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On the functional form of transcortin-recognizing subunit of transcortin membrane receptor

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Complex formation between transcortin and the 20 kDa sialoglycoprotein from the plasma membrane of human decidual endometrium (presumably a transcortin-recognizing subunit of transcortin membrane receptor) was studied using cross-linking reagents. The action of 1,5-difluoro-2,4-dinitrobenzene (DFDNB) on a solution of ^{125}I-labelled 20 kDa sialoglycoprotein and unlabelled transcortin resulted in the formation of two ^{125}I-containing species that corresponded to covalently linked complexes of one transcortin molecule and either 2 or 4 molecules of the labeled membrane sialoglycoprotein. Only the latter complex was observed when the endometrium membranes were incubated with [^{125}I]transcortin and treated with DFDNB. This suggests that the functional form of transcortin-recognizing subunit of the membrane receptor is a tetramer.

Transcortin; Progesterone; Plasma membrane receptor; Bifunctional reagents; Human decidual endometrium

1. INTRODUCTION

Specific steroid-binding globulins of human plasma, transcortin and sex-hormone-binding globulin, have been shown to specifically interact with the plasma membranes of human steroid hormone target tissues, liver [1], decidual endometrium [2–5], prostate [6,7] and placental syncytiotrophoblast [8]. This suggests that receptors for the steroid-binding globulins occur in these membranes. Using affinity chromatography of ^{125}I-labeled, sodium cholate-solubilized decidual endometrium membranes on transcortin–Sepharose, we have recently shown that these membranes contain a sialoglycoprotein with a minimum molecular mass of 20 kDa, which forms complexes with transcortin, and consequently serves the function of a transcortin-recognizing subunit of the transcortin receptor [5]. In the present work, we used cross-linking reagents for investigating the stoichiometry of transcortin complex formation with the 20 kDa sialoglycoprotein in solution and in the membrane.

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Abbreviations: DFDNB, 1,5-difluoro-2,4-dinitrobenzene; MBS, *m*-maleimidobenzoic acid *n*-hydroxysuccinimide ester; DMS, dimethyl suberimidate; SDS-PAGE, SDS-electrophoresis in polyacrylamide gel

2. MATERIALS AND METHODS

2.1. *Chemicals*

CNBr-activated Sepharose 4B and protein standards for electrophoresis were purchased from Pharmacia (Uppsala, Sweden), *m*-maleimidobenzoic acid *n*-hydroxysuccinimide ester (MBS), dimethyl suberimidate (DMS), 1,5-difluoro-2,4-dinitrobenzene (DFDNB), Iodogen, and Iodobeads were purchased from Pierce (Rockford, IL, USA). Other chemicals were obtained from Serva (Heidelberg, FRG).

2.2. *Proteins*

Transcortin was isolated and radioiodinated as previously described [4]. ^{125}I-Labeled 20 kDa subunit of transcortin receptor was isolated from the radioiodinated plasma membrane preparation of human decidual endometrium by affinity chromatography on transcortin–Sepharose [5]. Briefly, the membranes were isolated from homogenized endometrium by density-gradient centrifugation, radiolabeled using [^{125}I]sodium iodide and Iodobeads and solubilized with sodium cholate. The solubilizate was applied on a transcortin–Sepharose column. The column was thoroughly washed and the labeled 20 kDa subunit was then eluted with Krebs buffer containing 1 g/l transcortin and 2×10^{-6} M progesterone. (The steroid was included in all the buffers used throughout the purification procedure because it had been previously demonstrated that the transcortin-receptor interaction was steroid-dependent [5].) The eluate was freeze-dried, dissolved in a small volume of distilled water and filtered through a Bio-Gel P-6 (Bio-Rad, Richmond, CA, USA) column equilibrated with 0.01 M sodium phosphate buffer, pH 6.8, containing 0.1 g/l transcortin and 1×10^{-6} M progesterone. This affinity eluate was found to include only two proteins, unlabeled transcortin and ^{125}I-labeled 20 kDa subunit of the transcortin receptor (Fig. 1A).

2.3. *Cross-linking experiments*

To study the complex formation between transcortin and the 20 kDa receptor subunit in solution, one of the above bifunctional reagents dissolved in dimethyl sulfoxide was added to the affinity eluate to a final concentration of 0.2 g/l. The mixture was then incubated for 18 h at 4°C with continuous stirring. After that, 1 M Tris-

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HCl, pH 7.4, was added to the samples (one tenth of the sample volume). Samples were incubated at room temperature for 30 min and desalted by filtration through a Bio-Gel P-6 column.

To study the transcortin complexing with the membrane components, [^{125}I]transcortin was added to the unlabeled membrane suspension in Krebs buffer containing 5×10^{-6} M progesterone. Into a parallel set of samples, a 500-fold molar excess of unlabeled transcortin was introduced in order to evaluate nonspecific [^{125}I]transcortin interaction with the membrane components. The samples were incubated for 18 h at 4°C with shaking. Membranes were sedimented by centrifugation for 15 min at 4000 × g, and supernatant was discarded. The membrane pellets were twice washed with 0.01 M sodium phosphate buffer, pH 6.8, and suspended in the same buffer. DFDNB dissolved in dimethyl sulfoxide was added to the samples up to a final concentration of 0.2 g/l. The samples were incubated overnight at 4°C. Then the unreacted substances were removed by washing the membranes with Krebs buffer containing 1 g/l ovalbumin (3 times) and distilled water (3 times).

