Characterization of Hepatic Lactogen Receptor

SUBUNIT COMPOSITION AND HYDRODYNAMIC PROPERTIES*

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The structure of the membrane-bound and Triton X-100-solubilized female rat liver prolactin receptor has been studied by affinity cross-linking/sodium dodecyl sulfate-polyacrylamide gel electrophoresis, gel filtration, and sucrose/H2O and sucrose/D2O density gradient centrifugation. Hydrodynamic characterization revealed that the 125I-human growth hormone receptor-detergent complex represents a molecular species with a Stokes radius of 61 A, a sedimentation coefficient of 5.0 s, and a calculated molecular weight of 158,000. The molecular weight of the receptor was calculated to be 92,000. Three lactogenic hormone-binding species with M, values of 87,000, 40,000, and 35,000, respectively, were repeatedly found when detergent-solubilized preparations were analyzed using an affinity cross-linking technique. Estrogen treatment of female rats increased the intensity of these bands. Occasionally, an M, 165,000 hormone-binding species was also found. Two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis studies (first dimension, nonreducing; second dimension, reducing) demonstrated that disulfide- and nondisulfide-linked hormone-binding species with M, values of 40,000 and 35,000 are contained within the M, 87,000 species. It is concluded that the Triton X-100-solubilized female rat liver prolactin receptor has a molecular weight of about 90,000. This molecular species contains M, 40,000 and M, 35,000 hormone-binding subunits. It cannot be determined whether these subunits are combined with each other or with as yet undetected subunit(s) to make up the M, 90,000 species, or whether each one of these subunits is a proteolytic fragment of the M, 90,000 species.

The presence of specific binding sites for the lactogenic hormone prolactin in rat liver is well documented (1-4). Hepatic lactogenic receptors are present in both sexes, but at a much higher concentration in female liver (1, 3). This sex difference is regulated by gonadal steroids as well as hypothalamic and hypophyseal factors (5-11). A clear-cut role for prolactin and the lactogenic receptor in regulating liver function has not yet been demonstrated, although reports have appeared showing prolactin-induced responses in liver (12-16).

Characterization of the prolactin-binding entities in rat liver has shown that they are membrane-bound glycoproteins (17, 18). Structural data for the receptors have been obtained by the use of different techniques such as gel filtration and sucrose density gradient centrifugation as well as affinity cross-linking of iodinated hormone and receptor combined with SDS-PAGE1 and autoradiography. The latter method has revealed specific binding species with M, values ranging from 28,000 to 83,000 (19-23). No conclusive data have yet been presented ruling out a possible relation between the lower and higher molecular weight species. By hydrodynamic characterization of detergent-solubilized rat liver membrane preparations, molecular weights of 73,000 and 78,000 for the lactogenic receptor could be calculated (24, 25).

Using affinity cross-linking, molecular characteristics for the lactogenic receptor in rat tissues other than the liver have been examined. In the ovary two specific binders with molecular weights of 80,000 and 40,000, respectively, were found (26). Results from experiments employing a two-dimensional electrophoretic technique indicated that an M, 40,000 binder is contained within the M, 80,000 binder. Affinity cross-linking has also been used to investigate the molecular characteristics of lactogenic binders in Leydig cells, mammary gland, and kidney (23). The molecular weights presented for specific lactogenic binders were: 91,000, 81,000, 37,000 and 31,000 (Leydig cells); 93,000, 83,000, 30,000, and 28,000 (mammary gland); and 65,000 and 30,000 (kidney). It was also shown that the lactogenic binders in Leydig cells are similar to the binders in the ovary with respect to an M, 37,000 species being contained within the M, 81,000 species. In lactogenic hormone target tissues in other animals, such as pig and rabbit mammary gland, prolactin-binding species with molecular weights ranging from 28,000 to 69,000 have been described (22, 27-29).

Earlier studies in our laboratory have shown that the sexually differentiated content of prolactin receptors in rat liver is regulated by the sexually differentiated secretory rhythm of growth hormone (30). In order to study this regulation at a molecular level, a knowledge of the structure of the prolactin receptor in liver is needed. This knowledge would also help in the purification of this receptor.

In an attempt to elucidate the structure of the lactogenic receptor in rat liver, we have undertaken an investigation involving analysis of 125I-hGH-binding species by affinity cross-linking, gel filtration, and sucrose density gradient centrifugation.

