The binding of rat uterine cytosol oestrogen receptors to oligodeoxythymidylate–cellulose

Its relationship to a stable form of receptor complex with separate ligand- and oligonucleotide-binding sites

Leslie MYATT,*† Murdoch G. ELDER,* Chris NEETHLING†§ and Louis LIM†

*Institute of Obstetrics and Gynaecology, Hammersmith Hospital, Du Cane Road, London W12 0HS, U.K., and †Miriam Marks Department of Neurochemistry, Institute of Neurology, Queen Square, London WC1N 3BG, U.K.

(Received 25 August 1981/Accepted 16 September 1981)

The interaction of rat uterine cytosol oestrogen–receptor complexes with the synthetic acceptor oligo(dT)–cellulose was studied. Differences in the stability of receptor complexes and their ability to bind to oligo(dT)–cellulose on storage at 4°C or when exposed to increased temperatures indicated heterogeneity of steroid- and oligonucleotide-binding sites. Dilution, dialysis and (NH₄)₂SO₄ precipitation increased the interaction of receptor complexes with oligo(dT)–cellulose (a step termed activation). This increase may be the result of the removal of low-molecular-weight cytosol components which inhibit receptor activation, dimerization to the 5S form, which binds to oligo(dT)–cellulose, or interaction of 5S receptor with the oligonucleotide. Cytosol oestradiol–receptor complexes exhibited biphasic dissociation kinetics. All these manipulations resulted in an increase in the proportion of the slow-dissociating component equivalent to the increase in receptor binding to oligo(dT)–cellulose. In contrast, addition of 10 mM-sodium molybdate to cytosol decreased both oligo(dT)–cellulose binding and the proportion of receptor with slow dissociation kinetics. The inclusion of proteinase inhibitors did not affect interactions of receptor with oligo(dT)–cellulose nor the dissociation kinetics. These results suggest that oligo(dT)–cellulose binding may serve to quantify the proportion of cytosol receptor in an active form capable of nuclear interaction and to help to ascertain whether a receptor system is fully functional. This binding procedure could prove useful in the evaluation of oestrogen responsivity under normal and pathological conditions.

Oestrogen action is thought to be mediated via specific receptor proteins present in the cytosol of target cells which complex with the steroid and translocate into the nucleus (O'Malley & Means, 1974). Translocation requires activation of receptor to a state possessing increased affinity for the nucleus (Buchi & Vilee, 1976), and appears to occur concurrently with the transformation of receptor from a 4S into a 5S form, as analysed on sucrose density gradients (Jensen & DeSombre, 1972). Cytosol oestrogen receptors can be activated and transformed *in vitro* to a 5S form identical with 5S receptor extracted from nuclei. This cytosol 5S form can interact in a limited manner with nuclei in a

cell-free system (Haselbacher & Eisenfeld, 1976) or with synthetic acceptors such as DNA–cellulose or oligo(dT)–cellulose (Thrower *et al.*, 1976; Park & Wittliff, 1977). Formation of the 5S nuclear receptor may result from dimerization of two subunits rather than a conformational change in the 4S (cytosol) form (Myatt *et al.*, 1979). The dimerization could involve interaction of 4S receptor with another inactive 4S receptor or with another non-receptor subunit (Yamamoto, 1974; Thrower *et al.*, 1976).

The binding of 5S receptor to oligo(dT)–cellulose appears to be limited by receptor interactions with other cytoplasmic components (Myatt *et al.*, 1979) or by the availability of the second subunit involved in receptor dimerization (Haselbacher & Eisenfeld, 1976; Thrower *et al.*, 1976; White *et al.*, 1978; Myatt *et al.*, 1978). The availability of this subunit

† To whom reprint requests should be addressed.

