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Binding of uteroglobin to microsomes and plasmatic membranes

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Abstract **Microsomes and plasmatic membranes from rat liver bind radioactive uteroglobin (UG) in vitro with high affinity ($K_d = 1.7 \times 10^{-10}$ M). The binding is saturable and specific and dependent on previous reduction of UG with dithiothreitol. Microsomes from rat spleen or lung or from rabbit endometrium also possess a similar ability. Binding capacity is not affected by previous treatment of microsomes with phospholipase A_2 or peptide-*N*-glycosidase F but is lost after brief treatment with trypsin. The complex formed between UG and the binding component can be solubilized from microsomes with 5 mM CHAPS and it elutes with an apparent M_r of 90,000 in a Sephacryl 200 column. The complex is resistant to 8 M urea but is completely dissociated by Triton X-100. The UG-binding protein(s) has been partially purified from solubilized microsomes and membranes by affinity chromatography. The results are discussed in relation to a possible physiological effect of UG on cellular membranes.**

Key words: Membrane protein; Steroid-binding protein; Hydrophobic interaction; Affinity chromatography

1. Introduction

Uteroglobin (UG) is a small secretory protein (M_r = 15,000) synthesized in the epithelia of several organs of the rabbit and related species (see [1,2] for reviews). In the rabbit, the main source of UG is the endometrial epithelium where its gene can be induced by progesterone in early pregnancy. The protein is also synthesized in the lung [3], the epididymis [4] and the oviduct [5] under the control of glucocorticoids, androgens and estrogens, respectively. In other mammalian species, the counterpart of UG has been called CC10 (for lung Clara cell 10 kDa protein), although both proteins are very similar on the basis of amino acid sequence, quaternary structure, tissue expression and the organization of their genes. UG/CC10 has so far been identified in species of *Lagomorpha* [6], in human [7], rat [8,9], mouse and dog [10], suggesting a wide distribution among mammals and therefore an important function. Almost 30 years after the discovery of UG, the physiological role of this protein remains elusive. The better established property of UG, its ability to bind progesterone, was proposed as a transport mechanism of the hormone to the early embryo ([1,2], and references therein). Rat CC10 binds polychlorinated biphenyls [8] and, therefore, might be involved in processes of detoxification in lung. UG inhibits phospholipase A_2 in vitro [2] and might also act as an immunosuppressant in avoiding the rejection of the embryo [2]. More recently, UG has been shown to bind retinoids [11]. None of these properties has been conclusively related to a physiological function.

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In this study we show that UG binds specifically to microsomes and plasmatic membranes, apparently by interacting with a protein(s).

2. Materials and methods

$Na^{125}I$ was obtained from Amersham Int. Human growth hormone (hGH), ovine prolactin (oPRL), bovine insulin, cholamidopropyl-dimethylammoniopropanesulfonate (CHAPS) and phospholipase A_2 (EC 3.1.1.4) were from Sigma, USA. Peptide-*N*-glycosidase F (PNGase F; EC 3.2.2.18) was purchased from New England Biolabs. Homogeneous UG was purified as already described [12].

2.1. Preparation of microsomes, membranes and iodinated UG

Microsomes and membranes were obtained as described previously [13,14]. UG was labelled with $Na^{125}I$ and purified by gel-filtration chromatography [15]. The specific activity attained was about 10^7 cpm/μg of protein.

2.2. Binding assays

100 μl of microsomes or plasmatic membranes were incubated at 25°C for 1.5 h with ^{125}I-labelled UG either in the absence or in the presence of an excess (8 μg) of unlabeled UG (to serve as a control of non-specific binding). Both labeled and unlabeled UG were previously reduced with 10 mM dithiothreitol (DTT) for 15 min at 37°C [17]. Assays contained, in a final volume of 200 μl, 25 mM Tris, pH 7.5, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 0.1 M NaCl and 1 mM phenylmethylsulfonyl fluoride (PMSF). After incubation, the assays were centrifuged at 380 × *g* for 10 min. The pellets were washed three times by resuspension and centrifugation before radioactivity was counted in a γ-counter. Values corresponding to unspecific binding were subtracted. In some experiments microsomes were previously digested either for 15 min at 25°C with 4 μg/ml of trypsin, for 3 h at 37°C with PNGase F (4000 U/ml) or for 5 min at 37°C with phospholipase A_2 (1 μg/ml). After washing and centrifugation, microsomes were assayed for binding of UG. All determinations were done in duplicate.

