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Evidence for the involvement of the transcortin carbohydrate moiety in the glycoprotein interaction with the plasma membrane of human placental syncytiotrophoblast

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We have studied the interaction of human transcortin and the pregnancy-associated transcortin variant with the microvesicular membrane fraction derived from the human placental syncytiotrophoblast. Two classes of specific binding sites for these glycoproteins were found in this membrane preparation. One of these displays a relatively high binding capacity, $B_{max} = 140 \pm 60$ fmol transcortin per mg membrane protein, and a significantly higher affinity for transcortin, $K_d = (1.6 \pm 0.6) \cdot 10^{-10}$ mol/l, than for the pregnancy-associated variant, $K_d = (4.5 \pm 1.2) \cdot 10^{-9}$ mol/l. On the contrary, another class of the binding sites, occurring in the membranes at a far lower concentration: $B_{max} = 3.0 \pm 2.2$ fmol transcortin per mg membrane protein, shows a higher affinity for the pregnancy-associated transcortin variant, $K_d = (3.3 \pm 2.0) \cdot 10^{-12}$ mol/l, than for normal transcortin, $K_d = (2.5 \pm 0.7) \cdot 10^{-11}$ mol/l. Since the pregnancy-associated variant differs from normal transcortin with respect to its carbohydrate structures only (Avvakumov, G.V. and Strel'chyonok, O.A. (1987) Biochim. Biophys. Acta 925, 11–16), the results of the present work suggest that the transcortin carbohydrates are directly involved in the specific interaction of this serum hormone-binding globulin with the plasma membrane of the placental syncytiotrophoblast.

Introduction

During the last five years evidence has been presented that human serum transcortin and sex-hormone-binding globulin bind specifically to the plasma membranes of steroid hormone target tissues: liver [1], decidual endometrium [2,3] and prostate [4,5]. This suggests that, in addition to the well-known function of specific hormone-binding globulins to protect steroids from chemical and enzymatic attacks in the circulation and to regulate the levels of protein-bound and 'free' hormones [6], they can take part in a guided transport of steroid hormones to the target tissues.

A special transcortin variant appears in the maternal blood serum during pregnancy [7,8]. Its basic characteristics, parameters of steroid binding included, are virtually similar to those of transcortin from normal donor serum, suggesting the similarity of the polypeptide moieties of these two glycoproteins [9]. But their carbohydrate structures are different. The transcortin molecule contains two triantennary and three biantennary N-linked sugar chains of the *N*-acetyllactosamine type [10], whereas all five carbohydrate chains in

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the molecule of transcortin variant are triantennary oligosaccharides of the same type [8].

If hormone-binding glycoproteins would serve only a passive transport function in the circulation, the appearance of the special transcortin variant during pregnancy could hardly be expected. It is known [11] that carbohydrates are not involved in the steroid binding to transcortin and, as mentioned above, the variant displays the same affinity for steroids as normal transcortin. We have assumed that this variant can take part in the guided transport of steroids to some target tissue(s) developing during gestation. We have studied, therefore, the interaction of transcortin and its pregnancy-associated variant with the microvesicular membrane fraction derived from the syncytiotrophoblast brush border of human first-trimester placenta, a glucocorticoid target tissue [12]. The fact that transcortin and its pregnancy-associated variant have different carbohydrate structures gave us a unique opportunity to elucidate the role of the transcortin carbohydrates in the membrane–glycoprotein interaction.

Materials and Methods

Cortisol, methyl-α-D-glucopyranoside were purchased from Serva (Heidelberg, F.R.G.), human and bovine serum albumine, transferrin and orosomucoid were obtained from Sigma (St. Louis, MO, U.S.A.). The techniques described previously were used for the isolation of transcortin [13], pregnancy-associated transcortin variant [7], sex-hormone-binding globulin [14] and thyroxine-binding globulin [15] from human postpartum serum. Glycoproteins were radioiodinated using 1,3,4,6-tetrachloro-3α,6α-duophenylglycouril (Pierce, Rockford, IL, U.S.A.) [16]. The specific activity of the preparations of ^{125}I-labeled transcortin and its variant was 50–70 μCi/μg, their radiochemical purity was about 99%. The immunochemical purity tested with a monospecific antiserum was about 100%.

Microvesicular membrane fraction was prepared from the syncytiotrophoblast brush border as previously described [17]. For this purpose, normal placental tissue from therapeutic abortion (7–12 weeks of pregnancy) performed in a local hospital was used. The tissue was stored for approx. 3 h in Krebs buffer at 0–4 °C before isolation of the membrane fraction. The membrane vesicles obtained displayed a high activity of the plasma membrane marker enzyme 5′-nucleotidase [17] and a capacity to take up glucocorticoids [12].

