Immunorecognition of the active form of the oestrogen receptor by using a monoclonal antibody

Nora GIAMBIAGI and Jorge R. PASQUALINI*

C.N.R.S. Steroid Hormone Research Unit, Foundation for Hormone Research, 26 Boulevard Brune,
75014 Paris, France

(Received 12 February 1985/15 April 1985; accepted 24 April 1985)

In previous studies, two forms (α and β) of the oestrogen receptor, with different immunological characteristics, were observed in the cytosol fraction of fetal guinea-pig uterus, by using a monoclonal antibody to the human oestrogen receptor (D547Sp γ). Only the α form was recognized by the antibody, shifting its sedimentation coefficient in high-salt sucrose gradients. The present work investigated the effect of several factors (time, temperature, high salt concentrations and Na_2MoO_4) on the interconversion of these two forms. Only the β form was observed when cytosol was incubated with oestradiol for only 2–3 h, but 20 h later, 40–60% of the total oestradiol–receptor complexes were found as the α form. The transformation from the β to the α form was accelerated by temperature (25°C, 15 min) and exposure to high salt concentrations (0.4 M-KCl). On the other hand, Na_2MoO_4 completely blocked the transformation induced by time and temperature, but had little effect on that induced by KCl. The appearance of the α form always correlated with an increase in receptor binding to nuclei and DNA–cellulose. Finally, it was found that the isolated β form, recovered from the gradient, was transformed into the α form after overnight dialysis under reduced pressure. The present data suggest that the α form, which is recognized by the monoclonal antibody, is the activated form of the oestrogen receptor.

It is generally accepted that the primary action of steroid hormones is mediated by specific high-affinity receptor proteins. On binding to the hormone, cytosol receptors translocate to the nuclei (Jensen *et al.*, 1968; Giannopoulos & Gorski, 1971), where they interact with chromatin to modulate the transcription of specific genes (O'Malley *et al.*, 1976). From observations *in vitro*, translocation of the steroid–receptor complexes to the nucleus requires a change in the properties of the cytosol receptor, which increases its affinity for nuclear components (Jensen *et al.*, 1972; Grody *et al.*, 1982). This process, referred to as 'activation', can be achieved by various procedures, including heating (Jensen *et al.*, 1971), exposure to high ionic strength (Milgrom *et al.*, 1973; Bailly *et al.*, 1980), gel filtration, dilution or dialysis (Goidl *et al.*, 1977; Sato *et al.*, 1979).

Previous studies in our laboratory have demonstrated the presence of specific binding sites for

oestrogens in the fetal guinea-pig uterus (Pasqualini & Nguyen, 1976; Sumida & Pasqualini, 1979). Using a monoclonal antibody developed against the oestrogen receptor from a human breast-cancer cell line (Greene *et al.*, 1980b), we have shown that the oestrogen receptor measured in fetal uterine cytosol is composed of two forms (α and β), which can be differentiated on high-salt sucrose gradients, since only one (the α form) is bound by the antibody, shifting its sedimentation coefficient from 4.5S to 7.4S (Giambiagi & Pasqualini, 1982).

The present paper examines the different factors that influence the interconversion of these two forms of the oestrogen receptor, and the relationship between receptor activation and its binding to the monoclonal antibody.

Experimental

Biological material

Pregnant guinea pigs of the Hartley albino strain (55–65 days of gestation) were purchased from a commercial breeder (C.E.S.A.L., Vigneul-sous-

* To whom correspondence and requests for reprints should be addressed.

Montmedy, Meuse, France). After anaesthesia of the mothers with diethyl ether, the fetuses were obtained by laparotomy and the uteri removed and stripped of fat.

Chemicals

[6,7-³H]Oestradiol (sp. radioactivity 52 Ci/mmol) was obtained from New England Nuclear Corp. (Frankfurt, West Germany). Radioinert oestradiol was purchased from Steraloids (Touzart et Matignon, Vitry-sur-Seine, France). Double-stranded DNA (calf thymus)-cellulose (containing 3.8 mg of DNA/g of DNA-cellulose) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The monoclonal antibody D547Sp γ against the oestrogen receptor from MCF-7 human breast-cancer cells was given by Dr. E. V. Jensen (Zurich, Switzerland) and Dr. G. Greene (Chicago, IL, U.S.A.). Buffers were: 10 mM-Tris/HCl, 1.5 mM-EDTA, 0.5 mM-dithiothreitol, pH 7.4 (TED); TED + 0.25 M-sucrose, pH 7.4 (TEDS); TED + 0.4 M-KCl, pH 7.4 (0.4 M TKED); TED + 0.6 M-KCl, pH 7.4 (0.6 M TKED).

