ESTRADIOL-RECEPTOR-DNA INTERACTION: LIQUID POLYMER PHASE PARTITION

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Received 21 November 1975

1. Introduction

The interaction of hormone–receptor complexes with chromatin appears to be a crucial step in the mechanism of action of steroid hormones and numerous studies have strongly suggested some interaction between hormone-receptors and DNA [1–7]. The specificity of the interaction has been studied by sucrose gradients [4,8,9], DNA-cellulose chromatography [3,7,10], gel permeation chromatography [5,11], DNA-nitrocellulose filtration [10] and the more recent sedimentation partition chromatography [12]. With the exception of DNA-nitrocellulose filtration, these methods cannot accomodate the number of experimental points required for obtaining valid binding parameters. In addition, all but one (sedimentation partition chromatography) require the complete physical separation of the hormone-receptor–DNA complex from at least one of the reactants.

Albertson [13] has shown that macromolecules distribute between two aqueous polymer phases with characteristic partition coefficients defined as:

$$\alpha = \frac{\text{concentration of macromolecules in the top phase}}{\text{concentration of macromolecules in the bottom phase}}$$

For a given composition of polymers, the distribution of molecules between the phases is dependent on their size, and on the pH and ionic strength of the medium.

In this paper, the binding of calf uterine estradiol-receptor to calf thymus DNA was studied in a polymer system composed of 5% dextran T 500 and 4% polyethylene glycol 6000. Under the experimental conditions which are described, hormone-receptor is partitioned between the two phases while DNA and hormone-receptor–DNA complexes are found only in the dextran phase.

The results obtained show that: (1) hormone-receptor binds to native and denatured eucaryotic and procaryotic DNA. (2) The binding of increasing amounts of radioactive hormone-receptor (up to 0.5 nM) to a fixed concentration of DNA (100 μg/ml) is a non-saturable phenomenon. (3) If the same concentration of DNA is incubated with a fixed amount of receptor and increasing concentrations of estradiol, maximum binding is achieved with a concentration of estradiol which would saturate the amount of receptor present in the incubation medium and (4) not all hormone-receptor complexes have DNA binding capacity.

2. Materials and methods

2,4,6,7 [^{3}H] EII* (80–100 Ci/mmole) was obtained from Radiochemical Centre Amersham, polyethylene glycol 6000 from Farbwerke Hoechst, dextran T 500 from Pharmacia and nucleic acids and DNAse from Worthington Biochemicals.

All preparations were buffered in 20 mM Tris-HCl pH 8.0, 5 mM sodium bisulfite, 10% glycerol (Merck Selectipur).

EII-cytosol receptor was prepared by incubating the 105 000 *g* supernatant of calf uterus homogenate

* *Abbreviations:* PEG: polyethylene glycol 6000; DEX: dextran T 500; EII: estradiol; BSA: bovine serum albumin.

with ⩾ 5 nM radioactive EII (concentrations which saturate receptor sites) for 2 h at 0°C followed by 1 h at 20°C. Free or rapidly dissociated hormone was removed by a 15 min incubation (20°C) with a charcoal dextran suspension [14], and protein concentrations were measured by the Folin reaction [15]. Aliquots of the receptor preparations were incubated with DNA at 4°C for 4 h. EII binding protein concentrations are expressed by their hormone binding capacity.

Phase system: as the water soluble polymers are highly viscous, they are more accurately dispensed by weighing. A concentrated phase system is prepared by mixing 2 parts of 20% (w/w) DEX with 1 part of 30% (w/w) PEG and while completely emulsified, 2 g aliquots are transferred to glass stoppered centrifuge tubes and placed in the cold. From this point on, all manipulations are performed at 4°C. Three ml aliquots of the material to be partitioned are added to the tubes containing the 2 g stock system, giving a final phase composition of 5% DEX and 4% PEG.

The tubes are thoroughly mixed and left for 15–30 min before centrifugation at 2000 *g* for 10 min. Aliquots of the top and bottom phases are counted in 10 ml Unisol (Koch Light). The polymers are not miscible with toluene and precipitate in Bray's solution.