§ Present address: Department of Chemical Pathology, University of Stellenbosch, South Africa.

may govern the extent of nuclear binding of oestrogen receptor and biological response seen (White *et al.*, 1978). The ability of cytosol oestrogen receptor to bind *in vitro* to oligo(dT)-cellulose or DNA-cellulose (Thrower *et al.*, 1976) probably represents subunit availability. In the present paper we provide evidence for a distinction between the steroid-binding site and the oligonucleotide-binding site of receptor complexes. Additionally we show an association between receptor activation and its ability to bind to oligo(dT)-cellulose.

Experimental

Materials

[2,4,6,7(n)-³H]Oestradiol-17 β (sp. radioactivity 100 Ci/mmol) was supplied by The Radiochemical Centre, Amersham, Bucks., U.K. Oligo(dT)-cellulose was obtained from Collaborative Research, Waltham, MA, U.S.A., supplied by Uniscience, Cambridge, U.K. Sephadex LH-20 was from Pharmacia (G.B.), London W.5, U.K. Liquid scintillant Unisolve 1 was from Koch-Light, Colnbrook, Bucks., U.K. Female Wistar rats were supplied by Charles River (U.K.) and maintained in the animal unit, Royal Postgraduate Medical School. Phenylmethanesulphonyl fluoride and bacitracin were from Sigma Chemical Co., Poole, Dorset, U.K., and aprotinin was from Bayer Pharmaceuticals, Haywards Heath, Sussex, U.K.

Preparation of uterine cytosol and receptor assay

Mature female rats (70–80 days old) were used. Animals were killed by decapitation, uteri quickly removed, trimmed free of fat and mesentery and placed in ice-cold TED buffer [10 mM-Tris/HCl (pH 7.6)/1.5 mM-EDTA/1 mM-dithiothreitol]. All subsequent procedures were carried out at 4°C. Uteri were homogenized and a cytosol fraction was prepared as previously described (Myatt *et al.*, 1978). To quantify receptors, duplicate samples (0.2 ml) of cytosol were incubated for either 1 h or 18 h at 4°C with a saturating concentration (5 nM) of [³H]oestradiol with or without a 100-fold excess of diethylstilboestrol. Samples were then chromatographed on Sephadex LH-20 as previously described (Myatt *et al.*, 1978). Receptor content was estimated from the difference in macromolecular-bound radioactivity in those samples with or without diethylstilboestrol.

Binding of cytosol oestrogen-receptor complexes to oligo(dT)-cellulose

Duplicate cytosol samples were incubated with 5 nM-[³H]oestradiol at 4°C for the indicated times with or without 100-fold excess of diethylstilboestrol. These samples were then incubated with 100 mg of oligo(dT)-cellulose packed in a Pasteur

pipette and previously equilibrated with TED buffer (Thrower *et al.*, 1976) for 45 min at 4°C. Free [³H]oestradiol and oestrogen-receptor complexes not bound to oligo(dT)-cellulose were eluted with 15 ml of TED buffer. Bound complexes were eluted with 4 $\times$ 1 ml of 1.2 M-KCl/TED buffer. These fractions were collected and counted for radioactivity. Specific binding of receptor to oligo(dT)-cellulose was computed from the difference in the radioactivity of samples with and without diethylstilboestrol.

Effect of storage at 4°C or incubation at elevated temperature on binding of oestrogen receptor to oligo(dT)-cellulose

Cytosol oestrogen receptor was incubated with 5 nM-[³H]oestradiol with or without diethylstilboestrol at 4°C for periods from 1 to 70 h. At various times receptor content and binding to oligo(dT)-cellulose were determined. Additionally, cytosol was incubated overnight at 4°C with or without diethylstilboestrol. The incubation mixtures were then heated for 30 min at temperatures in the range 4–50°C, cooled to 4°C and chromatography on Sephadex LH-20 and oligo(dT)-cellulose was performed. Experiments were repeated with the inclusion of proteinase inhibitors [phenylmethanesulphonyl fluoride (0.5 mM), bacitracin (5 μ g/ml) and aprotinin (1000 units/ml)] in cytosol.

