Volume 267, number 1, 157-159 FEBS 08579 July 1990

Reconstitution of the 9 S estrogen receptor with heat shock protein 90

Kouichi Inano, Makoto Haino, Masaomi Iwasaki, Naomi Ono, Tsuneyoshi Horigome and Hiroshi Sugano

Department of Biochemistry, Faculty of Science, Niigata University, 2-Igarashi, Niigata 950-21, Japan

Received 10 May 1990

As a first step in the investigation of the reconstitution of steroid hormone receptor systems, we studied the reconstitution of 9 S estrogen receptor (ER) from purified vero ER, which is the estradiol binding subunit, and heat shock protein 90 (hsp 90) By using a phosphate buffer containing molybdate, thiocyanate, dimethylformamide, glycerol, etc., vero ER could be converted to 9 S ER with hsp 90, but not with the control protein, ovalbumin. Inactivation of ER during the reconstitution was suppressed partially by hsp 90, but not by ovalbumin. Like native 8 S ER, the reconstituted ER was sedimented at about 8.9 S and 4.6 S on glycerol gradient centrifugation in low and high salt buffers, respectively.

Estrogen receptor, Estrogen; Steroid hormone receptor, Hsp90, Heat shock protein, Reconstitution

1. INTRODUCTION

The transformation of a steroid hormone receptor through the binding of a specific hormone is one of the key steps during the course of steroid hormone signaling in target cells. Therefore, there have been many studies on the transformation mechanism and the structure of a receptor. In 1984, it was shown that non-transformed 8 S steroid hormone receptors contain vero receptors, which can bind specific hormones, and a 90 kDa protein [1]. Sanchez et al. demonstrated, using monoclonal antibodies, that the non-hormone-binding subunit of the 8 S glucocorticoid receptor is hsp 90, which is a very common cytosolic protein [2]. Later, it was also found in the progesterone [3], esterogen [4,5] and androgen [5] receptor systems. In an ER system, it was suggested that the non-transformed 8-9 S ER consisted of two vero receptors and two hsp 90 subunits, as judged by analysis of extensively purified 8 S ER [4]. On the other hand, reconstitution studies of 8 S steroid hormone receptors are still in very early stages. That is, 8 S receptor promoting factors have not yet been highly purified, these factors seeming to be different from hsp 90, though there have been some studies on the reconstitution of 8 S estrogen [6,7] and androgen [8] receptors. The reconstitution of any 8 S receptors from purified vero receptors and hsp 90 has not yet been reported. Therefore, we have investigated the reconstitution conditions for 8 S ER, from purified vero ER and hsp 90, as the first of studies on the transformation mechanism. With the simple mixing of these components, vero ER was not converted to 9 S ER. Then we examined many conditions which might facilitate the formation and stabilization of 9 S ER, and found conditions under which 9 S ER could be reconstituted.

Correspondence address: T. Horigome, Department of Biochemistry, Faculty of Science, Niigata University, 2-Igarashi, Niigata 950-21, Japan

Abbreviations: ER, estrogen receptor; hsp 90, heat shock protein with a molecular mass of about 90 kDa; E_2, 17β-estradiol; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Chaps, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; DMF, *N,N*-dimethylformamide

2. MATERIALS AND METHODS

2.1. *Materials*

The radioactive hormone, 17β-[6,7-^{3}H(N)]estradiol (sp. act. 48.3 Ci/mmol), was purchased from New England Nuclear. Antiserum against rat hsp 90 was a gift from Dr Takayuki Nemoto (Iwate Medical University, Morioka, Japan). The antibody has been previously characterized to react with hsp 90 [9] and non-transformed 9 S ER [5].

2.2. *Purification of vero ER and hsp 90*

Vero ER labeled with [^{3}H]E_2 (spec. act. 10 Ci/mmol) was purified from calf uterus using an estradiol-liked affinity resin, as previously described [10], except that the affinity column was washed once with a buffer containing 0.7 M KCl instead of 3 cycles of washing with 3 kinds of buffers after application of a sample. Vero ER was purified about 3000-fold by this method. Pure hsp 90 was obtained from a post-mitochondrial supernatant fraction of rat liver by successive DEAE-cellulose, hydroxyapatite and Sephacryl S-300 column chromatographies, as described previously [11].