Disposable polysterene gamma-counting tubes (approx. 3 ml volume) were used for the binding assay. Each sample contained 50–125 μg membrane protein in a final volume of 1 ml of 50 mM Tris-HCl (pH 7.4)/100 mM NaCl/10 mM $CaCl_2$ containing 0.2 mg/ml bovine serum albumin and $5 \cdot 10^{-7}$ M cortisol. At this concentration, cortisol saturated transcortin or its variant at all glycoprotein concentrations used (see Results). Increasing concentrations of ^{125}I-transcortin or ^{125}I-labeled pregnancy-associated transcortin variant were incubated with the membranes in the presence (nonspecific binding) or in the absence (total binding) of a 1000–2500-fold molar excess of the corresponding unlabeled glycoprotein. Specific binding was determined by subtracting nonspecific from total binding. In order to study the selectivity of the specific membrane binding, various competitors (see Table I) were added into the samples.

After incubation of the samples for 16 h at 0–2 °C with gentle shaking, the membranes were sedimented by centrifugation at 15000 × *g* for 30 min at 4 °C, and the supernatant was discarded. The membrane-bound ^{125}I-radioactivity was directly counted in the binding assay tubes with an RIA Gamma counter (LKB-Wallac, Turku, Finland). Parameters of the glycoprotein binding to the membranes were determined using the Lineweaver-Burk [18] and Hill [19] graphic analyses. These are given in the text as mean ± S.D. of an indicated number (n) of determinations from different membrane preparations.

Results

As seen from Fig. 1, the saturable binding of transcortin to the syncytiotrophoblast plasma membrane was observed at the glycoprotein concentrations below approx. 2 nmol/l. Analysis of the data from a series of the similar experiments ($n = 5$) gave the following values for apparent equilibrium binding parameters: maximum capacity, $B_{max} = 140 \pm 60$ fmol transcortin per mg membrane protein; dissociation constant, $K_d =$

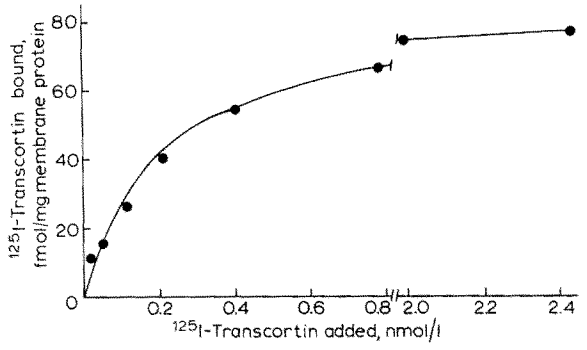

Fig. 1. Specific binding of ^{125}I-transcortin to the microvesicular membrane fraction from the human placental syncytiotrophoblast. See Materials and Methods for experimental details.

$(1.6 \pm 0.6) \cdot 10^{-10}$ mol/l; Hill coefficient, $H = 1.2 \pm 0.1$. So, the membranes display a high-affinity transcortin binding with a slight, positive cooperativity. Table I illustrates the high selectivity of the transcortin binding: no one of the human serum proteins used, except transcortin and its variant, competes with ^{125}I-transcortin for the membranes binding sites.

When studying the interaction of pregnancy-associated transcortin variant with the syncytiotrophoblast plasma membrane, we have found

TABLE I

INHIBITION OF THE SPECIFIC MEMBRANE BINDING OF ^{125}I-TRANSCORTIN AND THE ^{125}I-LABELED PREGNANCY-ASSOCIATED TRANSCORTIN VARIANT BY HUMAN SERUM PROTEINS

Inhibition was calculated as $(1 - B/B_0) \cdot 100\%$, where B and B_0 were the amounts of ^{125}I-labeled glycoprotein bound specifically to the membranes in the presence and in the absence of a competitor, respectively. Each sample contained either 0.65 nmol/l of ^{125}I-transcortin (first column) or 15 pmol/l of the ^{125}I-labeled transcortin variant (second column) and a 20-fold molar excess of a competing protein (a 1000-fold molar excess in the case of albumin). Values are means ± S.E. of three independent determinations.

