, our results also show that eight different monoclonal anti-(rabbit liver GH receptor) antibodies failed to react with the human receptor, while one antibody raised against rat liver receptor did immunoprecipitate the M_r 140000 complex. This antibody is also capable of immunoprecipitating the GH receptor and of inhibiting hGH binding in all three tissues: rat liver, rabbit liver and human lymphocytes [13]. Therefore, despite some major differences among the GH receptors of these species, some regions of the molecule close to the binding site are highly conserved.

The hGH receptor is believed to be a glycoprotein based on the effect of tunicamycin and some glycosidases on hGH binding [21,23,24] as well as on the ability of concanavalin A and wheat germ agglutinin to adsorb the solubilized receptor [16,20,21]. In the present study we have examined the nature of the oligosaccharide chains attached to the M_r 140000 receptor complex. Treatment with endoglycosidase F, an enzyme which cleaves *N*-linked oligosaccharides, induces a marked decrease in the apparent molecular size of the M_r 140000 band, demonstrating clearly the presence of asparagine *N*-linked carbohydrate chains. It is well known that all types of *N*-linked carbohydrate chains in mammalian cells are synthesized via a common high-mannose oligosaccharide ($Glc_3Man_9GlcNAc_2$) which is transferred co-translationally via a lipid carrier [25]. Subsequently these core oligosaccharides are subjected to the action of several glycosidases and glycosyltransferases and some of them may be converted to complex type [26]. In our experiments, treatment with endoglycosidase H, an enzyme which cleaves only high-mannose type oligosaccharides, induced no detectable change in the electrophoretic mobility of the hGH receptor; under the same experimental conditions, a clear decrease was observed in the apparent M_r of the insulin receptor, a glycoprotein known to contain both high mannose and complex type chains in its mature form [11]. On the other hand, treatment with neuraminidase clearly decreased the apparent M_r of the M_r 140000 complex. Therefore it can be concluded that the hGH receptor contains asparagine-linked oligosaccharides, most of which, if not all, are of the complex type. Furthermore a significant number of these complex type chains appear to contain sialic acid as the terminal sugar. This glycosylation pattern is also supported by our results with a panel of different immobilized lectins. The fact that wheat germ agglutinin was the most potent lectin in adsorbing the receptor, an effect which was abolished by prior neuraminidase treatment, together with the relative weak capacity of concanavalin A, lentil, ricin I and ricin II, are all suggestive of the presence of mostly sialylated complex type of carbohydrate chains.

The present results do not demonstrate or exclude the possible additional presence of *O*-linked type of oligosaccharide chains in the hGH receptor. The ^{125}I-hGH–receptor complex was weakly adsorbed after neuraminidase treatment by peanut agglutinin. This finding might suggest the presence of *O*-linked carbohydrate in the hGH receptor, since peanut agglutinin has a strong affinity for the disaccharide Gal-GalNAc, which is frequently present in *O*-linked oligosaccharide capped with sialic acid [27].

The comparison of the glycosylation pattern of the hGH receptor of human IM-9 lymphocytes with that of

another hormone receptor, the insulin receptor, which has been studied in some detail in the same cell type [9,11], shows interesting similarities and differences. Both receptors are *N*-glycosylated and, although presumably synthesized in a similar site and exported to a common destination, the plasma membrane, their degree of carbohydrate processing differs. The hGH receptor appears to be more extensively processed to complex type whereas the mature insulin receptor contains both complex and high-mannose chains [11]. This difference supports the view that the protein structure itself is the major determinant of the degree of carbohydrate processing.

Finally, the use of a monoclonal antibody which is capable of immunoprecipitating the hGH receptor, together with biosynthetic labelling techniques, should enable us to explore the life cycle of the hGH receptor with a greater scope than that allowed by the use of cross-linking bifunctional agents.

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