Preparation of uterine cytosol and nuclear fractions

Fetal uteri were homogenized in TEDS buffer with a Teflon/glass Potter-Elvehjem homogenizer. The homogenate was centrifuged at 900 *g* for 10 min. The pellet was washed twice by resuspension in TEDS buffer and centrifugation at 900 *g* for 10 min. The supernatants were pooled and centrifuged at 200 000 *g* for 30 min to obtain the cytosol fraction. The 900 *g* pellet was washed three times with TEDS buffer and resuspended in the same buffer to obtain the nuclear fraction. All procedures were carried out at 4°C.

Oestradiol-receptor complex

Portions of cytosol (containing 3–4 mg of protein/ml) were incubated with 10 nM-[³H]oestradiol with or without a 100-fold excess of unlabelled oestradiol for 2 h at 4°C. Unbound [³H]oestradiol was removed by 0.05% (w/v) dextran-coated 0.5% (w/v) charcoal (Korenman & Rao, 1968) for 10 min at 4°C, and specific binding was calculated by the difference between binding in the absence and in the presence of unlabelled oestradiol.

Oestradiol-receptor-monoclonal-antibody complex

Portions (100 μ l) of cytosol containing the oestrogen-receptor complex (3–4 pmol/ml) were incubated with 10 μ g of the monoclonal antibody for 1 h or 20 h at 4°C.

Nuclear and DNA-cellulose binding

Portions (0.5 ml) of cytosol containing the oestradiol-receptor complex were added to the nuclear pellet (100–150 μ g of DNA) or to 55 mg of

DNA-cellulose (containing 200 μ g of DNA) swollen in TED buffer and incubated for 1 h at 4°C. At the end of the incubation, the tubes were centrifuged at 900 *g* for 10 min. Unbound complexes were measured in the supernatant after dextran-coated-charcoal treatment. The nuclear or DNA-cellulose pellets were washed three times with TEDS buffer. To measure the nuclear-bound complexes, nuclei were extracted with 1 ml of 0.6 M TKED buffer for 1 h at 4°C, centrifuged at 15 000 *g* for 30 min, and samples of the supernatant were removed for radioactivity counting. To determine the complexes bound to DNA-cellulose, the pellets were suspended in 1 ml of TED buffer and samples were removed for radioactivity counting. Non-specific binding to nuclei or DNA-cellulose was determined with a cytosol which had been incubated with [³H]oestradiol in the presence of a 100-fold excess of radioinert oestradiol.

Sucrose density gradients

Portions (100 μ l) of dextran-coated-charcoal-treated oestradiol-receptor or oestradiol-receptor-monoclonal-antibody complexes were layered on 5 ml density gradients prepared with 10–30% (w/v) sucrose solutions in 0.4 M TKED buffer and centrifuged at 400 000 *g* for 2 h in a Beckman VTi 65 vertical rotor. Fractions (110–130 μ l) were collected and counted for radioactivity.

Protein and DNA assays

Protein was measured by the method of Lowry *et al.* (1951), and DNA was determined by that of Burton (1956).

Results

Effect of time and temperature on cytosolic oestrogen receptor binding to the monoclonal antibody

Previous results have shown that after overnight incubation at 4°C of the cytosolic oestradiol-receptor complex with the antibody, 40–60% of the total receptors was bound to it. This fraction was called the α form, and the remaining fraction, not recognized by the antibody, was called the β form. The sedimentation coefficient in high-salt sucrose density gradients of the α -form-antibody complex is 7.4 S and that of the β form 4.5 S (Giambiagi & Pasqualini, 1982). To determine if the existence of these two forms was a result of a time-dependent process, cytosol was incubated with [³H]oestradiol at 4°C for different periods (1 h, 4 h and 19 h) before being incubated for 1 h with the antibody. When analysed through high-salt sucrose gradients (Fig. 1a), no binding between receptor and antibody was detected after the 2 h incubation; all the specific radioactivity sedimented in the β -form zone (4.5 S).