2.3. Gel-filtration experiments

300 μl of microsomes were incubated with labeled UG and washed as described above. Pelleted microsomes were solubilized by stirring for 20 min at 25°C in 200 μl of 25 mM Tris-HCl, pH 7.5; 10 mM DTT, 5 mM CHAPS; 1 mM PMSF (buffer S). The mixture was centrifuged for 3 min at 10,000 × *g* and the supernatant was applied to a column of Sephacryl 200 (0.5 × 25 cm) equilibrated and developed at 4°C with buffer S. Fractions (0.3 ml) were assayed for radioactivity. Before chromatography, cytochrome *c* (200 μg) and [^{35}S]methionine (traces) were added to the samples as internal markers to correct for small differences of elution volumes between each individual chromatography. In some experiments urea or Triton X-100 were added at a final concentration of 8 M or 1%, respectively, before chromatography.

2.4. Affinity chromatography

UG was linked to CNBr-activated Sepharose and this matrix was poured onto a small column (300 μl of bed volume, 70 μg of linked UG) and equilibrated with buffer S. Prior to each chromatography the column was incubated for 15 min at 37°C in an oven to reduce the coupled protein. 1 ml of CHAPS-solubilized microsomes or membranes was slowly passed through the column which was then washed with buffer S. Bound material was eluted with 1% Triton X-100. As controls of non-specific binding to the matrix, duplicate samples were incubated with an excess of UG (30 μg) before chromatography.

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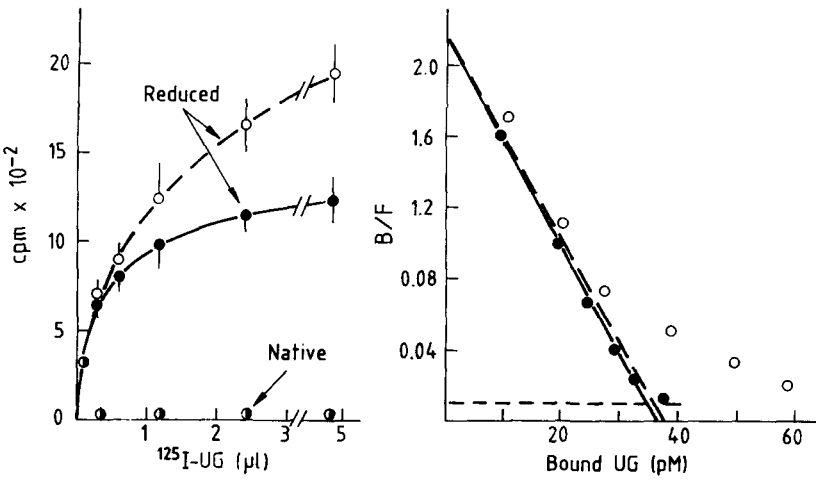

Fig. 1. (A) Saturation analysis of the binding of [^{125}I]UG to microsomes (○) and plasmatic membranes (●). Values are means ± S.D. for three independent experiments. (B) Scatchard plots of the data obtained in A.

3. Results

The saturation analysis of the binding of labeled UG to microsomes and plasmatic membranes is shown in Fig. 1A. Binding appeared saturable and was absolutely dependent on previous reduction of UG with DTT. Scatchard plots of the data (Fig. 1B) indicated the presence, in plasmatic membranes, of a single binding site with a K_d of 1.7×10^{-10} M. In microsomes, a similar site was observed together with an additional non-specific and non-saturable component. Microsomes from rabbit endometrium and from rat lung and heart also presented a similar binding ability (data not shown). The binding of labeled UG to microsomes could be specifically displaced by increased amounts of unlabeled UG but was not affected by other proteins such as cytochrome *c*, insulin, hGH or oPRL (Fig. 2). Treatment of microsomes with either phospholipase A_2 or PNGase F did not affect the binding but it was completely lost following brief treatment with trypsin (not shown).

