The strong hydrophobic domain of the activated estrogen receptor of porcine uterus

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Basic estrogen receptor (ER) molecule (*vero*-ER) of porcine uterus, which was previously shown to be the activated ER necessary to translocate from the cytoplasm into the nucleus, possesses a strongly hydrophobic nature. The strong hydrophobicity of *vero*-ER was concealed through binding with ER-binding factors (ERBFs). *Vero*-ER lost its strong hydrophobicity and its capability to bind with ERBFs after limited proteolysis by endogenous protease. The strong hydrophobic domain of *vero*-ER, indispensable for the nuclear translocation, was assumed to be located near the binding site with ERBFs.

Estrogen receptor *Hydrophobicity* *Activated receptor* *Receptor-binding factor* *Steroid hormone* *Hormone action*

1. INTRODUCTION

Steroid hormone receptors are extremely susceptible to modifications by the proteases present in the target tissues [1–3]. By utilizing antipain [4], a protease inhibitor of microbial origin, we showed previously that there is one basic estrogen receptor (ER) molecule (*vero*-ER) (M_r 82000) in the estrogen target tissues [5,6]. *Vero*-ER interacted specifically with the endogenous protein components designated as ER-binding factors (ERBFs) ['5S' ER-forming factor ('5S' ER-FF), (component A, M_r 58000); '6S' ER-FF, (component B, M_r 13700)$_6$; '7S' ER-FF, (component B)$_2$ · (component A); '8S' ER-FF, (component B)$_6$ · (component A)] to form ERs ['5S' ER, (*vero*-ER) · (component A); '6S' ER, (*vero*-ER) · (component B)$_6$; '7S' ER, (*vero*-ER) · (component B)$_2$ · (component A); '8S' ER, (*vero*-ER) · (component B)$_6$ · (component A)] with various molecular constitutions [6–8] (fig.1). Steroid hormone receptor in the cytoplasm undergoes activation to acquire the capability to translocate into the nucleus [9–12]. It was previously shown that under activation conditions of the cytoplasmic ER, *vero*-ER is dissociated from its original binding with ERBFs [8,13]. *Vero*-ER was shown to be the activate ER necessary to translocate from the cytoplasm into the nucleus [13,14]. It was recently proposed that a similar molecular mechanism may be applicable also for the activation of the glucocorticoid receptor [15]. We report here that *vero*-ER possesses an extremely hydrophobic domain, which is concealed through binding with ERBFs.

Abbreviations: ER, estrogen receptor; ERBF, estrogen receptor-binding factor; '5S' ER-FF, '5S' estrogen receptor-forming factor; TEMA buffer, 10 mM Tris–HCl, 1.5 mM EDTA, 1.5 mM 2-mercaptoethanol, 0.25 mM antipain, pH 8.0 at 2°C

This paper is dedicated to Professor F. Cramer on the occasion of his 60th birthday

2. MATERIALS AND METHODS

Antipain was purchased from Protein Research Foundation (Osaka). [2,4,6,7,16,17-^{3}H]Estradiol-17β (147 Ci/mmol) ([^{3}H]estradiol) was purchased

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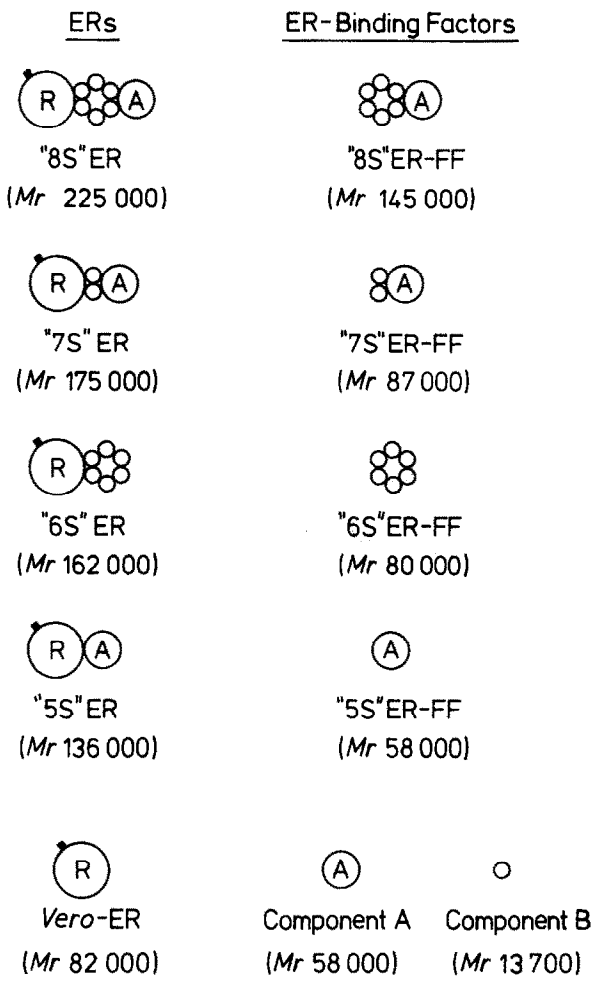