2.1. *Calculations*

The amount of [^{3}H] EII-receptor bound to DNA in the phase is calculated by the following equation:

$$[ER_b] = [ER_d] - [ER_p]/\alpha \text{ where } [ER_b], [ER_d], [ER_p]$$

represent the concentration of [^{3}H] EII-receptor bound to DNA, present in the DEX phase, present in the PEG phase, respectively, and α is the partition coefficient of radioactive EII-receptor in absence of DNA. The amount of hormone-receptor bound to DNA in the incubation medium can then be calculated, since at 4°C, the dissociation rate of the complexes (hormone-receptor–DNA) is very slow (unpublished observations) and negligible during the short time needed for separation of the phases.

3. Results

[^{3}H] EII-receptor, BSA and total cytosol proteins partition between the two phases (table 1). DNA is found exclusively in the DEX phase which also contains more than 90% of total yeast RNA. The partition of [^{3}H] EII and [^{3}H] EII-BSA is not influenced by the presence of DNA, indicating that there

Table 1
Partition of estradiol, estradiol-receptor, proteins and nucleic acids in the two phase system

	Concentrations	Partition Coefficient – DNA	+ DNA[a]
DNA (μg/ml)	20 – 1000	–	< 0.001
[^{3}H] EII (pM)	25 – 2500	1.40 ± .01[b]	1.40 ± .02
[^{3}H] EII-receptor (pM)[c]	1 – 350	0.80 ± .05	0.30 ± .02
[^{3}H] EII-BSA (pM)[d]	30 – 3000	1.06 ± .02	1.06 ± .01
BSA (mg/ml)	1.3 – 13	0.50 ± .04	–
Total cytosol proteins (mg/ml)	1.6 – 16	0.62 ± .06	–
Total yeast RNA (μg/ml)	20 – 1000	0.10 ± .01	–

[a] Incubation with DNA (100 μg/ml) for 4 h at 4°C.
[b] ± SD.
[c] Protein concentration, 1.6 - 16 mg/ml.
[d] Protein concentration, 0.08 - 8.0 mg/ml (radioactive EII indicates potential EII–BSA complexes).
Unlabelled proteins and nucleic acid concentrations in the phases were measured at 280 and 260 nm respectively and radioactivity as described in Materials and methods. Partition was performed as described (see Phase System) and the partition coefficient α calculated (see Calculations).

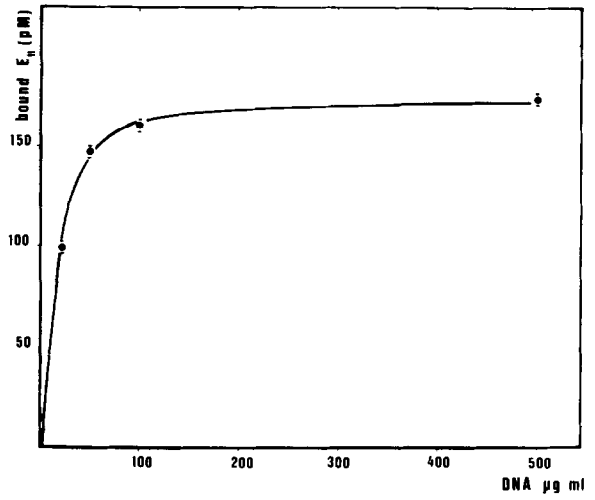

Fig.1.

is no detectable binding of hormone or eventual EII-BSA complex to DNA under these experimental conditions. After 4 h incubation with DNA (maximum binding is observed at this time), the difference in [^{3}H]EII-receptor partition coefficient, 0.3 vs 0.8 in absence of nucleic acid, is indicative of its binding to DNA. [^{3}H]EII-receptor prepared in the presence of a large excess of non-radioactive hormone showed no binding to DNA. It should be emphasized that protein partition coefficients can vary with different batches of polymers (that of receptor varies from 0.6 to 1.0), and α should therefore be determined for each new polymer preparation. No variation is observed for the distribution of [^{3}H]EII and DNA.