Fig. 1. Effect of time and temperature on the oestrogen receptor binding to the monoclonal antibody

(a) Samples of cytosol (0.5 ml) were incubated with 10 mM-[³H]oestradiol for 1 h (○), 4 h (▲) or 19 h (●) at 4°C; then the monoclonal antibody D547Spγ (10 µg/100 µl of cytosol) was added and the incubation was continued for 1 h at 4°C. (b) Samples of cytosol were incubated with 10 nM-[³H]oestradiol for 2 h at 4°C; then one was heated at 25°C for 15 min (●) and the other was kept at 4°C (○). After cooling, samples were re-incubated with the antibody for 1 h at 4°C. Unbound radioactivity was adsorbed with dextran-coated charcoal, and 0.1 ml of the supernatant was layered on sucrose gradients (10–30%, w/v; 0.4 M-KCl), which were centrifuged for 120 min at 400 000g in a vertical rotor (Beckman VTi 65). Non-specific binding was determined in parallel by adding a 100-fold excess of unlabelled oestradiol (□).

After the 5 h incubation, some binding to the antibody could be distinguished by a shoulder in the β-form peak, and 40–60% of the total receptors was bound to the antibody after the 20 h incubation; longer incubations did not increase these values. These data suggest that at 4°C there is a slow time-induced conversion from a form of the receptor initially present in the cytosol that is not recognized by the antibody (β form) into a form that it does recognize (α form).

The conversion into the α form was found to be also a temperature-dependent process. When the oestradiol–receptor complex was incubated at 4°C for 2 h, then warmed at 25°C for 15 min and re-incubated with the antibody for 1 h, the gradient profiles showed the presence of the α form, but it was absent from a similarly treated, but not warmed, cytosol (Fig. 1b).

Effect of high salt concentration on oestrogen receptor binding to the monoclonal antibody

Since modifications in the characteristics of the cytosol oestrogen receptor occur at high salt concentrations, the effect of KCl on the conversion of the β form into the α form was analysed. Fig. 2(a) shows that incubation of the oestradiol–receptor complex with 0.4 M-KCl for 2 h resulted in a significant conversion of the β form into the α form, which did not occur under similar experimental conditions but in the absence of KCl. The

conversion was even higher after 20 h of incubation (Fig. 2b). As shown in Fig. 2(b), the spontaneous conversion into the α form after 20 h in the absence of KCl was less than expected (see Fig. 1a). This could be explained by the fact that all samples were treated with dextran-coated charcoal, to remove unbound [³H]oestradiol, before the addition of KCl, since the charcoal adsorption procedure is not valid in high salt concentrations (Peck & Clark, 1977).

Effect of sodium molybdate on oestrogen receptor binding to the monoclonal antibody

Those factors that induce the conversion into the α form are also known to induce receptor activation, suggesting that the α form could be an activated form of the receptor. To explore this possibility further, we tested the effect of Na₂MoO₄, a well-known stabilizer of receptors and inhibitor of receptor activation.

Figs. 3(a) and 3(b) show that 10 mM-Na₂MoO₄ completely blocked both the time- and temperature-induced appearance of the α form; however, when Na₂MoO₄ was added after 20 h of incubation at 4°C or warming for 15 min at 25°C, no effect was observed. The effect of molybdate on the 0.4 M-KCl-induced conversion into the α form was somewhat different. When the oestradiol–receptor complex was prepared in a buffer containing 10 mM-Na₂MoO₄, only a partial inhibition of the

Fig. 2. *Effect of 0.4M-KCl on the oestrogen receptor binding to the monoclonal antibody*
Cytosol (2ml) was incubated with 10nM-[³H]oestradiol for 2h, and unbound radioactivity was adsorbed with dextran-coated charcoal. (a) Samples (0.5ml) were incubated without (○) or with (●) 0.4M-KCl for 1h, then the antibody was added and the incubation was continued for 1h. (b) Samples (0.5ml) were incubated without (○) or with (●) 0.4M-KCl for 19h, then the antibody was added and the incubation was continued for 1h. All procedures were carried out at 4°C. Samples were centrifuged through sucrose gradients as indicated in Fig. 1. Non-specific binding was determined in parallel by adding a 100-fold excess of unlabelled oestradiol (□).