1 The abbreviations used are: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; BSA, bovine serum albumin; CHAPS, 3-[3-cholamidopropyl]-dimethylammonio]-1-propanesulfonate; DSS, disuccinimidyl suberate; SDS, sodium dodecyl sulfate; HGH, human growth hormone; rPRL, ovine prolactin; bGH, bovine growth hormone; rGH, rat growth hormone; rPRL, rat prolactin; PBS, phosphate-buffered saline.
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\begin{figure}
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\includegraphics[width=\textwidth]{figure4.png}
\caption{Specificity of cross-linking of 125I-hGH to acid-precipitated redissolved receptors. Acid precipitated (pH 4.9) solubilized proteins were incubated with 125I-hGH (3 × 10^6 cpm) in the absence or presence of a 50- or 500-fold excess of different unlabeled hormones at 20 °C for 18 h. Cross-linking was performed with 0.5 mM DSS at 4 °C for 15 min. Aliquots were taken for SDS-PAGE, performed on 3-mm 7.5% polyacrylamide gels under reducing conditions. An autoradiogram of a dried gel is shown. The positions of the 14C-labeled molecular weight markers are shown on the left. The positions of the major labeled species are indicated on the right. rPRL, rat prolactin; bGH, bovine growth hormone.
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Materials and Methods

In this study hGH (Crescormon) and biosynthetic methionyl somatotropin (Somatonom) have been used for labeling specific lactogenic-binding sites in different preparations of female rat liver. No quantitative or qualitative differences were observed between these hormones. In the following text hGH will be used to designate both hormones.

Affinity Cross-linking Studies—The structure of the lactogenic receptor was studied by affinity cross-linking of 125I-hGH to binding species present in microsomal membranes or acid-precipitated, redissolved preparations of Triton X-100-solubilized membranes, followed by SDS-PAGE and autoradiography.

The specificity of cross-linking 125I-hGH to acid-precipitated, redissolved preparations was determined by competition studies with different unlabeled hormones (Fig. 4). The specific bands at Mr 109,000, 62,000, and 57,000 were completely reduced with hGH (Fig. 4, lanes B and C). A reduction of these bands was also seen with the higher concentration of rat prolactin (Fig. 4, lanes D and E). Bovine growth hormone did not affect the appearance of these bands (Fig. 4, lanes F and G). A weak band at Mr 187,000 was also seen in this experiment. It was reduced with hGH and a 500-fold excess.

Results

The results of the experiments are presented in Figs. 1-3, 10, and 11, and Tables 1 and 2. The positions of the major labeled species are indicated on the right. rPRL, rat prolactin; bGH, bovine growth hormone.

Cross-linking Studies of Solubilized Receptors—Acid-precipitated solubilized proteins (lanes A and B) were prepared from either untreated or estradiol (E2)-treated female rats as described under "Materials and Methods." These preparations (250 μg of protein) were incubated with 125I-hGH (2.5 × 10^6 cpm) at 20 °C for 18 h. Cross-linking was performed with 0.5 mM DSS at 4 °C for 15 min. Aliquots were taken for SDS-PAGE, performed on 1.5-mm 7.5% polyacrylamide gels under reducing conditions. An autoradiogram of a dried gel is shown. The positions of the 14C-labeled molecular weight markers are shown on the left. The positions of the major labeled species are indicated on the right.

Cross-linking of 125I-hGH to acid-precipitated microsomal membranes revealed three lactogenic hormone-binding bands at Mr 109,000, 62,000, and 57,000 and one somatogenic band at Mr 187,000. The same lactogenic bands are also seen when microsomal membranes are treated with the detergent CHAPS. Due to the absence of the somatogenic binding species, the acid-precipitated preparation of Triton X-100-solubilized microsomal membranes was used in this study.