2.3. *Reconstitution and analysis of 9 S ER*

Reconstitution was carried out at 4°C. Purified vero ER labeled with [^{3}H]E_2, 75 ng, and purified hsp 90 or the control protein, ovalbumin, 60 μg, were mixed in 0.4 ml of 18 mM Hepes-KOH buffer (pH 7.5 at 20°C) containing 2 mM Chaps, 7% (v/v) DMF, 0.5 M NaSCN and 12% (v/v) glycerol. Then the mixture was dialyzed against 10 mM potassium phosphate buffer (pH 7.5) containing 20 mM Na_2MoO_4, 2 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 5% (v/v) glycerol and

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

10 mM thioglycerol (dialyzing buffer) for 6 h. The reconstituted ER thus obtained was layered on a 10–35% (v/v) glycerol density gradient in 10 mM potassium phosphate buffer (pH 7.5) containing 20 mM Na_2MoO_4 and 10 mM thioglycerol, followed by ultracentrifugation at 140 000 × g for 15 h at 4°C. Sedimentation coefficients were determined using [^{14}C]ovalbumin (3.6 S) and [^{14}C]γ-globulin (6.6 S) as standard proteins.

2.4 *Measurement of the dissociation of E_2 from vero ER and reconstituted 9 S ER*

Vero ER and the reconstituted 9 S ER labeled with [^{3}H]E_2 were incubated at 28°C in 10 mM potassium phosphate buffer (pH 7.5) containing 67 nM nonradioactive E_2, 20 mM Na_2MoO_4, 2 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 5% (v/v) glycerol and 10 mM thioglycerol. At the times indicated in the figure, an aliquot was taken, incubated with hydroxyapatite at 0°C, and then washed with 10 mM Tris-HCl buffer (pH 7.6) containing 1.5 mM EDTA, 3 mM $MgCl_2$ and 10% (v/v) glycerol. Then radioactivity remaining on the hydroxyapatite was measured with a liquid scintillation counter.

3. RESULTS AND DISCUSSION

For reconstitution of 9 S ER from purified vero ER and hsp 90, we carried out many preliminary experiments with various conditions and finally chose the conditions given in section 2. Sodium molybdate and phosphate buffer present in the reconstitution medium are known to stabilize native non-transformed ER [12,13], and zinc chloride has been reported to be effective for reconstitution of the 8.6 S receptor in a crude androgen receptor system [14]. Therefore, we added these substances to the dialyzing buffer for the reconstitution of 9 S ER.

The glycerol gradient centrifugation pattern of the ER reconstituted from vero ER and hsp 90 is shown in Fig. 1. The reconstituted ER was sedimented at 9.2 S. Vero ER treated under the same conditions in the absence of hsp 90 was sedimented at 5.2 S. Moreover, when the reconstitution processes were performed with the control protein, ovalbumin, instead of hsp 90, the ER was sedimented at 5.2 S (Fig. 1, right). To know whether the reconstituted 9 S ER contains ksp 90, the reconstituted ER was preincubated with antiserum against hsp 90 and analyzed by glycerol density gradient. A large shift of the 9 S ER from 9.3 to 11.1 S was observed (data not shown). This result suggested that the reconstituted 9 S ER contained hsp 90. From these results, we concluded that 9 S ER was reconstituted from vero ER and hsp 90 under the reconstitution conditions. The sedimentation coefficients and recoveries of the reconstituted ER are summarized in Table I. The sedimentation coefficients of ER reconstituted in the presence and absence of hsp 90 were similar to those of the native non-transformed and transformed ER, respectively [4,15]. As can be seen in Table I, the recovery of ER was not so high, because a part of it became denatured and aggregated during the reconstitution processes, whereas the recoveries of ER processed with hsp 90 were higher than those without hsp 90 in all experiments. The average recovery in the presence of hsp 90 was 1.5-fold that in the case of the control protein, as can be seen in Table I. When

Table I

Sedimentation coefficients and recoveries of the reconstituted ER

Reconstitution	Sedimentation coefficient (S)	Recovery of ER[a] (%)
Hsp 90 (+)	8.9 ± 0.7 (n = 5)	33
Hsp 90 (−)[b]	4.9 ± 0.4 (n = 5)	22

[a] The recovery was calculated from the amount of ER recovered in fractions 1–20 of the sedimentation analysis was the same as in Fig. 1. The values are the means of 4 experiments