The complex formed between UG and the binding component(s) could be solubilized with the mild detergent CHAPS. After incubation with labeled UG, microsomes were solubilized with CHAPS and analyzed by gel-filtration as described in section 2. Fig. 3 shows that UG was associated to a macromolecule(s) forming a broad complex the main component of which eluted with an apparent M_r of approx. 90,000. Labeled UG could be displaced from the complex if the binding incubation was performed in the presence of an excess of the unlabeled protein. On the other hand, the complex was resistant to treatment with 8 M urea but was completely dissociated by Triton X-100 (Fig. 3).

Finally we partially purified the UG-binding molecule(s) by affinity chromatography of solubilized membranes on a UG-Sepharose column as described in section 2. The results (Fig. 4) indicated that the column matrix bound some proteins unspecifically, but a component of 32 kDa was clearly purified (lanes 2 and 2′) which was absent if the initial material was preincubated with an excess of UG (lanes 3 and 3′). Digestion of the bound material with proteinase K resulted in the complete disappearance of all the above observed bands (Fig. 4, lane 4), indicating that they were proteins.

4. Discussion

The results shown in this report demonstrated that microsomes and plasmatic membranes from rat liver bound UG with high affinity. This binding activity is probably widely distributed among membranes from other tissues and species since it was also found in heart, lung and endometrial microsomes either from the rabbit or the rat. The membrane-associated component responsible for the binding could be solubilized with a mild detergent, as is the case for many membrane-associated receptors. The fact that the complex formed between UG and its binding component was resistant to 8 M urea but dissociated following treatment with the detergent Triton X-100 suggests that the complex occurs through hydrophobic

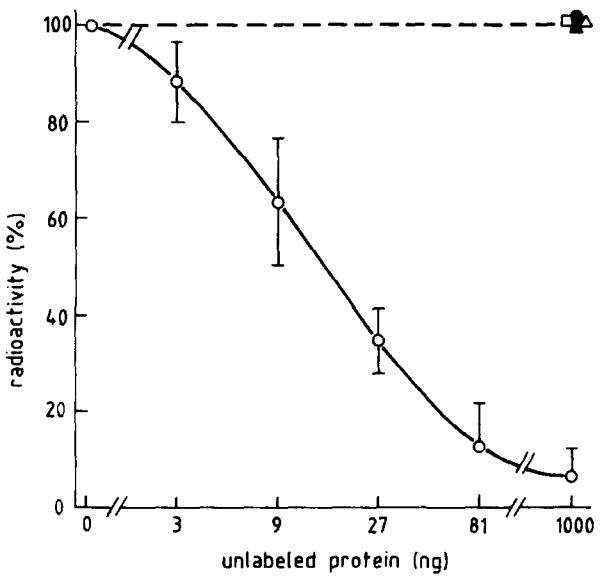

Fig. 2. Displacement of the binding of [^{125}I]UG to microsomes. Microsomes were incubated with labeled UG in the presence of the indicated amounts of the following unlabeled proteins: UG (○), cytochrome *c* (●), bovine insulin (△), hGH (▲) and oPRL (□). Values are means ± S.D. for three different experiments.

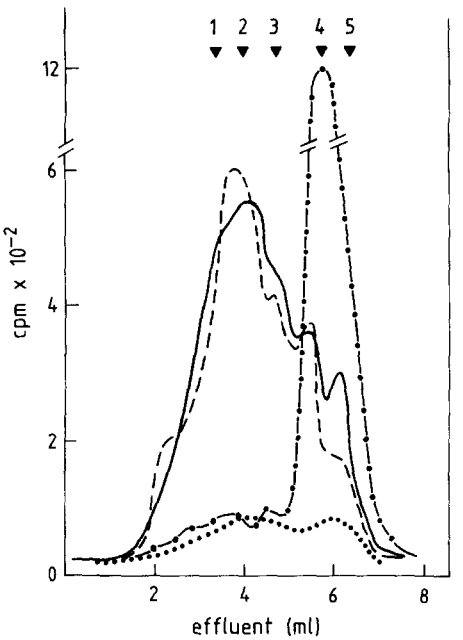

Fig. 3. Gel-filtration analysis of the complex ([^{125}I]UG-binding component). Microsomes were incubated with labeled UG either in the absence (——) or the presence (••••) of excess of unlabeled UG. Other samples were treated either with 8 M urea (– – –) or with 1% Triton X-100 (–•–), before chromatography. Arrowheads indicate the elution positions of the following markers: (1) IgG; (2) serum albumin; (3) ovalbumin; (4) UG; (5) cytochrome *c*.