Fig. 3. *Effect of Na₂MoO₄ on the oestrogen receptor binding to the monoclonal antibody*
(a) A sample of cytosol (0.5ml) was incubated with 10nM-[³H]oestradiol+10mM-Na₂MoO₄ for 19h at 4°C (▲); another was incubated with 10nM-[³H]oestradiol for 19h at 4°C, and then 10mM-Na₂MoO₄ was added (●). Both samples were re-incubated with the antibody for 1h. Unbound [³H]oestradiol was removed with dextran-coated charcoal, and samples were layered on sucrose gradients. (b) A samples of cytosol (0.5ml) was incubated with 10nM-[³H]oestradiol+10mM-Na₂MoO₄ for 2h at 4°C and heated for 15 min at 25°C (▲); another was incubated with 10nM-[³H]oestradiol for 2h at 4°C, heated for 15 min at 25°C and then 10mM-Na₂MoO₄ was added (●). Both samples were cooled and re-incubated with the antibody for 1h at 4°C. Unbound [³H]oestradiol was removed with dextran-coated charcoal and samples were layered on sucrose gradients. (c) A sample of cytosol (0.5ml) was incubated with 10nM-[³H]oestradiol+10mM-Na₂MoO₄ for 2h at 4°C, treated with dextran-coated charcoal to remove the free [³H]oestradiol and re-incubated with 0.4M-KCl for 2h (△); another two samples were incubated with [³H]oestradiol for 2h at 4°C and treated with dextran-coated charcoal, then one was re-incubated with 10mM-Na₂MoO₄+0.4M-KCl for 2h (▲) and the other was re-incubated with 0.4M-KCl for 2h and then 10mM-Na₂MoO₄ was added (●). Both samples were re-incubated with the antibody for 1h at 4°C and then layered on to sucrose gradients, and centrifuged as indicated in Fig. 1. Non-specific binding was determined in parallel by adding a 100-fold excess of unlabelled oestradiol (□).

KCl-induced conversion into the α form was found (Fig. 3c). No significant effect on this conversion was observed when 10mM- Na_2MoO_4 was added at the same time as, or after, the 2h exposure to 0.4M-KCl.

Activation of oestrogen receptor as determined by its binding to nuclei or DNA-cellulose

Since the observations on the formation of the immunorecognized α form seemed to indicate that it was induced by the same factors that induce receptor activation, this process itself was studied in more detail and compared with the formation of the α form. The extent of activation of the receptor was estimated by the increase in its binding to nuclei or DNA-cellulose at 4°C (Yamamoto & Alberts, 1972).

Table 1 shows that warming the oestradiol-receptor complex for 15min at 25°C increases its nuclear and DNA-cellulose binding; 10mM- Na_2MoO_4 blocked this increase when added before heating, but had no effect on the temperature-activated complex. The receptor was also partially activated with time, as indicated by the increase in DNA-cellulose binding from 8.6% after 2h at 4°C to 18.6% after 20h; 10mM- Na_2MoO_4 blocked the time-induced activation when it was present during the 20h incubation, but had no effect when added afterwards. These results can be compared with the observations shown in Fig. 1 and Figs. 3(a) and 3(b), and a correlation can be seen between the time- and temperature-induced activation of the receptor and the appearance of the α form.

Exposure to 0.4M-KCl for 2h also greatly increased the binding of the receptor to nuclei and DNA-cellulose (Table 2). To assess the nuclear and DNA-cellulose binding of the KCl-activated complexes, it was necessary to dilute the samples 4-

fold to lower the KCl concentration. The 4-fold dilution itself never increased nuclear or DNA-cellulose binding. After a 20h exposure to 0.4M-KCl, although the DNA-cellulose binding was increased, the values were less than those obtained after 2h, probably indicating some deactivation occurring during the 20h incubation. Nuclear and DNA-cellulose binding of the 0.4M-KCl-activated oestradiol-receptor complexes was only partially inhibited when they had been prepared in the presence of 10mM- Na_2MoO_4 . When 10mM- Na_2MoO_4 was added at the same time as, or after, the 2h exposure to 0.4M-KCl, it had relatively little effect on the KCl-induced activation. These findings can be correlated with the observations shown in Figs. 2 and 3(c). The KCl-activation of the receptor corresponds to the KCl-induced appearance of the α -form peak in the gradient profiles.