Competitor	Inhibition	
	trans-cortin	transcortin variant
Transcortin	96 ± 6	37 ± 5
Transcortin variant	12 ± 5	98 ± 4
Sex-hormone-binding globulin	3 ± 3	2 ± 3
Thyroxine-binding globulin	3 ± 3	1 ± 1
Transferrin	2 ± 1	2 ± 2
Orosomucoid	1 ± 1	2 ± 1
Albumin	6 ± 3	7 ± 4

two classes of specific binding sites (Fig. 2). One of these (Fig. 2B, nanomolar range of the glycoprotein concentrations) seems to be similar to the binding sites described above: $B_{max} = 150 \pm 80$

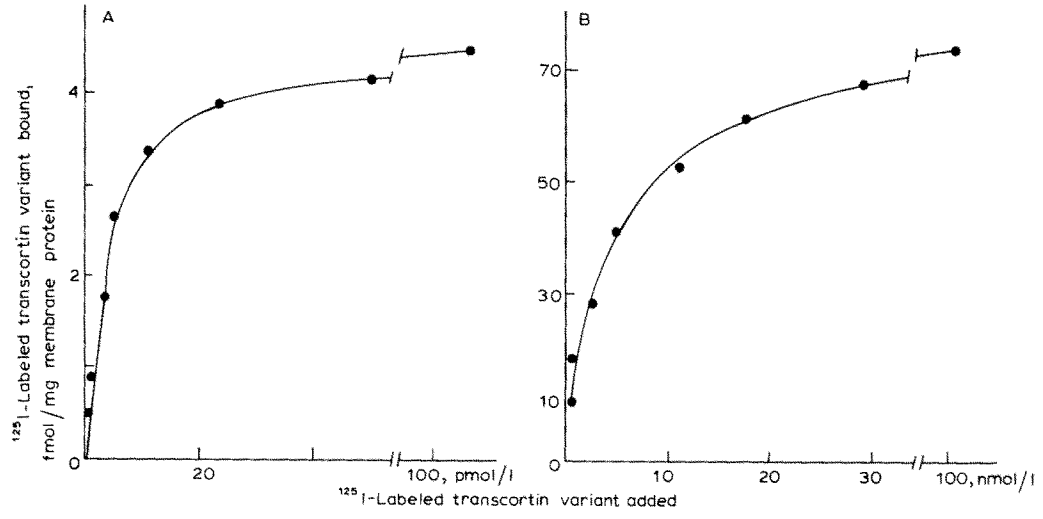

Fig. 2. Specific binding of the ^{125}I-labeled pregnancy-associated transcortin variant to the microvesicular membrane fraction from the human placental syncytiotrophoblast. (A) Picomolar range of the glycoprotein concentration, binding to relatively higher-affinity sites; (B) nanomolar range of the glycoprotein concentration, binding to relatively lower-affinity sites.

fmol transcortin variant per mg membrane protein, $H = 1.2 \pm 0.1$. These sites display a lower affinity to pregnancy-associated variant, $K_d = (4.5 \pm 1.2) \cdot 10^{-9}$ mol/l, as compared with normal transcortin (all three parameters were calculated from the data of three independent experiments). In accordance with this, pregnancy-associated variant was a less potent inhibitor of the ^{125}I-transcortin binding to these sites than transcortin (Table I).

Another class of the specific binding sites (Fig. 2A, picomolar range of the glycoprotein concentrations) shows a much higher affinity for transcortin variant, $K_d = (3.3 \pm 2.0) \cdot 10^{-12}$ mol/l, and a much lower capacity, $B_{max} = 3.3 \pm 2.1$ fmol transcortin variant per mg membrane protein, the binding reaction being non-cooperative: $H = 1.03 \pm 0.03$ ($n = 7$). Unexpectedly, we have found that normal transcortin could inhibit the specific membrane binding of transcortin variant to these high-affinity sites (see Table I). We therefore assumed that, in spite of our observations described above, transcortin could also bind to these sites. In a series of special experiments ($n = 3$) we used up to 700–900 μg membrane protein per assay tube (cf. Materials and Methods) and succeeded in determining the parameters of normal transcortin binding to these sites: $B_{max} = 3.0 \pm 2.2$ fmol transcortin per mg membrane protein, $K_d = (2.5 \pm 0.7) \cdot 10^{-11}$ mol/l. Thus, these sites display significantly lower affinity for transcortin than for its variant. It is peculiar that, in contrast to the transcortin-variant binding, the binding of normal transcortin to these sites is characterized by an apparent positive cooperativity: $H = 1.6 \pm 0.1$.

Discussion

Amongst the various biological functions attributed to the carbohydrate moieties of glycoconjugates, an important one is their ability to serve as specific determinants in biological recognition processes [20]. As most serum globulins, specific hormone-binding glycoproteins contain mainly N-linked oligosaccharide chains of the *N*-acetyllactosamine type [21]. It becames clear now that a unique structural organization of these oligosaccharide chains, a flexibility of the 'core' mannotriose unit and a comparative rigidity of the 'outer' antennae, allows them to adopt distinct conformations in each particular glycoprotein and even, likely, in each conformational state of a glycoprotein molecule (for a review see Ref. 22). So, these oligosaccharides might form unique spacial structures on the surface of glycoprotein molecules and, consequently, could serve a recognition function.