Dilution and dialysis of cytosol

Cytosol (approx. 5 mg of protein/ml) was prepared from a 1:7 (w/v) homogenate of mature rat uterus and then serially diluted with TED buffer to yield final dilutions of 1:14, 1:28, 1:56, 1:112. These dilutions were then incubated overnight at 4°C with 5 nM-[³H]oestradiol with or without diethylstilboestrol and then chromatographed on Sephadex LH-20 and oligo(dT)-cellulose. Another sample of cytosol was made into a series of mixtures containing 0–100% of a uterine cytosol from animals previously injected with 2.5 μ g of oestradiol 1 h before death to deplete the cytosol of oestrogen receptor. This depleted cytosol was first stripped of steroid by treatment with dextran-coated charcoal (0.5% charcoal/0.05% Dextran T₇₀) for 15 min at 4°C before use.

Cytosol was prepared from normal animals and dialysed overnight at 4°C against 500 vol. of TED buffer before labelling at 4°C with 5 nM-[³H]oestradiol with or without diethylstilboestrol and chromatography on Sephadex LH-20 or oligo(dT)-cellulose. Alternatively, cytosol was first labelled for 1 h at 4°C with 5 nM-[³H]oestradiol with or without diethylstilboestrol. The respective labelled cytosols were then correspondingly dialysed overnight at 4°C against 500 vol. of TED buffer containing 5 nM-[³H]oestradiol with or without diethylstilboestrol

before chromatography. Additionally a dialysis experiment was performed with phenylmethanesulphonyl fluoride (0.5 mM) in both the cytosol and the dialysis buffer.

Effect of $(\text{NH}_4)_2\text{SO}_4$ precipitation or gel filtration

Uterine cytosol from normal animals was incubated for 1 h at 4°C with 5 nM-[³H]oestradiol with or without diethylstilboestrol. Sufficient saturated $(\text{NH}_4)_2\text{SO}_4$ in TED buffer was added to give 30% or 50% saturation. After precipitation for 1 h at 4°C, the precipitate was collected by centrifugation at 10000 g for 15 min at 4°C. This pellet was then resuspended in the original volume of TED buffer containing 5 nM-[³H]oestradiol with or without diethylstilboestrol as appropriate, and incubated for 1 h at 4°C before chromatography.

Before gel filtration, cytosol was incubated overnight at 4°C with 5 nM-[³H]oestradiol with or without diethylstilboestrol. Samples (0.4 ml) were then chromatographed on columns (9 cm × 0.6 cm) of Sephadex LH-20. The void-volume fraction (1.0 ml, located by Blue Dextran) was collected and immediately relabelled with 5 nM-[³H]oestradiol with or without diethylstilboestrol in the respective tubes. After a 1 h incubation at 4°C, 0.2 ml portions of these samples were assayed for receptor content and ability to bind to oligo(dT)-cellulose.

Determination of receptor-dissociation kinetics

Cytosols or other preparations were incubated for 1 h at 4°C with 5 nM-[³H]oestradiol. Then 3.0 ml of labelled cytosol was added to the pellet of 1 ml of the dextran-coated charcoal solution for 5 min at 4°C. The cytosol was removed from the dextran-coated charcoal solution by centrifugation at 2000 g for 5 min at 4°C. The charcoal-treated cytosol was heated to 29°C and dissociation initiated by addition of 5 μM-oestradiol. Samples of cytosol were removed at various times (0–180 min) after initiation of the dissociation and added to 0.1 ml of dextran-coated charcoal solution, incubated for 5 min at 4°C and then centrifuged at 2000 g for 2.5 min at 4°C. A sample (0.2 ml) of the supernatant was then taken and assayed for radioactivity. The dissociation kinetics were also investigated with cytosol containing phenylmethanesulphonyl fluoride (0.5 mM) as proteinase inhibitor.