Transformation of the β form of the oestrogen receptor into the α form

In another series of experiments, the α and β forms of the receptor were separately recovered from the sucrose density gradient. Fractions 10-24 and 25-39 of the gradient were considered to contain the α and β forms respectively. Each form was recovered in 1.8ml of 0.4M-TKED/sucrose buffer, and concentrated overnight by dialysis under reduced pressure against TED buffer, with a Micro-ProDiCon system (Bibblock Scientific, Illkirch, France). A sample of this α form was re-submitted to sucrose-density-gradient analysis and found to sediment with the same 7.4S coefficient, indicating that it was not altered and remained bound to the antibody (Fig. 4a). A sample of the β form was re-incubated with the antibody for 1h and analysed on a sucrose density gradient. Fig. 4(a) indicates that most of the radioactivity

Table 1. Nuclear and DNA-cellulose binding of the time- and temperature-activated oestrogen receptor. Samples 1, 3, 4, 6 and 7 of cytosol (0.5ml) were incubated with 10nM-[^3H]oestradiol, and samples 2, 5 and 8 with 10nM-[^3H]oestradiol + 10mM- Na_2MoO_4 ('+ Mo'), for 2h or 20h (as indicated below), at 4°C. Samples 3, 4 and 5 were warmed at 25°C for 15min, and the rest was kept at 4°C. At the end of the incubation, 10mM- Na_2MoO_4 was added to samples 4 and 7. Samples were assayed for nuclear and DNA-cellulose binding as indicated in the Experimental section. The numbers of experiments are given in parentheses. The data are expressed as means $\pm$ S.E.M.; -, assays not performed.

Experimental conditions		Nuclear translocation (% of total receptor)	DNA-cellulose binding (% of total receptor)
1	4°C 2h	10.2 $\pm$ 1.9 (4)	8.6 $\pm$ 1.3 (3)
2 + Mo	4°C 2h	5.8 $\pm$ 1.5 (3)	6.0 $\pm$ 0.6 (3)
3	4°C 2h $\rightarrow$ 25°C	20.8 $\pm$ 4.4 (3)	17.8 $\pm$ 3.4 (3)
4	4°C 2h $\rightarrow$ 25°C + Mo	21.7 $\pm$ 2.5 (3)	22.2 $\pm$ 3.1 (3)
5 + Mo	4°C 2h $\rightarrow$ 25°C	6.5 $\pm$ 1.2 (3)	6.6 $\pm$ 0.5 (3)
6	4°C 20h	-	18.6 $\pm$ 2.7 (5)
7	4°C 20h $\rightarrow$ + Mo	-	24.0 $\pm$ 3.5 (3)
8 + Mo	4°C 20h	-	6.5 $\pm$ 1.2 (3)

Table 2. Nuclear and DNA-cellulose binding of the 0.4M-KCl-activated oestrogen receptor

Samples 1, 3, 4, 5, 7 and 8 of cytosol (0.5ml) were incubated with 10nM-[³H]oestradiol, and samples 2 and 6 with 10nM-[³H]oestradiol + 10mM-Na₂MoO₄ ('+ Mo'), for 2h and then treated with dextran-coated charcoal. Then 0.4M-KCl was added to samples 3, 4, 6 and 8, and 10mM-Na₂MoO₄ + 0.4M-KCl to sample 5; incubation was continued for 2h or 20h (as indicated below), and finally 10mM-Na₂MoO₄ was added to sample 4. Samples were diluted 4-fold with TEDS buffer or TEDS buffer + 10mM-Na₂MoO₄ (samples 2, 4, 5, 6) and assayed for nuclear and DNA-cellulose binding as indicated in the Experimental section. All procedures were carried out at 4°C. The numbers of experiments are given in parentheses. The data are expressed as means ± S.E.M.; -, assays not performed.

Experimental conditions				Nuclear translocation (% of total receptor)	DNA-cellulose binding (% of total receptor)
1	2h→	+	2h	5.8 ± 1.7 (3)	5.8 ± 1.1 (5)
2	+ Mo 2h→	+	2h	4.7 ± 2.3 (3)	5.0 ± 0.3 (3)
3	2h→	+ 0.4M-KCl	2h	54.2 ± 12.9 (3)	61.6 ± 4.2 (6)
4	2h→	+ 0.4M-KCl	2h→ + Mo	46.7 ± 7.4 (3)	62.4 ± 4.5 (4)
5	2h→ + Mo	+ 0.4M-KCl	2h	41.2 ± 7.6 (3)	57.5 ± 2.4 (4)
6	+ Mo 2h→	+ 0.4M-KCl	2h	35.7 ± 2.5 (3)	45.2 ± 7.6 (4)
7	2h→	+	20h	-	5.8 ± 3.7 (3)
8	2h→	+ 0.4M-KCl	20h	-	38.2 ± 11.8 (3)