[b] The reconstitution procedures were performed in the absence of hsp 90 as a control

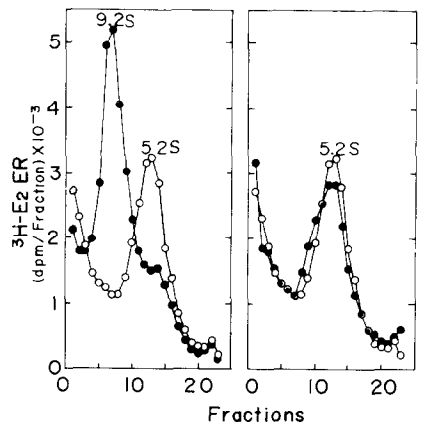

Fig. 1. Sedimentation analysis of reconstituted ER. 9 S ER was reconstituted from purified vero ER labeled with [^{3}H]E_2 and hsp 90, and analyzed by centrifugation on a 10–35% (v/v) glycerol gradient as described in section 2. (Left) Reconstitution was carried out in the presence (●) or absence (○) of hsp 90. (Right) As a control, reconstitution was carried out in the presence (●) or absence (○) of ovalbumin instead of hsp 90.

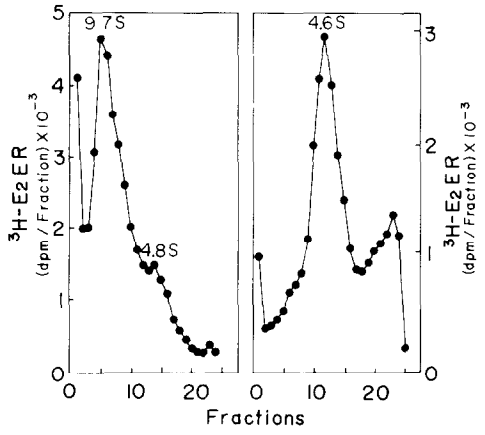

Fig. 2. Salt-transformation of reconstituted 9 S ER. (Left) Reconstituted ER labeled with [^{3}H]E_2 was analyzed by glycerol density gradient centrifugation in a low salt buffer as described in section 2. (Right) Reconstituted ER labeled with [^{3}H]E_2 was incubated in 10 mM Tris-HCl buffer (pH 7.6) containing 0.7 M KCl and 1 mM dithiothreitol at 4°C for 10 min, and then analyzed as left, but with the use of a density gradient containing 0.7 M KCl.

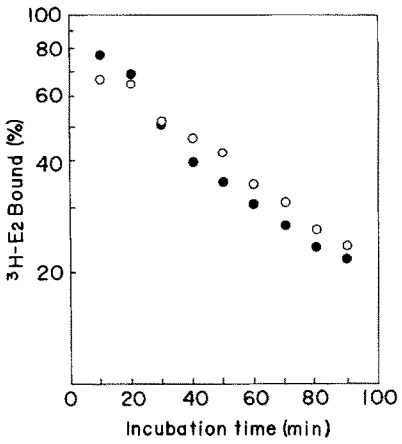

Fig. 3. Dissociation of E_2 from vero ER and reconstituted 9 S ER. Vero ER (○) and the reconstituted 9 S ER (●) labeled with [^{3}H]E_2 were incubated at 28°C, and then dissociation of [^{3}H]E_2 was measured as described in section 2.

ovalbumin was used instead of hsp 90, the recovery of ER was as low as that in the absence of hsp 90 (data not shown). These results may be a reflection of a putative function of hsp 90, that is, hsp 90 binds to denatured or unfolded proteins and thus prevents the aggregation of the proteins.

Some properties of the reconstituted 9 S ER were determined to characterize it. It is known that the native 8 S ER is transformed on incubation with a high salt concentration [15,16]. The reconstituted 9 S ER was incubated in a buffer containing 0.7 M KCl and then analyzed by glycerol gradient centrifugation in a high salt buffer (Fig. 2). The sedimentation coefficient decreased from 9.7 S to 4.6 S. This decrease suggested that the reconstituted 9 S ER dissociated into vero ER and hsp 90 like the native one.