The cross-linking studies presented in this article were performed with preparations of livers from estrogen-treated female rats. To examine the effect of estradiol treatment on the intensities of the individual bands seen upon autoradiography, preparations were obtained from livers of treated and untreated female rats. These preparations, containing equal amounts of protein, were incubated with equal amounts of 125I-hGH, cross-linked with 0.5 mM DSS, and subjected to SDS-PAGE under reducing conditions (Fig. 5). The intensity of the bands at Mr 109,000, 62,000, and 57,000 were markedly increased when the preparation was taken from livers of estrogen-treated female rats (Fig. 5, lanes A and B). A possible relationship between the different lactogenic hormone-binding species seen on autoradiograms was first investigated by comparing cross-linked samples run on SDS-PAGE under reducing or nonreducing conditions. It was shown that, in the absence of reductant, the intensities of the specific bands at Mr 62,000 and 57,000 were reduced and that the intensities of the bands at Mr 109,000 and 187,000 were increased as compared to the autoradiographic appearance of cross-linked samples run under reducing conditions (Fig. 6). This implies a possible relationship between the lower set of

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\footnote{3 L.-A. Haldosén and J.-Å. Gustafsson, manuscript submitted.}
To examine this further, the $^{125}$I-hGH-incubated and cross-linked acid-precipitated and redissolved preparation was investigated by two-dimensional SDS-PAGE in which the first and second dimensions were run in the absence or presence of reductant, respectively. As can be seen in the autoradiogram, the diagonal form contains three radioactive spots at $M_r$ 109,000, 62,000, and 57,000 (Fig. 7). Two spots with $M_r$ 62,000 and 57,000 are also seen straight below the spot at $M_r$ 109,000, implying that they are derived from the higher molecular weight spot. Unfortunately, in several experiments, we were unable to detect any spot at $M_r$ 187,000.

The presence of a radioactive spot at $M_r$ 109,000 in the two-dimensional SDS-PAGE could be the result of intermolecular cross-linking of possible subunits of lactogenic receptor in addition to cross-linking of $^{125}$I-hGH with subunit(s). To test this hypothesis the following experiment was performed. A $^{125}$I-hGH-incubated microsomal membrane preparation was cross-linked with DSS, and after Triton X-100 solubilization of membrane proteins the sample was divided into two portions. One of these was again subjected to cross-linking by DSS. SDS-PAGE was then run under reducing conditions. As can be seen in the autoradiogram (Fig. 8, lanes A and B), cross-linking before Triton X-100 solubilization produced a specific band at $M_r$ 62,000. However, if cross-linking was also performed after detergent treatment, a specific band at $M_r$ 109,000 was seen in addition to the $M_r$ 62,000 band (Fig. 8, lanes C and D). It should be noted that no band at $M_r$ 57,000 was seen in the Triton X-100-solubilized membrane preparation in this experiment. These results suggest that the binder giving rise to the $M_r$ 57,000 band is not exposed in the membrane preparation and thus cannot be cross-linked with $^{125}$I-hGH. Treatment of membranes with detergents has been shown to expose a population of masked or cryptic lactogenic receptors for binding (39-42).

To test this hypothesis, the $^{125}$I-hGH-incubated microsomal membrane preparation was treated with Triton X-100 after removal of unbound hormone by centrifugation. This preparation was divided into two samples, one of which was reincubated with $^{125}$I-hGH. Both samples were cross-linked with DSS and subjected to SDS-PAGE. The autoradiogram shows that reincubation of the Triton X-100-solubilized membrane preparation gives rise to an $M_r$ 57,000 band in addition to the $M_r$ 109,000 and 62,000 bands (Fig. 9, lane C). A specific band
from Scatchard analyses of $^{125}$I-hGH binding in Triton-phosphate-buffered saline containing 1% (v/v) Triton X-100. After 1 h preparations positions of the major labeled species are indicated on the was found at a pH of 4.9. Chromatofocusing of unlabeled rat was lowered, it was found that the solubilized proteins had a tions. An autoradiogram of a dried gel is shown. The positions of the with PI values of 5.0 and 5.9 (48). From these studies it might be concluded that the decreased solubility at pH values of 4-6 of the Triton X-100-solubilized specific binding seen in our study could be an isoelectric precipitation phenomenon. As judged from Scatchard analyses of $^{125}$I-hGH binding in Triton X-100-solubilized and acid-precipitated redissolved preparations, an increased binding capacity was found in the latter preparation. This could either be due to enrichment of lactogenic receptor, dissociation of endogenous hormone from the receptor, or a combination of both. Ovine prolactin specifically bound to rabbit mammary membrane receptor has been shown to dissociate in a pH-dependent manner with a pK of 4.7 (49).