Fig.1. Uterine *vero*-ER, ERBFs and the complexes [6,8].

from Amersham International (England). Phenyl-Sepharose CL-4B was from Pharmacia (Uppsala). Cytosol was prepared from fresh porcine uteri in TEMA buffer (10 mM Tris–HCl, 1.5 mM EDTA, 1.5 mM 2-mercaptoethanol, 0.25 mM antipain, pH 8.0 at 2°C) and labeled with [^{3}H]estradiol as in [5]. *Vero*-ER (sedimentation coefficient 4.5 S; Stokes radius 44 Å) freed from ERBFs was prepared from the labeled cytosol as in [5] by a two-step gel filtration on Sephadex G-150 columns carried out in TEMA buffer in the presence of 0.4 M KCl, and then in the presence of 0.4 M NaSCN. Partial proteolysis of *vero*-ER into *secto*-ER (sedimentation coefficient 4.5 S; Stokes radius 35 Å) or '3.8S' ER (sedimentation coefficient 3.8 S; Stokes radius 32 Å) by the endogenous protease was carried out as in [5,6]. Partially purified '5S' ER-FF, '6S' ER-FF and '8S' ER-FF were prepared from the cytosol of porcine uterus as in [6,7]. Reconstructed '5S' ER, '6S' ER and '8S' ER were prepared from *vero*-ER and the corresponding ERBFs ('5S' ER-FF, '6S' ER-FF and '8S' ER-FF) as in [6,7]. Sucrose density gradient centrifugation of ERs was carried out in 5–20% sucrose gradients in TEMA buffer as in [5].

3. RESULTS AND DISCUSSION

We showed previously that *vero*-ER of the uterine cytosol is dissociated from ERBFs in high-salt (0.4 M KCl) conditions, but is complexed with '8S' ER-FF in low-salt conditions [6–8]. ER of the cytosol of porcine uterus sedimented at 8 S in TEMA buffer, and at 4.5 S in TEMA buffer containing 0.4 M KCl (fig.2A). When the ER of the uterine cytosol labeled with [^{3}H]estradiol was ap-

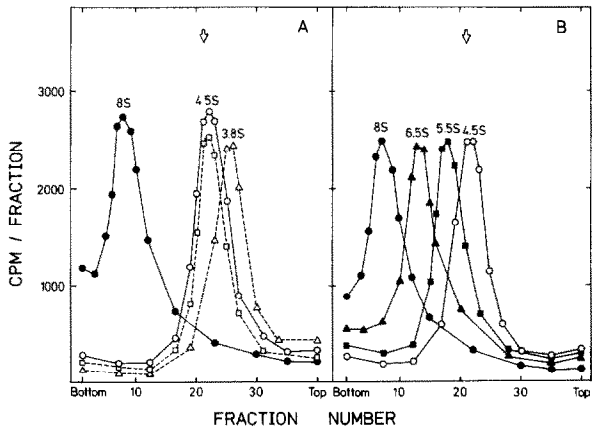

Fig.2. Sedimentation analysis of cytosolic and reconstructed ERs. (A) The cytosolic ER and the proteolyzed ERs were analyzed by sucrose density gradient centrifugation in TEMA buffer in the presence or absence of 0.4 M KCl. Labeled cytosol in TEMA buffer (—●—); labeled cytosol in TEMA buffer containing 0.4 M KCl (—○—); *secto*-ER in TEMA buffer (---□---); '3.8S' ER in TEMA buffer (---△---). (B) Reconstructed ERs were analyzed by sucrose density gradient centrifugation in TEMA buffer. *Vero*-ER (—○—); reconstructed '5S' ER (—■—); reconstructed '6S' ER (—▲—); reconstructed '8S' ER (—●—). The arrows mark the peak of [^{14}C]BSA (4.6 S) as an internal marker.

