SOLUBILIZATION AND PARTIAL PURIFICATION OF A GROWTH HORMONE RECEPTOR FROM RABBIT LIVER

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1. Introduction

Specific binding sites (receptors) for growth hormone (GH) have been identified in microsomal membranes obtained from rabbit liver [1–3]. Although receptors for a number of polypeptide hormones have been solubilized and partially purified [4–10] successful purification of the growth hormone receptor has not been achieved. This report deals with the solubilization, partial purification and characterization of the rabbit liver receptor.

2. Methods

2.1. *Iodination of growth hormone*

Human growth hormone (Kabi, Sweden) was labelled with ^{125}I by a modification of the lactoperoxidase method of Thorell and Johansson [11]. The ^{125}I-GH was separated from unreacted ^{125}I and aggregated ^{125}I-GH on a Sephadex G-100 column (90 × 1 cm) equilibrated in 50 mM Tris-HCl (pH 7.5), 0.1% bovine serum albumin (BSA). The purified material was stored at −20°C.

2.2. *Preparation of liver microsomes and solubilization of receptors*

Livers from 30 day pregnant rabbits were homogenized in 0.3 M sucrose with a Potter-Elvejhem or Braun multimix homogenizer. The resultant homogenate was subjected to differential centrifugation [1] and the microsomal fraction stored in 50 mM Tris-HCl pH 7.5 + 10 mM $CaCl_2$ at −20°C. For solubilization the microsomes were washed with Tris-HCl pH 7.5 and Triton X-100 (1 mg/mg protein) added. After stirring for 30 min. the mixture was centrifuged for 200 000 g × h and the supernatant stored in aliquots at −20°C.

2.3. *Binding of ^{125}I-GH to soluble receptors*

The Triton X-100 extract (50–100 μg protein) was incubated for 18–24 h at room temperature with ^{125}I-GH (300–500 pg) in Tris-HCl pH 7.5, 0.1% BSA, 10 mM $MgCl_2$ γ-globulin (final conc. 0.025%) and polyethylene glycol (final conc. 12.5%) were added [4] and the tubes centrifuged. The supernatant was removed and the radioactivity in the pellet counted in a γ-counter. All reported data (specific binding) are the mean of duplicate samples and corrected for 'non-specific' binding by subtracting from total binding that bound in the presence of 10 μg GH.

2.4. *Affinity chromatography of the soluble receptor*

Human GH (Kabi) was dialyzed to remove glycine (added as a stabilizer) lyophilized, dissolved in 0.1 M $NaHCO_3$ (pH 8.6) and coupled to the *N*-hydroxy-succinimide ester of 3,3′-diaminodipropyl-amino-succinyl agarose (Affi-Gel 10, Bio-rad). The mixture was shaken at 4°C for 4 h and 100 ml 1 M glycine added. The gel was washed with 200 ml 6 M guanidine hydrochloride, 500 ml 8 M urea, and 2 litres 0.1 M $NaHCO_3$, pH 8.5. Efficiency of coupling averaged 85%. Preliminary chromatographic studies were performed in mino-columns (~ 1 ml gel) to optimize binding and elution conditions. Columns were equilibrated with Tris-HCl 50 mM, 10 mM $MgCl_2$, 0.1% Triton X-100 of pH 7.0–9.5. Extract was added at the same pH. After washing with 10–20 bed volumes of buffer, receptor was eluted with 5 M $MgCl_2$ [4]. The later fractions were dialyzed against 200–300 volumes of

Tris-HCl pH 7.5. Preparative purification was performed with 5–10 ml bed volume of gel.

3. Results

Treatment of liver microsomes with Triton X-100 solubilized 70–80% of ^{125}I-GH binding activity. Demonstration of the presence of solubilized receptors was performed by the two independent methods of polyethylene glycol precipitation (table 1) or Sephadex G-100 chromatographic separation of receptor bound and free ^{125}I-GH (fig.1). Good agreement was found between the two measurements of ^{125}I-GH binding, and displacement with increasing amounts of unlabelled hormone.

Table 1
Comparison of separation of receptor bound and free ^{125}I-GH by gel chromatography and polyethylene glycol (PEG) precipitation methods

Growth hormone concentration (ng/ml)	^{125}I-Growth hormone (% bound)	
	Gel chromatography	PEG
0	79	66
20	64	42
100	32	18
10 000	9	8

PEG separation as described under methods; gel chromatography as in fig.1.