When the redissolved precipitate was incubated with $^{125}$I-hGH and subjected to cross-linking with DSS and run on SDS-PAGE, three lactogenic bands with $M_r$ 109,000, 62,000, and 57,000 were seen. Assuming a 1:1 binding ratio and an $M_r$ of 22,000 for hGH, the $M_r$ values of the unoccupied binders would be 87,000, 40,000, and 35,000, respectively (Fig. 12).

The finding of increased intensity of the $M_r$, 109,000 band and decreased intensity of the $M_r$, 62,000 and 57,000 bands in unreduced samples as compared to reduced samples suggested a direct relationship between the bands. In line with this notion, two-dimensional electrophoresis indicated that two binders with $M_r$ values of 40,000 and 35,000 are contained within the $M_r$, 87,000 binder (Fig. 12) and that these binders are disulfide-linked each to either other or to another protein present in the $M_r$, 87,000 binder. In addition, two labeled species with $M_r$ values of 62,000 and 57,000 were demonstrated along the diagonal seen in the autoradiogram, indicating that these binders with $M_r$ values of 40,000 and 35,000 are not disulfide-linked. From these results it might also be deduced that there exist two populations of $M_r$, 87,000 binders, one in which the $M_r$, 40,000 and 35,000 subunits are disulfide-linked and one population in which they are not.

When running $^{125}$I-hGH-incubated and cross-linked microsomal membrane preparations on SDS-PAGE, under both reducing or nonreducing conditions, only one specific binder with an $M_r$ of 40,000 is seen. Due to the presence of a binder with an $M_r$ of 40,000 that is disulfide-linked, it can be concluded that detergent treatment exposes a binder with this molecular weight in addition to the nondisulfide-linked $M_r$, 40,000 binder present in the membrane.

The two-dimensional electrophoresis experiment also indicated that there might exist an $M_r$, 87,000 binder from which no subunits can be released under reducing conditions, but as seen in Fig. 8, the existence of this "refractory" binder is probably due to intermolecular cross-linking between subunits. It is interesting to note that this intramolecular cross-

![Fig. 9. Effect of reincubation of Triton X-100-treated $^{125}$I-hGH-incubated microsomal membranes. A microsomal membrane preparation was incubated with $^{125}$I-hGH (3.5 x $10^5$ cpm) in the absence or presence of a 100-fold excess of unlabeled hGH at 20 °C for 18 h. The samples were then divided into two sets of tubes and centrifuged for 20 min at 10,000 x g in an Eppendorf centrifuge to remove unbound $^{125}$I-hGH. The pellet was resuspended in phosphate-buffered saline containing 1% (v/v) Triton X-100. After 1 h $^{125}$I-hGH (3.5 x $10^5$ cpm) was added to one set of the solubilized preparations (lanes C and D). Unlabeled hGH was added to the tube which contained solubilized microsomal membranes incubated with $^{125}$I-hGH in the presence of unlabeled hGH (lane D). To the other set of tubes an equivalent volume of buffer was added (lanes A and B). After 6 h of incubation cross-linking was performed with 0.5 mM DSS at 4 °C for 15 min. Aliquots were taken for SDS-PAGE, performed on 1.5-mm 7.5% polyacrylamide gels under reducing conditions. An autoradiogram of a dried gel is shown. The positions of the $^4$C-labeled molecular weight markers are shown on the left. The positions of the major labeled species are indicated on the right.

Fig. 12. A compilation of values for molecular weights, sedimentation coefficient, and Stokes radius for $^{125}$I-hGH-specific binder(s) in different preparations of rat liver as obtained by various methods. The abbreviations used in this figure are: G.F., gel filtration on Sephacryl S-400; S.D.G. (H,O), sucrose/H,O density gradient centrifugation; S.D.G. (H$_2$O/D$_2$O), sucrose/H$_2$O and sucrose/D$_2$O density gradient centrifugation; 61A, fraction of Sephacryl S-400 chromatography (elute that was further analyzed); 2D, two-dimensional SDS-PAGE; R.I., reincubation with $^{125}$I-hGH; L, lactogenic binding activity; S, somatogenic binding activity.
linking does not occur when microsomal membrane preparations are used. Thus, detergent treatment either brings subunits into favorable position for cross-linking, exposes suitable sites for cross-linking due to disruption of membrane vesicles, or removes membrane lipids surrounding the proteins which might hinder cross-linking. The results in Fig. 8 also indicate that the M, 40,000 binder present in membrane preparation is associated with an unlabeled subunit to which it can be cross-linked only when Triton X-100-solubilized (Fig. 13a).