Effect of sodium molybdate on receptor-dissociation kinetics and on oligo(dT)-cellulose binding capability

Cytosol was prepared from adult animals as previously described but in TED buffer containing 10 mM-sodium molybdate (TEDM buffer). The cytosol was incubated with 5 nM-[³H]oestradiol with or without diethylstilboestrol at 4°C for 1 h. Oestrogen-receptor dissociation kinetics and binding to

oligo(dT)-cellulose were measured as previously described. The oligo(dT)-cellulose columns had been equilibrated with TEDM buffer before binding studies.

Results

Oestrogen-receptor-complex stability and interaction with oligo(dT)-cellulose

The oestrogen-receptor complex was stable when stored at 4°C (Fig. 1) and when exposed to temperatures up to 35°C. Above this temperature rapid exponential dissociation of complexes was observed, with no detectable binding of steroid at 55°C (Fig. 2). This agrees with a previous report (Puca *et al.*, 1971). In contrast, the binding of receptor-oestradiol complexes to oligo(dT)-cellulose was decreased with storage at 4°C (half-life, $t_{1/2}$, approx 45 h; Fig. 1). In addition, interaction of complexes with oligo(dT)-cellulose also decreased with increases in temperature in a sigmoidal manner, with a sharp decrease between 20 and 40°C. The differential effects of increasing temperature on (a) loss of the receptor-oestradiol binding and (b) interaction of receptor-oestradiol complexes with oligo(dT)-cellulose can be easily distinguished (Fig. 2). Loss of 50% ligand binding and complex interaction occurred at 43°C and 28°C respectively. Addition of proteinase inhibitors did not

Fig. 1. *Stability of ligand-binding and oligonucleotide-binding sites of oestrogen receptor in vitro at 4°C*

A uterine cytosol was prepared, labelled with 5 nM-[³H]oestradiol with or without diethylstilboestrol and incubated at 4°C. At the indicated times the amount of receptor labelled with [³H]oestradiol and its capability for binding to oligo(dT)-cellulose were measured as described in the Experimental section. All values shown are duplicates which agree to within 5% (10000 c.p.m. is approx. 40 fmol of receptor/mg of protein). ●, [³H]Oestradiol specifically bound to oestrogen receptor; ○, [³H]oestradiol-labelled receptor binding to oligo(dT)-cellulose.

Fig. 2. Temperature-stability profiles for ligand- and oligonucleotide-binding sites of oestrogen receptor

A uterine cytosol was prepared and incubated overnight with 5 nM-[³H]oestradiol with or without diethylstilboestrol. Samples of this cytosol were then incubated for 30 min at the indicated temperatures. After cooling back to 4°C, the amount of receptor labelled with [³H]oestradiol and its capability for binding to oligo(dT)-cellulose were measured as described in the Experimental section. The values at each temperature were then expressed as a percentage of the value measured at 4°C. Mean values $\pm$ S.E.M. for three separate experiments are shown. ●, [³H]Oestradiol specifically bound to oestrogen receptor; ○, [³H]oestradiol-labelled receptor binding to oligo(dT)-cellulose.

protect against selective loss of binding to oligo(dT)-cellulose (results not shown).

Heating cytosol from mature animals did not result in 'activation' of receptors, as has been reported for cytosol from rat mammary gland (Park & Wittliff, 1977). Interestingly, during the initial part of the incubation (0–5 h) at 4°C (Fig. 1) the ability of oestrogen-receptor complexes to bind to oligo(dT)-cellulose increased from 28 to 37%. Buchi & Villee (1976) observed a similar time-dependent increase in the uptake of cytosol receptor into nuclei when incubated at 0°C.

Effects of (a) dilution, (b) dialysis, (c) precipitation with (NH₄)₂SO₄ or (d) gel filtration on receptor-complex interactions with oligo(dT)-cellulose

(a) When cytosol was serially diluted with TED buffer the amount of measurable receptor complexes at each dilution decreased proportionately. The ability of these receptor complexes to interact with oligo(dT)-cellulose was found to increase on dilution (Fig. 3a). The decrease in protein concentration in the incubation mixtures may have affected the interaction of receptor complexes with cytosol components and hence altered oligo(dT)-cellulose binding. However, when a receptor-depleted cytosol was used as diluent to maintain protein concentration there was still an increase in ability of receptor complexes to bind to oligo(dT)-cellulose (Fig. 3b).