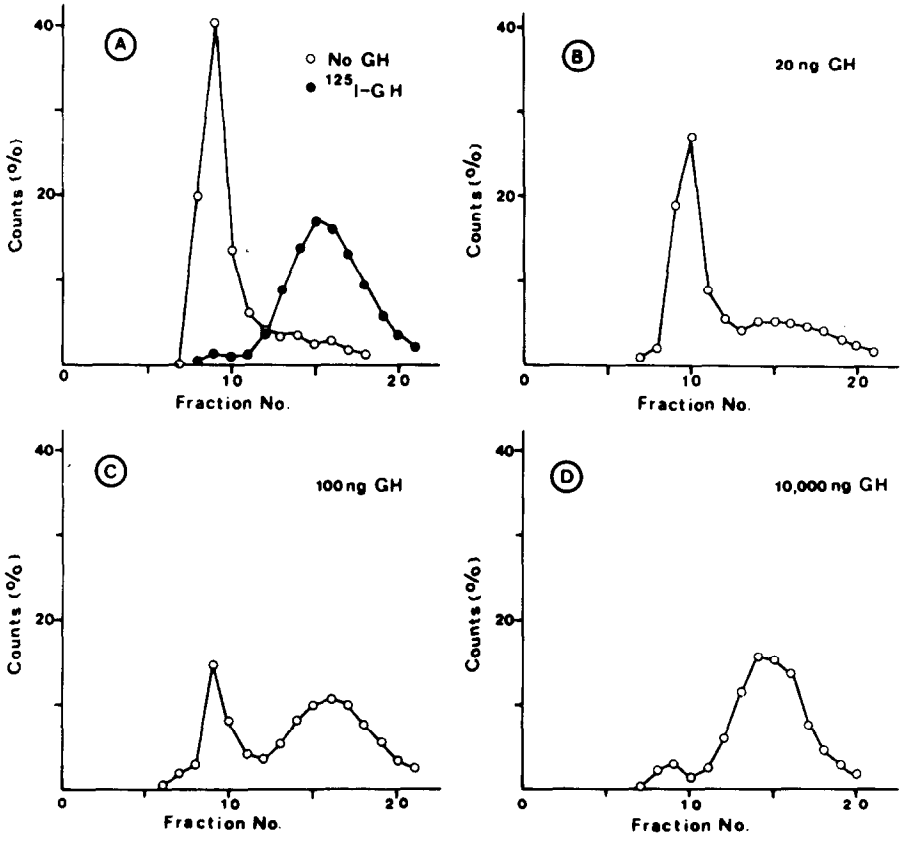

Fig.1. Separation of receptor bound and free ^{125}I-GH on Sephadex G-100. Triton extract (200 μg protein) was incubated with ^{125}I-GH (45 000 cpm) for 16 h at 22°C. Incubation was performed in the absence of unlabelled GH (A) or with 20 ng (B), 100 ng (C) or 10 μg (D) GH. ^{125}I-GH incubated in the absence of receptor eluted as in (A). An aliquot (100 μl) was applied to a Sephadex G-100 column (30 × 0.5 cm) and eluted with 50 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 0.1% Triton. Fractions (0.3 ml) were collected and counted in a γ-counter. The ordinate axis is represented as % of recovered counts in the fraction.