The possibility exists that the M, 35,000 binder could be a proteolytically modified or a less glycosylated form of the M, 40,000 binder and that each one of these binders is combined with an unlabeled subunit with an M, around 50,000 and not seen by cross-linking (Fig. 13, c and d). If this were the case, one would expect that this would generate two bands with M, values of around 110,000. To improve resolution of higher molecular weight species we have run SDS-PAGE of cross-linked samples on 10% polyacrylamide gel but only found one band (not shown). It is possible that the resolution obtained with 10% gels is not enough and that there exists two populations of higher molecular weight binding species.

It can be concluded, both from data in Fig. 9 and from the two-dimensional electrophoresis experiment, that the M, 35,000 binder is contained within an M, 87,000 species and that the 35,000 binder is not available for cross-linking with 125I-hGH in the membrane preparation but only in Triton X-100-solubilized preparations. These results and the findings presented in Fig. 8, showing the presence of the M, 87,000 and 40,000 binders only when cross-linking was performed before and after Triton X-100 solubilization of incubated microsomal membranes, give further support to the notion that each one of the M, 35,000 and 40,000 binders is combined with an unlabeled subunit or subunits to make up the M, 87,000 species (Fig. 13, c and d).

If the M, 87,000 binder is composed of a combination of the M, 40,000 and 35,000 species, one question concerns the localization of the actual binding site for 125I-hGH. Both the M, 40,000 and 35,000 binders interact with 125I-hGH and are cross-linked with DSS. It seems unlikely that the M, 87,000 binder contains binding sites for two molecules of 125I-hGH, as this would generate a labeled band at a higher M, than 109,000. Covalent binding of hGH to purified prolactin receptor from rat ovary and analysis on SDS-PAGE of the complexes showed that each one of the purified proteins of M, 41,000 and 88,000 bound one molecule of hGH (47). The M, 41,000 protein has earlier been shown to be contained within the M, 88,000 protein (26). An explanation for the labeling of both subunits could be that one of these, e.g. the M, 40,000 binder, contains the actual binding site and that the M, 35,000 binder is close enough to the M, 40,000 binder to allow contact with 125I-hGH in Triton X-100-solubilized preparations, such that one part of the M, 40,000-35,000 hGH complex will be cross-linked between the M, 40,000 binder and 125I-hGH and one part of the same complex cross-linked between the M, 35,000 binder and 125I-hGH, giving rise to M, 62,000 and 57,000 bands, respectively (Fig. 13b). The contention that the M, 40,000 binder contains the actual binding site is supported by the fact that its autoradiographic intensity is higher than that of the M, 35,000 binder. The difference in intensity could also be explained by the fact that the M, 40,000 binder is a subunit of a receptor population that is more abundant than a receptor population in which the M, 35,000 binder is present (Fig. 13, c and d).

Another possibility is that the M, 40,000 and 55,000 binders represent proteolytical fragments of an M, 87,000 species which dissociate when run on SDS-PAGE (Fig. 13, c and d). This would agree with the results of Mitani and Dufau (47), who observed M, 41,000 and M, 88,000 proteins in purified preparations of rat ovary prolactin receptors as analyzed by SDS-PAGE run under reducing conditions, although these authors included protease inhibitors in their purification procedure.

Analysis by two-dimensional electrophoresis of 125I-hGH-incubated and cross-linked Triton X-100-solubilized rat ovary and Leydig cell membranes has revealed lower molecular weight species in the M, 35,000-40,000 region that were contained within a higher molecular weight species with an M, of 80,000-90,000 (23, 26). Thus, it seems that, at least in ovary, Leydig cells, and liver, similarities exist between the rat lactogenic receptors, although the reported number and molecular weights of the binding species in the different tissues are not the same.

In order to describe the hydrodynamic properties and the amount of detergent bound to the 125I-hGH receptor-detergent complex and to determine—by another method than SDS-PAGE—a molecular weight for the lactogenic receptor, a method described by Clarke was used (35).