Fig. 4. Isolation by density-gradient centrifugation of the β form and transformation to the α form

(a) A sample of cytosol (0.3 ml) was incubated with 10 nM-[³H]oestradiol and the antibody for 20 h at 4°C, and after dextran-coated-charcoal treatment 0.2 ml of the supernatant was centrifuged through the sucrose gradient (see the Experimental section). Fractions 10–24 (α) and 25–39 (β) of the gradient were recovered separately and concentrated (from 1.8 ml to 0.4 ml) overnight by dialysis against TED buffer under reduced pressure at 4°C by using a Micro ProDiCon system. Portions (0.2 ml) of the recovered α form (●) and of the recovered β form, re-incubated with the antibody for 1 h (▲), were analysed through sucrose gradients. (b) Samples (200 μ l) of cytosol were incubated with 10 nM-[³H]oestradiol for 20 h, treated with dextran-coated charcoal, diluted to 2 ml with TED/sucrose (□) or 0.4 M TKED/sucrose buffers (■) and concentrated to 0.4 ml by overnight dialysis against TED buffer under reduced pressure at 4°C as in Fig. 4(a). Samples (200 μ l) were incubated with the monoclonal antibody for 1 h and analysed by centrifugation through sucrose gradients.

sedimented in the α zone, suggesting a complete transformation of this β form into the α form.

To investigate if this conversion was spontaneous or induced by the treatment, 200 μ l samples of [³H]oestradiol-labelled cytosol were diluted to 2 ml with either TED/sucrose or 0.4 M TKED/sucrose buffers and concentrated overnight by dialysis under reduced pressure against TED, incubated for 1 h with the antibody and analysed on sucrose density gradients. In both cases, a great conversion into the α form was found, especially when 0.4M-

KCl was present in the dilution buffer (Fig. 4b), indicating that the experimental conditions (dilution, exposure to KCl, time and dialysis) to which the β form was submitted for isolation and analysis could be responsible for its conversion into the α form.

Discussion

Two forms (α and β) of the cytosol oestrogen receptor from fetal guinea-pig uterus were

differentiated on high salt sucrose gradients by their selective binding to the monoclonal antibody D547Spy. This IgG-class monoclonal antibody, raised against a partially purified preparation from MCF-7 human breast-cancer cells, reacts specifically with the oestrogen receptor from primates and other mammals, such as rat and calf. Its affinity is lower for rat and calf receptors than for MCF-7 receptors, although, in the presence of an excess of antibody, binding is complete, whereas no interaction occurs with the hen oviduct receptor. These findings indicate the conservation of a common antigenic determinant across the mammalian species (Greene *et al.*, 1980b; Greene & Jensen, 1982). This antigenic determinant, which is recognized by the D547Spy antibody, appears to be localized in an intermediate region of the MCF-7 receptor, neither close to the steroid-binding domain nor close to the DNA-binding domain (Greene *et al.*, 1984). As shown here and in previous work (Giambiagi & Pasqualini, 1982; Giambiagi *et al.*, 1984), this monoclonal antibody also recognized the oestrogen receptor from fetal guinea-pig uterus; however, the cytosol receptor was only partially bound, even in the presence of an excess of antibody (Giambiagi & Pasqualini, 1982), revealing the existence of two forms of the receptor with different immunological characteristics. This antibody did not recognize the progesterone receptor from fetal guinea-pig uterus (Giambiagi & Pasqualini, 1982), confirming the specificity studies described by Greene *et al.* (1980b).

The first suggestion that the α form could be an activated form of the receptor appeared from the different behaviour of the α and β forms during the translocation process. When cytosol was incubated with nuclei at 25°C, the α form disappeared rapidly from the cytosol, whereas the β form was only slightly affected; the receptor extracted from the nuclei increased with the time of incubation and was always totally bound by the antibody (Giambiagi *et al.*, 1984).

The present data show that there is a spontaneous time-dependent partial transformation of the β to the α form, suggesting that the two forms are not independent, but that the α form originates from the β form. Under these conditions a spontaneous partial activation of the receptor was also shown by the increase in its binding to DNA-cellulose. The spontaneous activation of steroid receptors in long incubations at 4°C has been reported by different authors (Milgrom *et al.*, 1973; Müller *et al.*, 1983). The conversion into the α form was accelerated by increasing the temperature, which also induced a partial activation of the receptor. This temperature-induced activation of steroid receptors is a well-described phenomenon (Grody *et al.*, 1982).