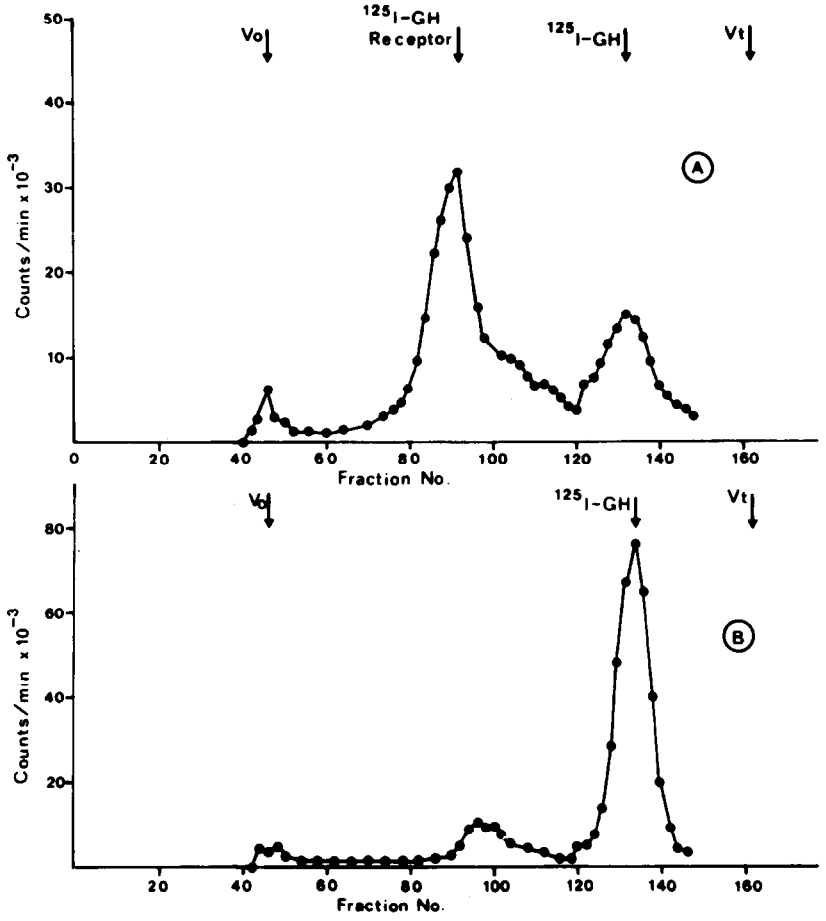

Fig. 2. Gel-filtration on Sepharose 6B (90 × 1.5 cm) of solubilized growth hormone receptors following incubation with ^{125}I-GH (500 000 cpm) for 24 h at 22°C in the absence (A) or presence (B) of 20 μg GH. Elution was performed with 50 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 0.1% Triton. Vo = Void volume (Blue Dextran) Vt = Total volume (^{125}I).

Gel-filtration on Sepharose 6B of the Triton X-100 extract following equilibration with ^{125}I-GH showed two major peaks corresponding to a ^{125}I-GH-receptor complex and free ^{125}I-GH (fig.2A), and a smaller peak in the void volume. The ^{125}I-GH-receptor peak possessed a shoulder on the descending limb. Inclusion of 10 μg GH in the incubation medium failed to displace the void volume peak and resolved the shoulder into a definite peak of 'unspecifically' bound ^{125}I-GH (fig.2B). Calibration of the column with a number of proteins of known mol. wt. gave an estimate of the mol. wt of the ^{125}I-GH complex of 200 000. The growth hormone receptor is therefore similar in nature to the receptor for other polypeptide hormones [4–9] in its susceptibility to Triton X-100 extraction. The approximate mol. wt., although requiring verification by measurement of sedimentation constants, is similar to that of gonadotrophin [6,7], prolactin [4], and parathyrin [8] receptors but smaller than that for insulin [9].

The solubilized receptor showed significant binding to GH coupled to Affi-Gel 10 over the pH range 7.0 to 9.5 (fig.3). However, maximum binding occurred at pH 7.0 (not shown) and 7.5 and 67% of applied ^{125}I-GH binding activity was readily eluted with 5 M $MgCl_2$. At pH 8.5 and 9.0 only 43% and