(b) There was no loss of oestrogen-receptor complexes on dialysis. The proportion of complexes able to bind to oligo(dT)-cellulose was increased

Fig. 3. Effect of cytosol dilution on the binding of oestrogen-receptor complexes to oligo(dT)-cellulose

(a) A uterine cytosol was prepared [1:7 (w/v) homogenate] and diluted as indicated. These diluted cytosols were incubated overnight at 4°C with 5 nM-[³H]oestradiol with or without diethylstilboestrol and the binding of [³H]oestradiol to receptor and binding of receptor complexes to oligo(dT)-cellulose measured. (b) The indicated mixtures of a normal uterine cytosol and a receptor-depleted cytosol, from animals injected with 2.5 μ g of oestradiol 1 h before death, were made (see the Experimental section). The various mixtures were incubated overnight at 4°C with [³H]oestradiol with or without diethylstilboestrol. The binding of [³H]oestradiol to receptor (●) and binding of receptor complexes to oligo(dT)-cellulose (○) were then measured.

(Table 1). Proteinase inhibitors did not prevent this increase on dialysis (results not shown).

(c) After precipitation of oestrogen–receptor complexes with $(\text{NH}_4)_2\text{SO}_4$, the recovery of complexes was 81% at 30% saturation and 53% at 50% saturation (Table 1). Proportionally more of these recovered complexes were able to bind to oligo(dT)–cellulose, although there was obviously loss of the second subunit necessary for the 5 S interaction upon precipitation, since the amount of bound receptor was lower than that found after dialysis. There were differences in the recovery of oestrogen–receptor complexes and of the second subunit with precipitation at either 30% or 50% $(\text{NH}_4)_2\text{SO}_4$ saturation.

(d) There was no loss of $[^3\text{H}]$ oestradiol binding after gel filtration of cytosol on Sephadex LH-20 (Table 1), but the oestradiol–receptor complexes had a decreased ability to bind to oligo(dT)–cellulose. This decrease occurred regardless of whether or not cytosol receptor was labelled with $[^3\text{H}]$ oestradiol before gel filtration.

Receptor-dissociation kinetics

The dissociation curve of receptor–oestradiol complexes of adult rat uterine cytosol could be resolved into two components (Fig. 4). The faster-dissociating component had a dissociation rate constant (k_{-1}) of $0.26 \pm 0.08 \text{ min}^{-1}$ and the slower component had a rate constant (k_{-2}) of $2.42 \pm 0.78 (\times 10^{-3}) \text{ min}^{-1}$ (mean $\pm$ s.d., $n = 4$) at 29°C when labelled with $[^3\text{H}]$ oestradiol. The presence of a proteinase inhibitor in cytosol did not alter the dissociation kinetics (results not shown). The proportion of the slowly dissociating component in cytosol was approx. 30–35%, agreeing closely with that proportion of cytosol receptor which normally bound to oligo(dT)–cellulose. Both dialysis and

$(\text{NH}_4)_2\text{SO}_4$ precipitation of receptor also increased the proportion of the slower dissociating form of receptor (Fig. 4), again in close agreement with the increase in binding of receptor complexes to oligo(dT)–cellulose seen after these treatments (Table 1).