We next examined the kinetics of the dissociation of E_2 from reconstituted 9 S and vero ERs. When [^{3}H]E_2-ER complexes were incubated at 28°C, almost the same dissociation rate was observed for the two preparations (Fig. 3). There have been many reports that the rate of dissociation of E_2 from native 8 S ER is faster than that from 4 S or 5 S ER [17–19]. But Redeuilh et al. demonstrated that there was a state of 8–9 S ER which showed dissociation of E_2 as slow as that in the case of 4 S ER, and suggested that there were small but functionally significant changes in the structure of the 8–9 S ER remaining in a non-DNA-binding form, whereas the rate of E_2 dissociation was lowered [19]. The slow dissociation of E_2 from the reconstituted 9 S ER might be due to a similar structural change. Other properties of the reconstituted ER are being investigated further.

As described above, we reconstituted 9 S ER from vero ER and hsp 90. The results were not consistent with those of Murayama et al. [6], because they reconstituted 8 S ER from one vero ER (82 kDa), one component A (58 kDa) and six components B (13.7 kDa), and their system did not contain any component with a molecular mass larger than that of vero ER, such as hsp 90 [7]. Our reconstitution results are consistent with the analytical results for purified 8–9 S ER [4,5], that is, 8–9 S ER is composed of vero ER and hsp 90.

The reconstitution method described in this paper will be applicable to the analysis of other steroid hormone and dioxin [20] receptor systems, because all these systems contain 8–10 S receptors with hsp 90 as a component.

Acknowledgements: The authors wish to thank Dr Takayuki Nemoto for the generous gift of antiserum against rat hsp 90 and Masahide Yamamoto for his technical assistance.

REFERENCES

[1] Joab, I., Radany, C. Renoir, M , Buchou, T., Catelli, M.G., Binart, N., Mester, J. and Baulieu, E.E. (1984) Nature 308, 850–853.
[2] Sanchez, E R., Toft, D.O., Schlesinger, M.J. and Pratt, W.B. (1985) J. Biol. Chem. 260, 12398–12401.
[3] Schuh, S., Yonemoto, W., Brugge, J., Bauer, V.J., Riehl, R.M., Sullivan, W.P. and Toft, D.O. (1985) J. Biol. Chem. 260, 14292–14296.
[4] Redeuilh, G., Moncharmont, B , Secco, C. and Baulieu, E.E. (1987) J. Biol. Chem. 262, 6969–6975.
[5] Nemoto, T., Ohara-Nemoto, Y. and Ota, M. (1987) J. Biochem. 102, 513–523.
[6] Murayama, A., Fukai, F., Hazato, T. and Yamamoto, T. (1980) J. Biochem. 88, 963–968.
[7] Murayama, A , Fukai, F. and Yamamoto, T. (1980) J. Biochem. 88, 969–976.
[8] Colvard, D.S. and Wilson, E.M. (1981) Endocrinology 109, 496–504.
[9] Ohara-Nemoto, Y., Nemoto, T., Sato, N. and Ota, M. (1988) J. Steroid Biochem. 31, 295–304.
[10] Horigome, T., Golding, T.S., Quarmby, V.E., Lubahn, D.B., McCarty, K., Sr, and Korach, K.S. (1987) Endcrinology 121, 2099–2111.
[11] Iwasaki, M., Saito, H., Yamamoto, M., Korach, K.S., Horigome, T. and Sugano, H. (1989) Biochim. Biophys. Acta 992, 1–8.
[12] Shyamala, G. and Leonard, L. (1980) J. Biol. Chem. 255, 6028–6031.
[13] Mauck, L.A., Day, R.N. and Notides, A C. (1982) Biochemistry 21, 1788–1793.
[14] Wilson, E.M. (1985) J. Biol. Chem. 260, 8683–8689.
[15] Redeuilh, G., Secco, C., Baulieu, E.E. and Richard-Foy, H. (1981) J. Biol. Chem. 256, 11496–11502
[16] Muller, R.E., Traish, A.M. and Wotiz, H.H. (1983) J. Biol. Chem. 258, 9227–9236.
[17] Weichman, B.M. and Notides, A.C. (1977) J. Biol. Chem. 252, 8856–8862.
[18] Muller, R.E., Traish, A.M., Hirota, T., Bercel, E. and Wotiz, H.H. (1985) Endocrinology 116, 337–345.
[19] Redeuilh, G., Secco, C., Mester, J. and Baulieu, E.E. (1987) J. Biol. Chem. 262, 5530–5535.
[20] Perdew, G.H. (1988) J. Biol. Chem. 263, 13802–13805.