High salt concentrations also induced the transformation to the α form and, as was described for several steroid receptor systems (Milgrom *et al.*, 1973; Bailly *et al.*, 1980; Müller *et al.*, 1983), had a great effect on receptor activation. However, the activation after 20 h exposure to 0.4 M-KCl was less than after 2 h, indicating a possible deactivation of the activated complex which did not affect its ability to bind to the antibody. Activation by high-salt treatment proved to be the most effective, probably because this procedure avoids exposing the receptor to elevated temperature or long incubations. Thus the observations presented here show a correlation between receptor activation and the conversion of the β into the α form, which is consistent with the hypothesis that the α form is the activated form of the receptor.

Additional support for this proposal was provided by the use of Na₂MoO₄. In agreement with the findings by other investigators (Leach *et al.*, 1979; Nishigori & Toft, 1980; Noma *et al.*, 1980; Shyamala & Leonard, 1980; Müller *et al.*, 1983), Na₂MoO₄ completely blocked the temperature-induced activation, but had no effect once the receptor was activated. The transformation from the β to the α form induced by brief heating was also completely inhibited by Na₂MoO₄ only when it was added before heating. Similarly, both activation and transformation to the α form induced by long incubation at low temperature and ionic strength were blocked when Na₂MoO₄ was present from the beginning of incubation, but were not affected when it was added afterwards.

On the other hand, we found only a weak inhibition of the KCl-induced activation when Na₂MoO₄ was present during preparation of the oestradiol-receptor complex, and only a partial diminution of the α form. This partial inhibitory effect of Na₂MoO₄ on the high-salt-induced activation was also observed by other authors (Nishigori & Toft, 1980; Redevilh *et al.*, 1981; Mauck *et al.*, 1982; Müller *et al.*, 1983). The different effect of Na₂MoO₄ on activation induced by temperature or high ionic strength suggests that these processes of activation are not identical. In any case, the complete or partial inhibition of activation was always accompanied by a complete or partial inhibition of the transformation to the α form.

Finally, it was observed that the β form, isolated and recovered from the sucrose gradient, could be completely transformed into the α form by the dilution and dialysis to which the samples were submitted. Dilution, in a time-dependent process (Bailly *et al.*, 1977; Goidl *et al.*, 1977), and dialysis (Sato *et al.*, 1979, 1980) have been reported to induce receptor activation by removing low- M_r components of the cytosol. We have also observed

that overnight dialysis induced conversion of the β into the α form and an increase in the receptor binding to DNA-cellulose (N. Giambiagi & J. R. Pasqualini, unpublished work). Low- M_r inhibitors present in the cytosol could be responsible in part for the incomplete nuclear and DNA-cellulose binding and transformation to the α form.

The present work gives evidence that the form recognized by the monoclonal antibody is the activated form of the oestrogen receptor of fetal guinea-pig uterus. The activation process involves conformational changes in the receptor, which apparently make the antigenic determinant recognized by the antibody more accessible. Differences in reactivity of monoclonal antibodies with different forms of the oestrogen receptor have been described for an IgM-class monoclonal antibody to the calf nuclear oestrogen receptor, which has more affinity for the nuclear than for the cytosol form of this receptor (Greene *et al.*, 1980a). Subsequently, this monoclonal antibody was found to interact more strongly with the activated than with the non-activated cytosol form of the calf oestrogen receptor, and also to have a different affinity for the receptor bound to oestradiol or to 4-hydroxytamoxifen (Borgna *et al.*, 1984). On the other hand, no differences have been reported in the binding of the D547Spy monoclonal antibody used in our studies to nuclear or activated and non-activated cytosol forms of the human oestrogen receptor, except for a slight difference in sedimentation coefficient between the nuclear and cytosol receptor-antibody complex. However, the guinea-pig receptor must be different enough from the human receptor so that the same antibody will selectively bind to only the activated form. The possibility of discriminating between activated and non-activated forms of the oestrogen receptor with a monoclonal antibody will be very useful for further studies on the receptor-activation process.

Part of the expense of this work was defrayed by the Centre National de la Recherche Scientifique, France (Unité Associée du C.N.R.S. no. 548) and by the Fondation pour la Recherche Médicale Française. We express our sincere thanks to Dr. E. V. Jensen (Zurich, Switzerland) and Dr. G. L. Greene (Chicago, U.S.A.) for the gift of monoclonal antibody D547Spy.