Receptor that bound to oligo(dT)–cellulose and was removed by high salt existed mainly as the

Fig. 4. Dissociation kinetics of uterine oestrogen–receptor complexes

Oestrogen receptor from a labelled normal uterine cytosol (O), a labelled cytosol dialysed overnight against 500 vol. of TED buffer containing 5 nM- $[^3\text{H}]$ oestradiol (●), or a labelled cytosol precipitated at 50% saturation with $(\text{NH}_4)_2\text{SO}_4$ and resuspended in TED buffer containing 5 nM- $[^3\text{H}]$ oestradiol (■) were treated with dextran-coated charcoal and the receptor-dissociation kinetics measured at 29°C as described in the Experimental section. The first-order dissociation rate constants were determined from the slopes of a plot of $\log (\% \text{ of } [^3\text{H}] \text{ oestradiol bound})$ versus time of incubation. The rate constant of the first component (k_{-1}) was determined after removing the contribution of the second component (k_{-2}). The magnitude of the second component was measured by extrapolation back to zero time.

Table 1. Effect of dialysis, $(\text{NH}_4)_2\text{SO}_4$ precipitation, gel filtration or addition of molybdate on the binding of $[^3\text{H}]$ oestradiol to oestrogen receptor and the binding of $[^3\text{H}]$ oestradiol-labelled complexes to oligo(dT)–cellulose

The binding of $[^3\text{H}]$ oestradiol to receptor and the binding of $[^3\text{H}]$ oestradiol-labelled receptor to oligo(dT)–cellulose was measured before and after the indicated manipulations as described in the Experimental section. All results are expressed as a percentage of the receptor content measured before treatment.

Treatment	Cytosol oestrogen–receptor complexes measured		Receptor complexes binding to oligo(dT)–cellulose		Recovery of activating factor After treatment
	Untreated	Treated	Untreated	Treated	
Dialysis	100	100	38	81	213
$(\text{NH}_4)_2\text{SO}_4$ precipitation					
(a) 30% satn.	100	84	38	54	118
(b) 50% satn.	100	53	38	65	89
Gel filtration					
(a) Labelled complexes	100	100	32	18	56
(b) Unlabelled complexes	100	100	32	14	44
Cytosol + 10 mM-molybdate	100	100	23	4	18

Fig. 5. Dissociation kinetics of receptor isolated from oligo(dT)-cellulose and in cytosol treated with molybdate

The dissociation kinetics of uterine oestrogen receptor that bound to oligo(dT)-cellulose and was eluted by 0.4M-KCl (O) and oestrogen receptor from a cytosol containing 10mM-sodium molybdate (●) were determined as described in the Experimental section. The first-order rate constants k_{-1} and k_{-2} were determined as previously described (Fig. 4), as was the magnitude of the k_{-2} component.

slower-dissociating form (Fig. 5). However, rather than existing solely as the fast-dissociating form, the receptor complexes that did not bind to oligo(dT)-cellulose also showed dissociation kinetics with two components with proportions similar to those found in unfractionated cytosol.

Effects of molybdate on receptor-dissociation kinetics and oligo(dT)-cellulose binding

In the presence of 10mM-molybdate most of the receptor (90%) was found as the fast-dissociating form (Fig. 5). The proportion of slow-dissociating form found was roughly equivalent to that proportion of receptor in molybdate-treated cytosol that was able to bind to oligo(dT)-cellulose (Table 1). The rate constants of the receptor forms in the presence of molybdate were k_{-1} 0.20min^{-1} and k_{-2} $2.21 \times 10^{-3}\text{min}^{-1}$, which were within the range for untreated cytosol.

Discussion

The 5S receptor which binds to oligo(dT)-cellulose is indistinguishable from that which can be extracted from nuclei (Thrower *et al.*, 1976). The apparent limited binding of cytosol receptor to oligo(dT)-cellulose is directly related to the amount of 5S receptor that can be formed in cytosol by interaction of 4S receptor and its complementary subunit. Only 5S receptor appears to bind to oligo(dT)-cellulose with 4S receptors in the non-

binding fraction (Thrower *et al.*, 1976). Studies of the stability of steroid binding to oestrogen receptor and capability for binding to oligo(dT)-cellulose (Figs. 1 and 2) indicate that the steroid- and oligonucleotide-binding sites were distinct within the 5S receptor complex. These sites may also be distinguished by photo-oxidation of receptor complexes (Feldman *et al.*, 1980).