Gel permeation chromatography of a 125I-hGH-incubated acid-precipitated redissolved preparation yielded a single peak of radioactivity, representing the hormone receptor-detergent complex, with a Stokes radius of 61 Å. Cross-linking and SDS-PAGE under reducing conditions of this species revealed three bands with M, values of 109,000, 62,000, and 57,000 (Fig. 12). On the basis of these results and the results from two-dimensional electrophoresis it can be concluded that the M, 87,000 binder precipitates in an intact form. Combining the data obtained by gel permeation chromatography and sucrose density gradient analysis the molecular weight of the solubilized receptor was calculated as 92,000.

The derived values for the frictional and axial ratios indicate that the hormone receptor-detergent complex has an elongated structure. This explains in part its behavior on gel chromatography where it co-elutes with ferritin, which has a molecular weight of 443,000 (50). Several investigators have used only gel chromatography to determine the M, of the Triton X-100-solubilized lactogenic receptor and obtained values ranging from 220,000 to 380,000 (18, 51, 52). In those studies the amount of detergent and the shape of the hormone receptor-detergent complex have not been taken into consideration.
The results presented here should be compared with the results of Jaffe (25) and Rae-Venter and Dao (24), who have used the same technique to characterize the Triton X-100-solubilized lactogenic receptor from rat liver. They obtained sedimentation coefficients, partial specific volumes, and Stokes radii of 5.0 and 4.7 $s$, 0.791 and 0.766 ml/g, and 54 and 50 Å, respectively. The molecular weights of the lactogenic receptor calculated from these data were 78,000 and 73,000.

In conclusion, the present study has shown that the Triton X-100-solubilized female rat liver prolactin receptor has a molecular weight of about 90,000. This species contains $M$, 40,000 and $M'$, 35,000 hormone-binding subunits. It cannot be decided whether these subunits are combined with each other or with undetected subunit(s) to make up the $M$, 90,000 species, or whether each represents a proteolytic fragment of the $M$, 90,000 species. Results are presented which indicate that some of the $M$, 40,000 and 35,000 subunits are (also) disulfide-linked within the $M$, 90,000 species. Clearly, further studies including purification are needed to clarify the structure of the prolactin receptor.

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Characterization of Hepatic Lactogen Receptor

**EXPERIMENTAL MATERIAL TO**

**Characterization of hepatic lactogen receptor**

**Biologic composition and hydrodynamic properties**

Larvato, A. and J. A. Cathers

**MATERIALS AND METHODS**

**Antigens** - Normal female Sprague-Dawley rats (vaccination, Stockholm, Sweden). 8-10 weeks of age were used throughout the study. In general, the animals were injected intraperitoneally with 50 μg of chick embryo oviduct extract (Pergamol-Depot, Sterling-Mi Berlin, FRG) once weekly for 2 weeks before the start of the experiment. In some experiments, as indicated in the text, rats were untreated.

**Chemicals** - Triton X-100, BSA, bovine immunoglobulin G, ergot alkaloids, and progesterone were purchased from Sigma Chemical Co., St Louis, MO. Propylthiouracil 6.5-hydroxystrophanthidin (PHT, 6,5-BSH) and theophylline were purchased from Sigma Chemical Co., Poole, Dorset, U.K. All other chemicals were purchased from Sandoz Ltd., Basel, Switzerland or Merck, Darmstadt, Federal Republic of Germany, or Pharmacia, Uppsala, Sweden. All other specific activity 5-125I and 125I-specific activity in microorganisms were obtained from The Radiochemical Centre, Amersham, England and Fluka AG, Buchs, Switzerland, respectively. Reagents for SDS-PAGE were purchased from Sigma Chemical Co., St Louis, Missouri. All other chemicals were of analytical grade from either Sigma or Merck.

**RESULTS**

Acid precipitation of Triton X-100-solubilized membrane protein. When lowering the pH of Triton X-100-solubilized rat liver microsome preparations in order to remove more detergent, no precipitation was seen that accompanied the formation of precipitates and the precipitation of the precipitates was not further investigated. Acid precipitation was seen as a phase difference of the sedimentation rate of the precipitates when the supernatant was centrifuged at 17,000 rpm at 4°C. The precipitate formed at a pH of 4.0 was used for subsequent experiments.