It should be noted that as early as in 1972 Winterburn and Phelps [23] offered a hypothesis that sugar chains of serum hormone-binding glycoproteins impart 'antigenic properties' to low-molecular-weight, hydrophobic hormones, thus favouring their recognition by the responsive cells. Although this hypothesis has yet been neither supported nor disproved, some circumstantial evidence favours it. Thus, individual features of the carbohydrate structures of three major hormone-binding glycoproteins from human serum, namely, transcortin, sex-hormone-binding globulin and thyroxine-binding globulin, offered a chemical basis for this hypothesis [21]. Moreover, it has been noted [24] that the pregnancy-associated transcortin variant binds to specific transcortin-binding sites in human liver plasma membrane less tightly than normal transcortin.

A comparative study of transcortin and its pregnancy-associated molecular variant [9] revealed that the polypeptide chains of both glycoproteins are, likely, identical, whereas their carbohydrate moieties are different. Transcortin contains three biantennary and two triantennary oligosaccharide chans of the *N*-acetyllactosamine type [10]. All five sugar chains of the transcortin variant are triantennary oligosaccharides of the same type [8]. This fact gave us a unique opportunity to elucidate the involvement of the transcortin carbohydrates in the glycoprotein binding to the plasma membrane of a hormone-target tissue without any chemical or enzymatic modification of the sugar chains, which could result in a partial transcortin denaturation.

The following reasons prompted us to use human placental syncytiotrophoblast obtained at 7–12 weeks of pregnancy. First, placenta is a glucocorticoid target tissue. A specific transport system for the glucocorticoid uptake from the maternal blood is located in the brush border of the syncytiotrophoblast plasma membrane [12]. In

human serum, glucocorticoids are largely bound to transcortin [6]. So, it could be expected that, complexed with cortisol, transcortin and/or its variant bind specifically to syncytiotrophoblast membrane. Second, we assumed that transcortin variant, appearing in the maternal blood at the end of the first trimester of pregnancy, takes part in steroid transport into a tissue(s) developing during gestation [9]. Consequently, the presence of specific binding sites for transcortin variant in syncytiotrophoblast plasma membrane could be expected. Third, normal trophoblast tissue can be obtained in rather large amounts, and the microvesicular membrane fraction that originates from the plasma membrane brush border can be prepared easily and quickly [17].

Two classes of specific binding sites for transcortin and its variant were found in the microvesicular fraction of syncytiotrophoblast membrane. Elucidation of the exact mechanisms of the binding reactions and their physiological significance must await further investigation. In the frameworks of the present paper, it is essential that both classes of the binding sites discriminate between transcortin and its variant. This suggests that the transcortin carbohydrate moiety takes part in the glycoprotein–membrane interaction.

When considering the way whereby the sugar chains modulate the transcortin membrane binding, it should be emphasized that enhanced glycosylation (addition of extra 'antennae' to the sugar chains of transcortin variant as compared with those of transcortin) affects in opposite manners the glycoprotein interaction with the two classes of binding sites. One class of the sites binds transcortin more tightly than its variant: the K_d values are $(1.6 \pm 0.5) \cdot 10^{-10}$ and $(4.5 \pm 1.2) \cdot 10^{-9}$ mol/l, respectively. An approximately 30-fold lesser affinity for transcortin variant than for normal transcortin could be explained as follows. The binding sites recognize certain areas on the surface of protein core of the transcortin molecule. Bulky triantennary oligosaccharide chains might shield these areas, thus hindering their interaction with the binding sites. A weaker interaction of transcortin variant with the transcortin-binding sites in human liver plasma membrane [24] could be similarly explained.

Such 'negative' role of carbohydrates in the glycoprotein–membrane interaction could not, however, explain the fact that another class of the binding sites displays a significantly higher affinity for transcortin variant, $K_d = (3.3 \pm 2.0) \cdot 10^{-12}$ mol/l, than for normal transcortin, $K_d = (2.5 \pm 0.7) \cdot 10^{-11}$ mol/l. In this case, the more the transcortin molecule is glycosylated the more tightly it binds to the membranes. Consequently, this class of the binding sites recognizes and binds oligosaccharide chains or their fragments having proper spacial orientation. Substitution of biantennary sugar chains for triantennary ones in the transcortin variant molecule probably results in a better fitting of the interacting segments of the molecules of transcortin and membrane binding component(s). Thus, the results obtained indicate that the carbohydrate chains of serum hormone-binding glycoproteins can be involved in specific binding of these glycoproteins to the plasma membranes of hormone target tissues.

Very recently, the data supporting the above conclusion have been obtained in this laboratory. It has been found that enzymatic desialylation of human sex-hormone-binding globulin completely inhibits its specific binding to human endometrium plasma membrane [25].

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