Using oligo(dT)-cellulose binding of receptor complexes as an index, we find that dilution, dialysis or $(\text{NH}_4)_2\text{SO}_4$ precipitation increased 'activation' of receptor, in agreement with other workers studying the oestrogen receptor (Sato *et al.*, 1978, 1979) and other steroid receptors (Bailly *et al.*, 1977; Goidl *et al.*, 1977; Saffran *et al.*, 1976). The increased binding to oligo(dT)-cellulose observed after these treatments may be the result of removal of low-molecular-weight components which inhibit either the interaction of the two subunits necessary to form 5S receptor or the interaction of 5S receptor with oligo(dT)-cellulose. The removal of a low-molecular-weight inhibitor by dialysis has been reported to facilitate oestrogen-receptor transformation from 4S to 5S in mouse Leydig-cell-tumour cytosol (Sato *et al.*, 1978). The differences in the recovery of cytosol receptor complexes and of their capability to bind to oligo(dT)-cellulose on precipitation at 30 or 50% saturation with $(\text{NH}_4)_2\text{SO}_4$ could represent either differential removal of inhibitory components or heterogeneity between the cytosol receptor and the factor with which it dimerizes when binding to oligo(dT)-cellulose. The decrease in oligo(dT)-cellulose binding of oestrogen-receptor complexes that we observed after gel filtration on Sephadex LH-20 was possibly due to partial separation of cytosol receptor from components necessary for interaction with oligo(dT)-cellulose, such as was suggested to occur on Sephadex G-100 chromatography (Thrower *et al.*, 1976). Interestingly, the presence of oestradiol appears to stabilize receptor and the capability for oligo(dT)-cellulose binding during gel filtration (Table 1) and $(\text{NH}_4)_2\text{SO}_4$ precipitation (results not shown).

It has been suggested (Weichman & Notides, 1980) that the biological activity of oestrogens and ability to maintain nuclear retention of receptors is related to their ability to maintain receptor in the activated state, i.e. the slow-dissociating form. This 'activated' receptor sediments as 5S on sucrose density gradients (Weichman & Notides, 1980). In our experiments the cytosol receptor fraction that binds to oligo(dT)-cellulose gave a single-component dissociation curve corresponding to the slower-dissociating form (activated or 5S). Nevertheless, the non-binding fraction still exhibited a two-component dissociation curve with similar proportions of each component to that seen in

cytosol of mature animals. The proportion of slower-dissociating form in cytosol was equivalent to that receptor which bound to oligo(dT)-cellulose. The increase in oligo(dT)-cellulose binding of receptor after dialysis or $(\text{NH}_4)_2\text{SO}_4$ precipitation of cytosol matched the increase in the proportion of slower-dissociating form of receptor in these treated cytosols. These results indicate that either dimerization of receptor resulted in the slower dissociation kinetics for steroid (by inducing some change in the steroid-binding site), or alternatively that only an activated (slow-dissociating) form of 4S receptor was capable of interaction with the complementary subunit to form the 5S species. Oligo(dT)-cellulose binding may have facilitated this latter interaction. Removal of low-molecular-weight inhibitory compounds could contribute to the 'activation' of receptor. On addition of 10 mM-molybdate to cytosol the oestrogen receptor was found predominantly in the faster-dissociating form, as previously described (Shyamala & Leonard, 1980). These workers suggested that molybdate acts by preventing the dissociation of the low-molecular-weight inhibitor, a step necessary for activation of receptor. The concomitant low extent of oligo(dT)-cellulose binding found in molybdate-treated cytosols (Table 1) further supports a relationship between activation and transformation of receptor. Molybdate was also found to inhibit uptake of cytosol oestrogen receptor by nuclei in a cell-free system (Noma *et al.*, 1980). In all of these experiments the inclusion of proteinase inhibitors was without effect, suggesting that selective proteolysis is not a factor to be considered in alterations of oligonucleotide-binding capability and receptor-dissociation kinetics.