interactions. This may be related to the ability of UG to interact with several hydrophobic compounds such as steroids [1,2], polychlorobiphenyls hydrocarbons [8] and retinoids [11]. The exact nature of the UG-binding component is not known but all the evidence shown in this study suggests that it is a protein. Thus, neither phospholipase A_2 nor PNGase digestion of microsomes had effect on UG-binding activity but it was lost after brief treatment with trypsin. In addition, the material purified by affinity chromatography was completely digested by proteinase K. Since it has been reported that UG inhibits phospholipase A_2 [2], an enzyme located in cellular membranes, it might be that the complex observed in the gel-filtration experiments was UG bound to this enzyme. This possibility can be ruled out, however, since, in similar experiments, phospholipase A_2 did not form any detectable complex with [^{125}I]UG (not shown). The UG-binding molecule appears to be a minority component of the liver membrane since the material purified by affinity chromatography could only be observed by silver-staining and its protein contant was too low to be accurately estimated. However, a rough calculation indicated that it might account for approx. one-ten thousandth of the total membrane protein.

It is striking that the properties (or possible functions) of UG are dependent on the reduced state of the protein. Thus, in addition to the results shown here, binding of UG to progesterone [1,2] or retinoids [11] is greatly enhanced by treatment with reducing agents. This implies that UG must be in a reduced form to exert these possible functions in physiological conditions. The oxidation–reduction state of UG in the physiological milieu is unknown but there are some observations suggesting such reduction. Thus, incubation of fresh uterine secretions with either labelled progesterone or retinoids resulted in a relatively important binding of these compounds to UG. However, when UG was further handled and purified, the binding capacity of the protein was considerably reduced and needed treatment with DTT to be recovered (our unpublished observations).

The interaction of UG with membranes described here invites speculation about a physiological role of this protein involving a membrane-mediated mechanism. This is particularly attractive in view of some previous reports. One of these reports assigned to UG a growth factor-like activity on the development of the early embryo [16]. Another study has indicated a receptor-like mechanism for the specific transport of UG inside the blastocyst [17]. Also, both a possible binding of UG to the

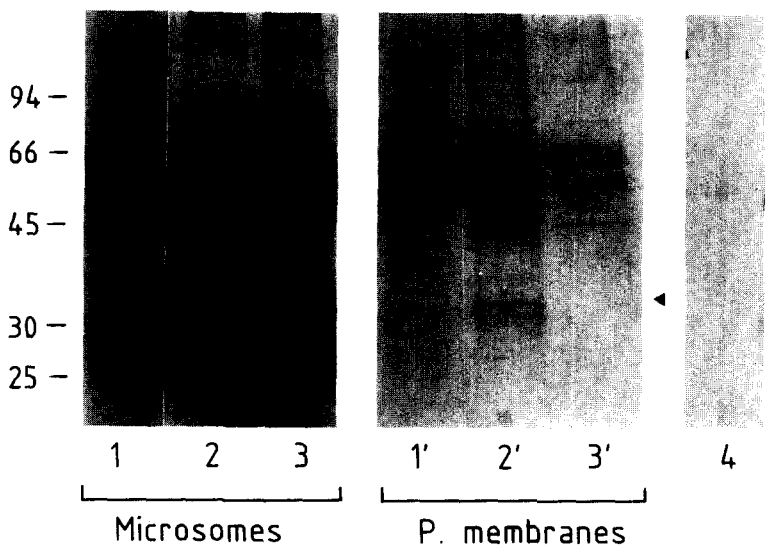

Fig. 4. Purification of the UG-binding proteins by affinity chromatography. Samples were analyzed by SDS-PAGE (8% acrylamide). (1 and 1') Starting material; (2 and 2') material bound to the column and eluted with Triton X-100; (3 and 3') same as 2 and 2' but the starting material was incubated with excess of UG before chromatography; (4) same as 2 but digested with proteinase K. The arrowheads point to the 32 kDa band. The M_r of the markers (kDa) is given on the left. Lanes 1 and 1' were stained with Coomassie blue; the remaining ones were silver stained.

microvilli membranes of endometrial cells [18] and the uptake of the protein by these cells (perhaps by a receptor-mediated mechanism) [19] have been suggested. The results shown here can open new perspectives on the physiological function of UG.

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