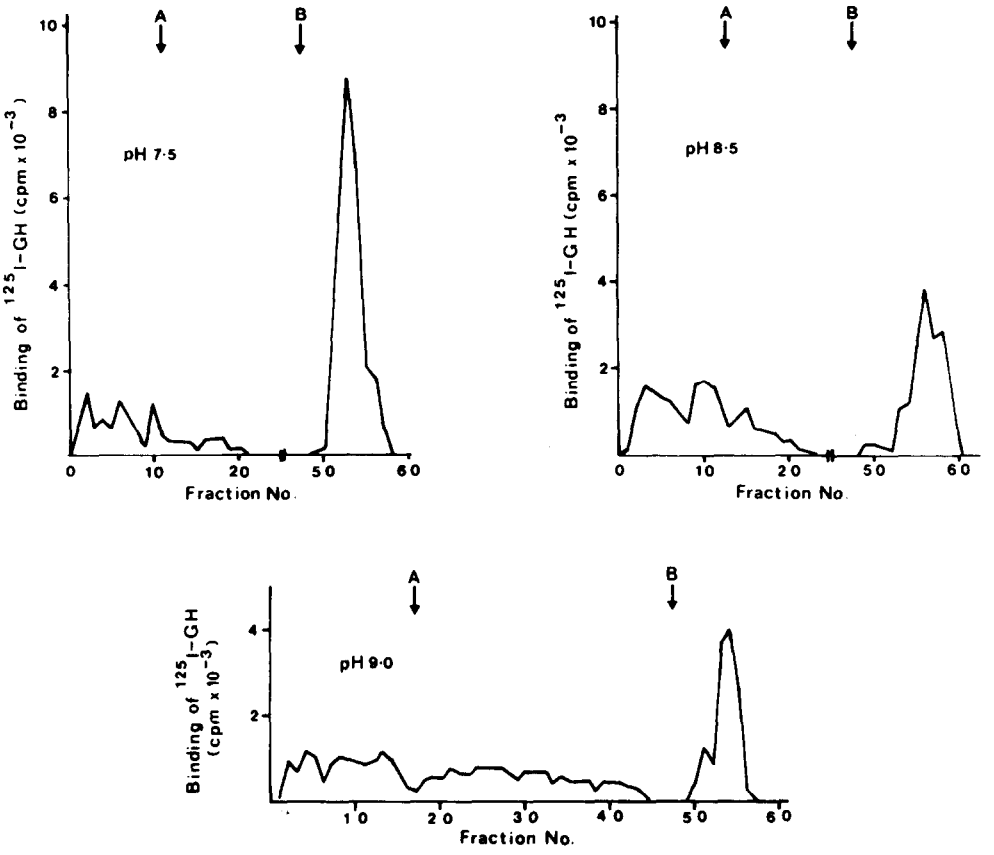

Fig.3. Affinity chromatography of growth hormone receptors on mini-columns (1 ml bed volume) of GH coupled to *N*-hydroxysuccinimide ester of 3,3'-diaminopropylaminosuccinyl agarose. Triton X-100 extract was applied at the indicated pH and the column washed with the same buffer (A). Elution of bound receptor with 5 M $MgCl_2$ commenced at B. Data is expressed as total ^{125}I-GH binding ability (cpm) per fraction.

Table 2
Equilibrium association constants and binding capacities of the GH receptor at various stages of purification

	Equilibrium association constant K_a (M^{-1})	Binding capacity fmoles/mg protein
Membranes	4.0×10^9	330
Triton X-100 extract	2.2×10^9	552
Unbound to affinity column	2.6×10^9	54
Eluted with 5 M $MgCl_2$	4.2×10^9	–

21%, respectively, of applied binding activity were recovered. At pH 9.0 binding to the column was of low affinity since displacement could be achieved by repeated washing with 10 mM $MgCl_2$ alone.

Displacement curves obtained with the crude Triton X-100 extract, the unbound material, and eluted purified receptor (fig.4B), and Scatchard [12] analysis (fig.2B) of the data, revealed a single order of binding sites with an increase in the equilibrium association constant (K_a). The K_a of purified material was the same as that of membrane bound receptors (table 2). Since the protein content of purified material was too low to accurately measure the binding capacity is at present uncertain. The apparent decrease in association constant on solubilization is in agreement with Dufau et al. [7] and may be due to a change in conformation on dissociation from the membrane. However, since purification and reduction in the amount of Triton X-100 present restores the value to its original level, it is possible that micelle formation may be the cause of the apparent reduction.

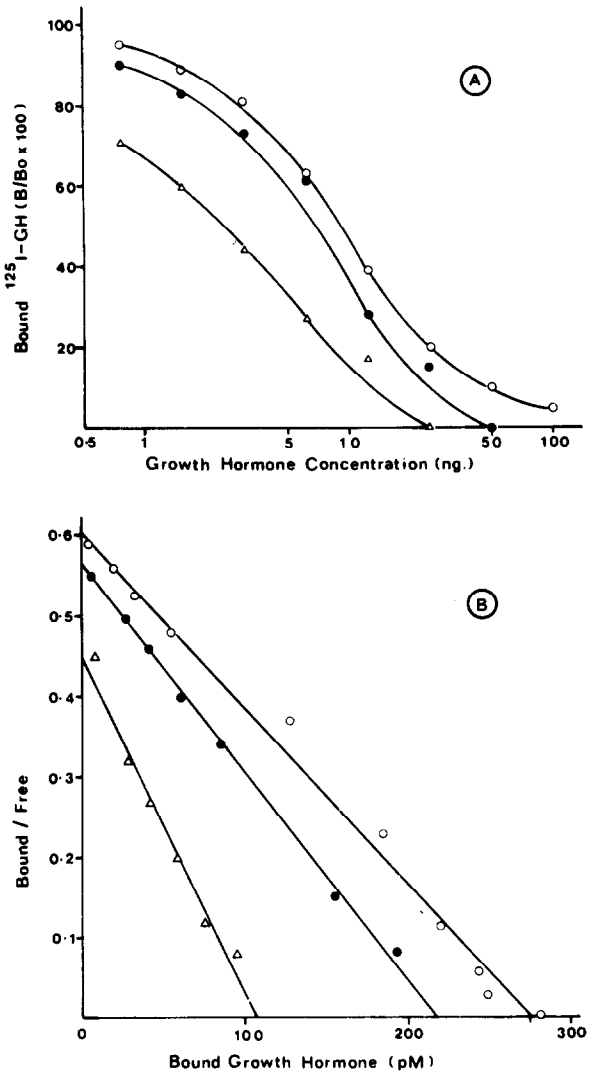

Fig.4. Displacement curves (A) and Scatchard analysis of the data (B) with crude Triton X-100 extract of liver microsomes (○), material not bound to affinity columns at pH 7.5 (●) and eluted, purified receptors (△). Incubation was performed for 24 h at 22°C with ^{125}I-GH (200 pg) and increasing concentrations of unlabelled GH. Separation by the polyethylene glycol method.

Elucidation of the mode of action of hormones requires detailed examination of each stage of the process from binding to the membrane to the final event. A purified receptor is more amenable for studying the chemical events occurring during interaction of hormone and receptor and the present techniques provide a simple method for the preparation of such material.

Acknowledgements

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Note added in proof

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