plied to a phenyl-Sepharose column in TEMA buffer in the presence of 0.4 M KCl, ER was attached to the column (8×10^5 cpm ER/ml phenyl-Sepharose). The attached ER could not be eluted from the column with TEMA buffer. However, when the ER of the uterine cytosol labeled with [^{3}H]estradiol was applied to a phenyl-Sepharose column in the absence of KCl, ER passed straight through the column. The results seemed to suggest the presence of a strong hydrophobic domain in *vero*-ER, which is concealed through binding with '8S' ER-FF. Accordingly, we analyzed in detail the hydrophobicity of *vero*-ER freed from ERBFs and the ERs reconstructed from *vero*-ER and ERBFs. In fig.2B are shown the sedimentation patterns of *vero*-ER and the reconstructed ERs. *Vero*-ER freed from ERBFs was attached to the phenyl-Sepharose column when applied under the low-salt conditions (TEMA buffer) (fig.3A). The attached *vero*-ER could not be eluted even with TEMA buffer containing 50% ethylene glycol. To elute *vero*-ER from the column, it was necessary to utilize ethanol as a polarity-reducing agent. *Vero*-ER could be eluted quantitatively from the column with TEMA buffer containing 30% ethanol (fig.3A). [^{3}H]Estradiol activity of the eluate did not diminish throug the dialysis against TEMA buffer, indicating that the dissociation of [^{3}H]estradiol from the receptor during the elution process was negligible. These results demonstrated the extremely strong hydrophobicity of *vero*-ER.

In contrast, reconstructed '5S' ER, '6S' ER and '8S' ER passed straight through the phenyl-Sepharose column when applied in TEMA buffer (fig.3B). This indicated that the strong hydrophobic domain of *vero*-ER is concealed through binding with ERBFs. The results also suggested that the hydrophobic domain of *vero*-ER is located near the binding site with ERBFs.

We reported previously that the binding site of *vero*-ER with ERBFs is easily proteolyzed by endogenous proteases [1,5,6]. The proteolyzed ERs [*secto*-ER (M_r 65000); '3.8S' ER (M_r 50000)] did no longer interact with ERBFs [1,5,6], and did not translocate into the nucleus [14]. Both *secto*-ER and '3.8S' ER passed straight through the phenyl-Sepharose column under the low-salt conditions (fig.3C), indicating that the strongly hydrophobic domain is removed by limited proteolysis of *vero*-ER. These observations also supported the

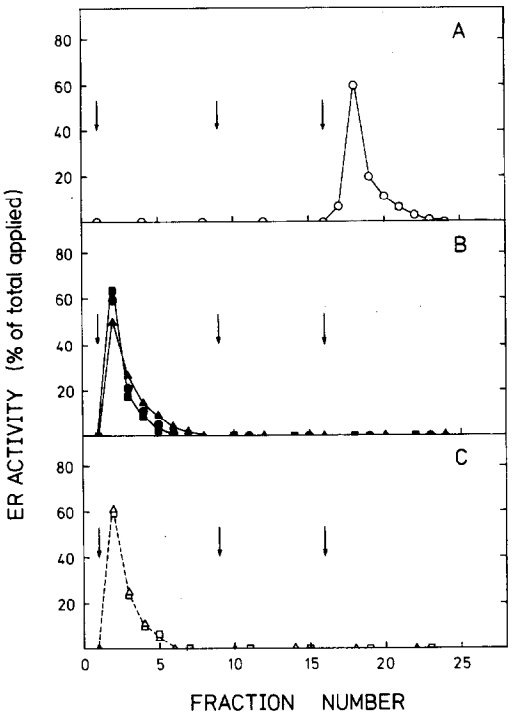

Fig.3. Analysis of the hydrophobicity of *vero*-ER, the complexes of *vero*-ER with ERBFs and the proteolyzed ERs with a phenyl-Sepharose column. 4×10^5 cpm of ERs in 2 ml of TEMA buffer were applied (beginning at the first arrow) to 0.5 ml (0.9×0.8 cm) columns pre-equilibrated with TEMA buffer. The columns were then washed 7-times with 2 ml TEMA buffer, and subsequently 7-times with 2 ml TEMA buffer containing 50% ethylene glycol (beginning at the second arrow). The columns were finally washed 7-times with 2 ml of TEMA buffer containing 30% ethanol (beginning at the third arrow). The ER activities of the eluates are expressed as % of the total amount applied: (A) *vero*-ER (—○—); (B) reconstructed '5S' ER (—■—); reconstructed '6S' ER (—▲—); reconstructed '8S' ER (—●—); (C) *secto*-ER (---□---); '3.8S' ER (---△---).

assumption that the binding site of *vero*-ER with ERBFs and the strongly hydrophobic domain are situated in sterically close proximity.

We showed previously that *vero*-ER translocates from the cytoplasm into the nucleus without suffering any proteolysis [16]. The strongly hydrophobic domain of *vero*-ER is expected to play indispensable roles in the subsequent reactions of the receptor in the nucleus to trigger the biological response of the target cell.

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