To study the requirement of transcortin for the cross-linking of 20 kDa receptor subunit, we have carried out the following experiment. A preparation of the plasma membranes was divided into 2 parts. After treating one of them with DFDNB in the absence of transcortin, both parts were radioiodinated, solubilized, and the radiolabeled transcortin-binding components were isolated as above in parallel chromatography runs using 2 similar transcortin-Sepharose columns.

The samples of the affinity eluates prepared from both intact and DFDNB-treated membranes, affinity eluate treated with various cross-linking reagents, and membrane suspension cross-linked with transcortin were analyzed by SDS-PAGE [9].

3. RESULTS AND DISCUSSION

We have recently demonstrated [5] that purified 20 kDa subunit of the transcortin receptor, dissolved in a buffer without detergents, tended to form an oligomer. But the functional form of this receptor subunit in the membrane remained unknown. To elucidate the stoichiometry of its complexing with transcortin, we have used 3 bifunctional reagents, MBS, DMS and DFDNB.

At the first stage of the work, we have studied the cross-linking of transcortin with the purified 20 kDa receptor subunit in solution. With this aim, we treated the affinity eluate containing this subunit labeled with ^{125}I and a great excess of unlabeled transcortin (complexed with progesterone, which was a requirement for the transcortin interaction with the membrane receptor [5]) with the above bifunctional reagents. It has been previously demonstrated that, in this eluate, the 20 kDa receptor subunit is tightly complexed with transcortin [5]. As seen from Fig. 1A, the eluate contained only 2 proteins, unlabeled transcortin and radioiodinated receptor subunit.

SDS-PAGE of the eluate treated with MBS and DMS revealed that these bifunctional reagents were ineffective in cross-linking transcortin-membrane receptor subunit complex: only a radioactive band corresponding to the ^{125}I-labeled 20 kDa protein was observed.

When the affinity eluate was treated with DFDNB, 2 other radioactive bands were found (Fig. 1B). These bands corresponded to protein species with molecular masses of 92 and 136 kDa. Based upon the transcortin molecular mass of 55 kDa [10], we have concluded that these species represent covalently linked complexes of transcortin with 2 and 4 protomers of the receptor subunit, respectively.

Effective cross-linking of transcortin and the receptor subunit with DFDNB indicates close contact between the 2 proteins, since this bifunctional reagent can link reactive groups located at the distance of 3-5 Å [11]. We cannot as yet explain the ineffectiveness of the two other bifunctional reagents. A possible explanation is that the hydroxyl groups of the sugar residues of transcortin and/or the receptor subunit were involved in the cross-linking with DFDNB whereas the polypeptide chains of the two glycoproteins were at a relatively large distance from one another. Such explanation would be consistent with our findings on the involvement of transcortin sugar chains in the interaction with the other membrane receptors [12].

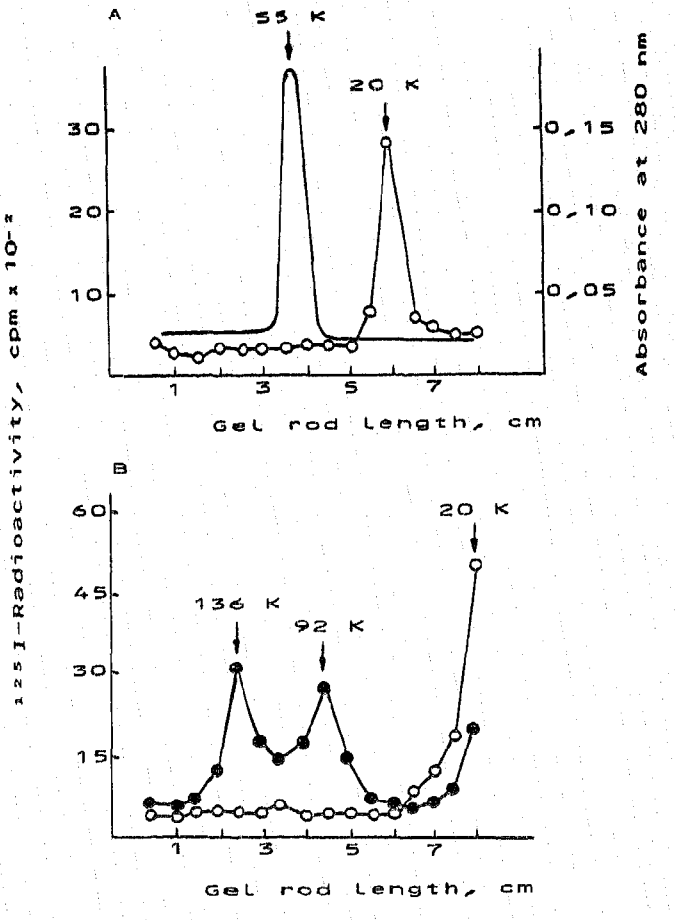

Fig. 1. (A) SDS-PAGE in 15% gel of the affinity eluate containing ^{125}I-labeled membrane 20 kDa receptor subunit and unlabeled transcortin complexed with progesterone: ○ = profile of radioactivity, — = absorbance at 280 nm. (B) SDS-PAGE in 5% gel of the same eluate with (●) and without (○) the treatment with DFDNB.