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**FIG. 1.** Precipitation of Triton X-100-solubilized membrane protein. Triton X-100-solubilized rat liver microsomes were prepared as described in Materials and Methods. The preparation was divided into seven micro-precipitates, and 10 μg of protein was added to each precipitate aliquot. The pH was measured continuously and the addition of 5% trichloroacetic acid was stopped when the pH was observed to decrease to less than 4.5. The precipitate was collected by centrifugation at 17,000 rpm for 10 minutes and the supernatant was assayed for protein concentrations by the method of Lowry et al. (17), with BSA as standard.
Characterization of Hepatic Lactogen Receptor

TABLE 1

Characterization of Hepatic Lactogen Receptor

Preparation | Apparent Kd (nM) | % Max bound (Pentoxifylline) | Correlation coefficients
--- | --- | --- | ---
Acid-precipitated | 1.5 | 5 | 0.93
Hepatic lactogen receptor

The specificity of 125I-labeled binding to acid-precipitated, red blood cell membranes was measured in a competition assay. Significant competition was shown with kiloimmolar concentrations of unlabeled hormone at pH 7.4. The IC50 value was determined by gel-filtration chromatography. The binding data was analyzed according to Scatchard (16).

Figure 3. Specificity of 125I-labeled binding to acid-precipitated, red blood cell membranes was measured in a competition assay. Significant competition was shown with kiloimmolar concentrations of unlabeled hormone at pH 7.4. The IC50 value was determined by gel-filtration chromatography. The binding data was analyzed according to Scatchard (16).

Figure 4. Gel-filtration chromatography of Sepharose 4-B of 125I-labeled acid-precipitated red blood cell membranes was measured in the presence of unlabeled hormone (Figure 3). The peak of radioactivity that was shown when the sample was incubated with 125I-labeled and unlabeled unlabeled hormone was collected in a glass vial and analyzed by gel-filtration chromatography. The peak of radioactivity at the position where the standard proteins were eluted was also determined as described in text (16).

Figure 5. Sepharose 2-400 chromatography of Triton X-100-solubilized, acid-precipitated, red blood cell receptor-dense complex. About 2 mg of acid-precipitated (1 mg) protein (1 mg) was incubated with 125I-labeled in the absence (0-8) or presence (0-9) of unlabeled hormone at pH 7.4. For 15 h and applied to a Sepharose 2-400 column (4 x 60 cm) equilibrated in Tris-Triton X-100 buffer and eluted with the same buffer at a flow rate of approximately 30 ml/h, one mg fractions were collected and assayed for radioactivity. The column was calibrated with blue dextran (2,000 kDa) and the following standard proteins: ovalbumin (116 kDa), aldolase (150 kDa), bovine serum albumin (66 kDa), and myoglobin (21 kDa).

Figure 6. Sucrose density gradient analysis of 125I-labeled red blood cell membranes was measured in a competition assay. Significant competition was shown with kiloimmolar concentrations of unlabeled hormone at pH 7.4. The IC50 value was determined by gel-filtration chromatography. The peak of radioactivity that was shown when the sample was incubated with 125I-labeled and unlabeled hormone was collected in a glass vial and analyzed by gel-filtration chromatography. The peak of radioactivity at the position where the standard proteins were eluted was also determined as described in text (16).

TABLE 2

Parameter

Debye's R (A) | 6.182 (5)

Partial specific volume, νm (g/ml) | 0.7640 (5) (3) (3)

Osmotic coefficient, ωS (o)/ω | 0.9500.16 (3) (3)

Triton X-100 bound (mg of complex) | 0.27

Molecular weight, Mw | 111,300

Hormone-receptor complex | 111,300

Receptor | 92,300

Fractional mass, f/m | 2.51

Area ratio, a | 319

The values given are the standard deviations for the number of determinations shown in parentheses.

Three sucrose gradient centrifugations were performed in 0.5 and 0.3 M sucrose. The values given for V and m are the standard deviations for the number of determinations shown in parentheses.

The weight fraction of Triton X-100 in the 125I-labeled hormone-receptor complex was calculated with the assumption that the observed partial specific volume represents the average of the partial specific volumes of the complex and the protein. The equation used was the following: νm = (1/vp + v) ωS/ω, where ω and ωS are the weights of protein and protein plus protein, respectively, and νm is the partial specific volume of the complex.

The molecular weight of the 125I-labeled hormone-receptor complex was calculated by substracting the molecular weight of Triton X-100 from the molecular weight of the 125I-labeled hormone-receptor complex.