Oestrogenic responses may well be limited by the availability of the second subunit with which 4S cytosol receptor may interact. In some oestradiol-independent mammary tumours (Shyamala, 1972), cytosol but not nuclear receptors are present, and in some breast tumours which show poor objective response to endocrine therapy only the 4S cytosol receptor is present and 5S receptor cannot be formed (Kute *et al.*, 1978). Furthermore, Yamamoto *et al.* (1976) described a range of cloned mouse lymphoma cells whose nuclear glucocorticoid-receptor binding paralleled the ability of cytosol receptor to bind to DNA-cellulose. Our observations suggest that the biological potency of various

oestrogens could be equally assessed by oligo(dT)-cellulose binding or by analysis of the dissociation kinetics of receptor complexes as suggested by Weichman & Notides (1980).

We thank the Brain Research Trust for financial support.

References

- Bailey, A., Sallas, N. & Milgrom, E. (1977) *J. Biol. Chem.* **252**, 858–863
- Buchi, K. & Vilee, C. A. (1976) *J. Steroid Biochem.* **7**, 539–544
- Feldman, M., Kallos, J., Miller, A. & Hollander, V. P. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 2133
- Goidl, J. A., Cake, M. H., Dolan, K. P., Parchman, G. & Litwack, G. (1977) *Biochemistry* **16**, 2125–2130
- Haselbacher, G. K. & Eisenfeld, A. (1976) *Biochem. Pharmacol.* **25**, 2571–2581
- Jensen, E. V. & DeSombre, E. R. (1972) *Annu. Rev. Biochem.* **41**, 203–230
- Kute, T. E., Heidemann, P. & Wittliff, J. L. (1978) *Cancer Res.* **38**, 4307–4313
- Myatt, L., Chaudhuri, G., Elder, M. G. & Lim, L. (1978) *Biochem. J.* **176**, 523–529
- Myatt, L., Elder, M. G. & Lim, L. (1979) *Biochem. Soc. Trans.* **7**, 15–16
- Noma, K., Nakao, K., Sato, B., Nishizawa, Y., Matsumoto, K. & Yamamura, Y. (1980) *Endocrinology* **107**, 1205–1211
- O'Malley, B. W. & Means, A. R. (1974) *Science* **183**, 610–620
- Park, D. C. & Wittliff, J. L. (1977) *Biochem. Biophys. Res. Commun.* **78**, 251–258
- Puca, G. A., Nola, E., Sica, V. & Bresciani, F. (1971) *Biochemistry* **10**, 3769–3779
- Saffran, J., Loeser, B. K., Bohnett, S. A. & Faber, L. E. (1976) *J. Biol. Chem.* **251**, 5607–5613
- Sato, B., Huseby, R. A. & Samuels, L. T. (1978) *Endocrinology* **102**, 545–555
- Sato, B., Nishizawa, Y., Noma, K., Matsumoto, K. & Yamamura, Y. (1979) *Endocrinology* **104**, 1474–1479
- Shyamala, G. (1972) *Biochem. Biophys. Res. Commun.* **46**, 1623–1630
- Shyamala, G. & Leonard, L. (1980) *J. Biol. Chem.* **255**, 6028–6031
- Thrower, S., Hall, C., Lim, L. & Davison, A. N. (1976) *Biochem. J.* **160**, 271–280
- Weichman, B. M. & Notides, A. C. (1980) *Endocrinology* **106**, 434–439
- White, J. O., Thrower, S. & Lim, L. (1978) *Biochem. J.* **172**, 37–46
- Yamamoto, K. R. (1974) *J. Biol. Chem.* **249**, 7068–7075
- Yamamoto, K. R., Gehring, U., Stampfer, M. R. & Sibley, C. H. (1976) *Recent Prog. Horm. Res.* **32**, 3–32