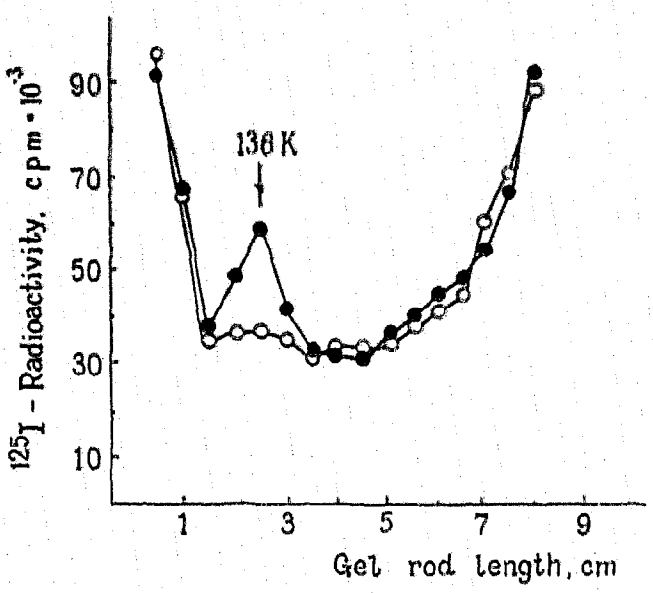

Fig. 2. SDS-PAGE in 5% gel of the endometrium membranes incubated with [^{125}I]transcortin in the presence (○) and in the absence (●) of excess unlabeled transcortin and treated with DFDNB.

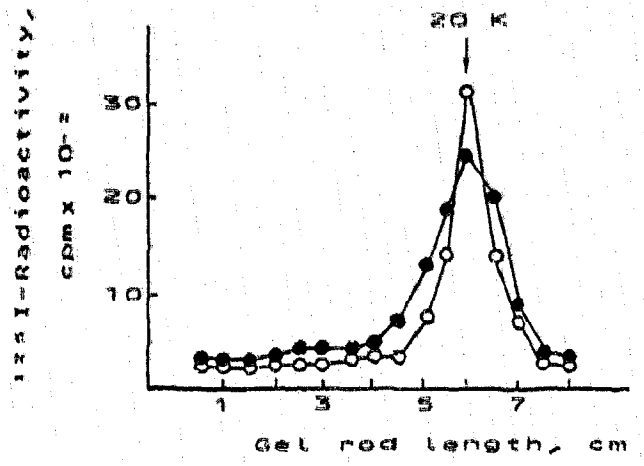

Fig. 3. SDS-PAGE in 5% gel of ^{125}I-labeled transcortin-binding component isolated from the radioiodinated plasma membranes pretreated with DFDNB (●) in comparison with the same component isolated without the pretreatment of the membranes with the cross-linking reagent (○).

In order to determine the functional form of the transcortin-recognizing receptor subunit in the membrane, a suspension of the unlabeled membranes was incubated with [^{125}I]transcortin-progesterone complex either in the absence or in the presence of excess unlabeled transcortin (to evaluate nonspecific cross-linking) and treated with DFDNB. In this case, SDS-PAGE (Fig. 2) revealed only one discrete radioactive band which corresponded to a protein species with a molecular mass of 136 kDa. Only this discrete band disappeared when excess unlabeled transcortin was present in the samples. This means that this band corresponds to a specific complex of labeled transcortin with the component(s) of transcortin receptor. It is evident that, as in the case of the cross-linking in solution, this complex consists of one molecule of transcortin and 4 molecules of 20 kDa receptor subunit.

The transcortin requirement for the covalent cross-linking of the 20 kDa receptor subunit was finally proved by an experiment in which we compared the molecular masses of ^{125}I-labeled receptor components isolated by affinity chromatography on transcortin-Sepharose from the intact and DFDNB-treated radioiodinated membranes. Fig. 3 shows that the pretreatment of the membranes with the cross-linking reagent had no effect on the SDS-electrophoresis pattern of the purified receptor components: the only observed radioactive band corresponded to a monomeric form of the 20 kDa subunit. This means that, although one transcortin molecule interacts with 4 20 kDa molecules, the latter can be cross-linked with DFDNB only to transcortin but not to one another.

Collectively, the data obtained in this work give evidence that the functional form of the transcortin-recognizing subunit of transcortin membrane receptor is a tetramer.

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