References

- Bailly, A., Sallas, N. & Milgrom, E. (1977) *J. Biol. Chem.* **252**, 858–863
- Bailly, A., Le Fevre, B., Savouret, J. F. & Milgrom, E. (1980) *J. Biol. Chem.* **255**, 2729–2734
- Borgna, J.-L., Fauque, J. & Rochefort, H. (1984) *Biochemistry* **23**, 2162–2168
- Burton, K. A. (1956) *Biochem. J.* **62**, 315–323
- Giambiagi, N. & Pasqualini, J. R. (1982) *Endocrinology (Baltimore)* **110**, 1067–1069
- Giambiagi, N., Pasqualini, J. R., Greene, G. L. & Jensen, E. V. (1984) *J. Steroid Biochem.* **20**, 397–400
- Giannopoulos, G. & Gorski, J. (1971) *J. Biol. Chem.* **246**, 2524–2529
- Goidl, J. A., Cake, M. H., Dolan, K. P., Parchman, L. G. & Litwack, G. (1977) *Biochemistry* **16**, 2125–2130
- Greene, G. L. & Jensen, E. V. (1982) *J. Steroid Biochem.* **16**, 353–359
- Greene, G. L., Fitch, F. W. & Jensen, E. V. (1980a) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 157–161
- Greene, G. L., Nolan, C., Engler, J. P. & Jensen, E. V. (1980b) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5115–5119
- Greene, G. L., Sobel, N. B., King, W. J. & Jensen, E. V. (1984) *J. Steroid Biochem.* **20**, 51–56
- Grody, W. W., Schrader, W. T. & O'Malley, B. W. (1982) *Endocr. Rev.* **3**, 141–163
- Jensen, E. V., Suzuki, T., Kawashima, T., Stumpf, W. E., Jungblut, D. W. & DeSombre, E. R. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **59**, 632–638
- Jensen, E. V., Numata, M., Brecher, P. I. & DeSombre, E. R. (1971) *Biochem. Soc. Symp.* **32**, 133–159
- Jensen, E. V., Mohla, S., Gorell, T., Tanaka, S. & DeSombre, E. R. (1972) *J. Steroid Biochem.* **3**, 445–458
- Korenman, S. G. & Rao, B. R. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **61**, 1028–1033
- Leach, K. L., Dahmer, M. K., Hammond, N. D., Sando, J. J. & Pratt, W. B. (1979) *J. Biol. Chem.* **254**, 11884–11890
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mauck, L. A., Day, R. N. & Notides, A. C. (1982) *Biochemistry* **21**, 1788–1793
- Milgrom, E., Atger, M. & Baulieu, E.-E. (1973) *Biochemistry* **12**, 5198–5205
- Müller, R. E., Traish, A. M. & Wotiz, H. H. (1983) *J. Biol. Chem.* **258**, 9227–9236
- Nishigori, H. & Toft, D. (1980) *Biochemistry* **19**, 77–83
- Noma, K., Nakao, K., Sato, B., Nishizawa, Y., Matsumoto, K. & Yamamura, Y. (1980) *Endocrinology (Baltimore)* **107**, 1205–1211
- O'Malley, B. W., Schwartz, R. J. & Schrader, W. T. (1976) *J. Steroid Biochem.* **7**, 1151–1159
- Pasqualini, J. R. & Nguyen, B.-L. (1976) *C. R. Hebd. Seances Acad. Sci. Ser. D* **283**, 413–416
- Peck, E. J. & Clark, J. H. (1977) *Endocrinology (Baltimore)* **101**, 1034–1043
- Redevilh, G., Secco, C., Baulieu, E.-E. & Richard-Foy, H. (1981) *J. Biol. Chem.* **256**, 11496–11502
- Sato, B., Nishizawa, Y., Noma, K., Matsumoto, K. & Yamamura, Y. (1979) *Endocrinology (Baltimore)* **104**, 1474–1479
- Sato, B., Noma, K., Nishizawa, Y., Nakao, K., Matsumoto, K. & Yamamura, Y. (1980) *Endocrinology (Baltimore)* **106**, 1142–1148
- Shyamala, G. & Leonard, L. (1980) *J. Biol. Chem.* **255**, 6028–6031
- Sumida, C. & Pasqualini, J. R. (1979) *Endocrinology (Baltimore)* **105**, 406–413
- Yamamoto, K. R. & Alberts, B. M. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2105–2109