When a fixed amount of [^{3}H]EII-receptor (350 pM) is incubated with increasing concentrations of DNA (fig.1), the plateau obtained indicates that only about 48% of incubated EII-receptor is bound to DNA. This value fluctuates between 30–60% for different receptor preparations, and in no case is 100% binding observed. These results which are in agreement with other published data [6,16,17], suggest that some of the [^{3}H]EII-receptor complexes do not have DNA binding capacity. Incubation of fixed receptor protein containing increasing concentrations of radioactive EII with a fixed amount of DNA results in a plateau (fig.2a). In contrast, incubation of increasing amounts of [^{3}H]EII-receptor with the same amount of DNA shows no saturation of the DNA binding sites (fig.2b). In all experiments, the removal of free hormone and dissociation of non specific hormone complexes was obtained by charcoal treatment (see Materials and methods). Consequently, the EII concentrations given in the figures represent receptor concentration. The plateau in fig.2a reflects the depletion, from a given cytosol population, of hormone-receptor complexes which possess DNA binding capacity. The hormone concentration at which maximum DNA binding is achieved corresponds to that which would saturate an equivalent amount of cytosol-receptor (data not shown). The data of fig.2b simply indicate that the capacity of DNA for binding hormone-receptor complexes is very large, and that no saturation is obtained in the range of reactants used in these experiments.

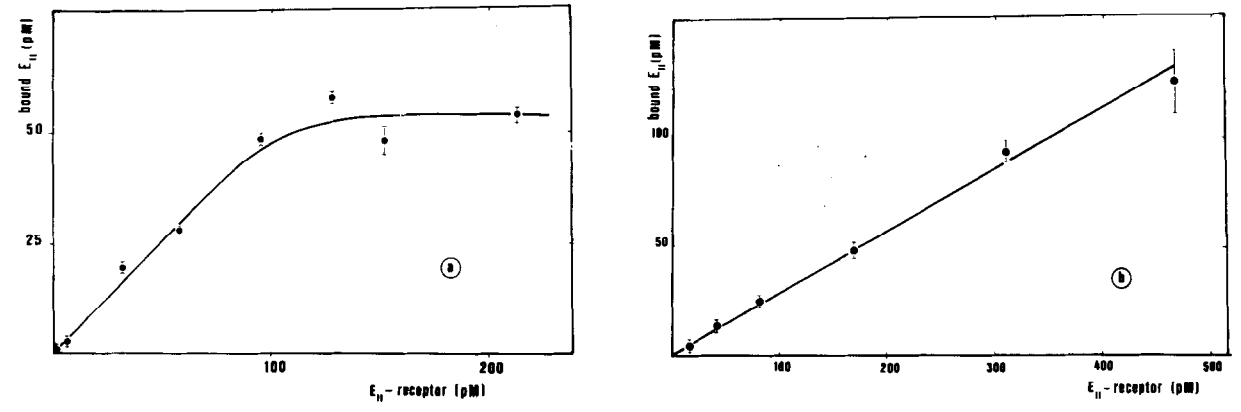

Fig.2.

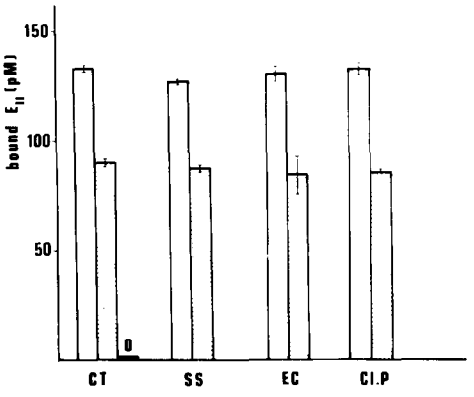

Fig.3.

No significant differences were found for hormone-receptor complex binding to either native or denatured DNA from different species and no binding was observed to DNA pretreated by DNAse (fig.3).

4. Conclusions

The aqueous polymer phase system used here for studying hormone-receptor–DNA interaction presents certain advantages over more established techniques. (1) It allows the manipulation of a large number of experimental points, (2) rapid execution-separation of the reactants is accomplished in less than 30 min and (3) it permits the study of hormone-receptor–DNA interactions at equilibrium. The possibility of varying pH and ionic strength as well as polymer composition, provides much versatility in the use of this technique.

The results reported here and which are in agreement with previously published data on hormone-receptor–DNA interactions [6,8,10–12,17] suggest that the method can be a useful tool for more detailed studies of the binding of hormone-receptors to DNA.

Acknowledgements

This work was partially supported by the Délégation Générale à la Recherche Scientifique et Technique, the Ford Foundation and Roussel-Uclaf.

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