A COMPARISON OF HEPARIN AGAROSE AND DNA CELLULOSE FOR THE CHARACTERIZATION AND PARTIAL PURIFICATION OF ANDROGEN RECEPTORS FROM RAT PROSTATE

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1. Introduction

In the rat prostate a receptor for androgenic steroids is present in cytoplasmic and nuclear fractions. Before the transfer of the cytoplasmic steroid–receptor complex to the nuclear acceptor sites a presumably temperature-dependent conformational change is needed. Some characteristic differences between the cytoplasmic and nuclear receptor have been reported [1,2], e.g., a difference in sedimentation value and in isoelectric point. A detailed knowledge of the different forms of the androgen receptor can only be obtained after extensive purification of the receptor.

From recent studies on the interaction of the uterine oestradiol receptor with heparin agarose [3], it appeared that the cytoplasmic, but not the nuclear receptor was strongly adsorbed by heparin agarose and could be purified several fold by selective elution from heparin agarose.

The results to be presented show that both cytoplasmic and nuclear forms of the androgen receptor from rat prostate can be bound to heparin agarose and that heparin is effective in preventing the aggregation of nuclear receptors. In contrast, DNA cellulose interacted strongly with cytoplasmic androgen receptors, but did not bind nuclear receptors.

2. Materials and methods

2.1. *Steroid*

5α-[1,2,4,5,6,7-^{3}H]dihydrotestosterone (114 Ci/mmol) was obtained from the Radiochemical Centre, Amersham.

2.2. *Cytoplasmic and nuclear receptors*

Cytoplasmic and nuclear receptors were prepared from rat prostates 1 day after castration. A buffer (pH 7.4) containing 0.01 M Tris–HCl, 1.5 mM EDTA, 1.5 mM dithiothreitol, with 10% glycerol (TEDG buffer) was used in all experiments. Prostate tissue was homogenized in 3 vol. TEDG buffer and the 100 000 × *g* cytosol fraction was isolated [4]. Cytosol fractions were incubated for 2 h at 0°C with 20 nM labelled 5α-dihydrotestosterone (DHT), in the presence and absence of a 100-fold non-radioactive DHT. For preparation of nuclear extracts prostate tissue was incubated for 1 h at 37°C in Eagle's minimal essential medium with 2×10^{-8} M [^{3}H]DHT. The tissue was homogenized as above and the 700 × *g* pellet was prepared. The pellet was washed with TEDG buffer with 0.2% Triton X-100 and a nuclear extract was prepared by extraction with 0.4 M KCl as in [5].

2.3. *Estimation of dihydrotestosterone receptors*

Agar gel electrophoresis (90 min at 0°C in a cooled apparatus) and measurement of radioactivity were performed essentially as in [5,6]. Protein was determined using a modification of the Lowry procedure [7]. The receptor-bound steroid was analysed with thin-layer chromatography [5] and was found to be essentially unmetabolized DHT.

2.4. *Heparin agarose and DNA cellulose*

Heparin agarose was prepared by coupling of heparin (Sigma, grade II 10 mg/ml) with CNBr-activated Sepharose (Pharmacia) using established procedures [8]. Any remaining active groups were reacted with ethanol amine.

Heparin content (7 mg/g agarose) was calculated from the free heparin concentration (estimated according to [9]) before and after the coupling reaction.

DNA cellulose was prepared according to [10]. DNA content (475 μg/g cellulose) was estimated after hydrolysis [11].

The DHT–receptor preparations (2–5 ml in TEDG buffer) were incubated with 0.5 ml 10% suspension of heparin agarose or with 0.5 ml 20% suspension of DNA cellulose. After the incubation free steroid and receptor-bound steroid were eluted either in a batch-wise procedure, or continuously after transfer of the suspension into small columns (disposable Pasteur pipette with glass wool support connected to a small peristaltic pump).

3. Results

[^{3}H]DHT-loaded cytoplasmic and nuclear receptor preparations were transferred to a medium with 0.08 M KCl and were incubated with either heparin agarose or DNA cellulose at 4°C for 2 h. Subsequently the receptor adsorbent mixtures were transferred to small columns. Unbound DHT was eluted with buffer solution and the steroid receptor complexes were eluted with 0.6 M KCl (fig.1). Cytoplasmic as well as nuclear receptor preparations were retained by heparin agarose. In contrast only cytoplasmic, but not the nuclear receptor was adsorbed by DNA cellulose. The salt concentration needed for a rapid elution of the receptors from heparin agarose columns was 0.3–0.4 M KCl (fig.2), but rapid elution was also achieved with a heparin solution (3 mg/ml). The presence of [^{3}H]DHT–receptor complexes in the eluates was evaluated by agar gel electrophoresis (fig.3). After elution from the adsorbent 70–80% of the steroid was still bound to the cytoplasmic receptor. Also the nuclear receptor was retained and saturable binding protein was present in preparations eluted from heparin agarose. A remarkable difference was found between

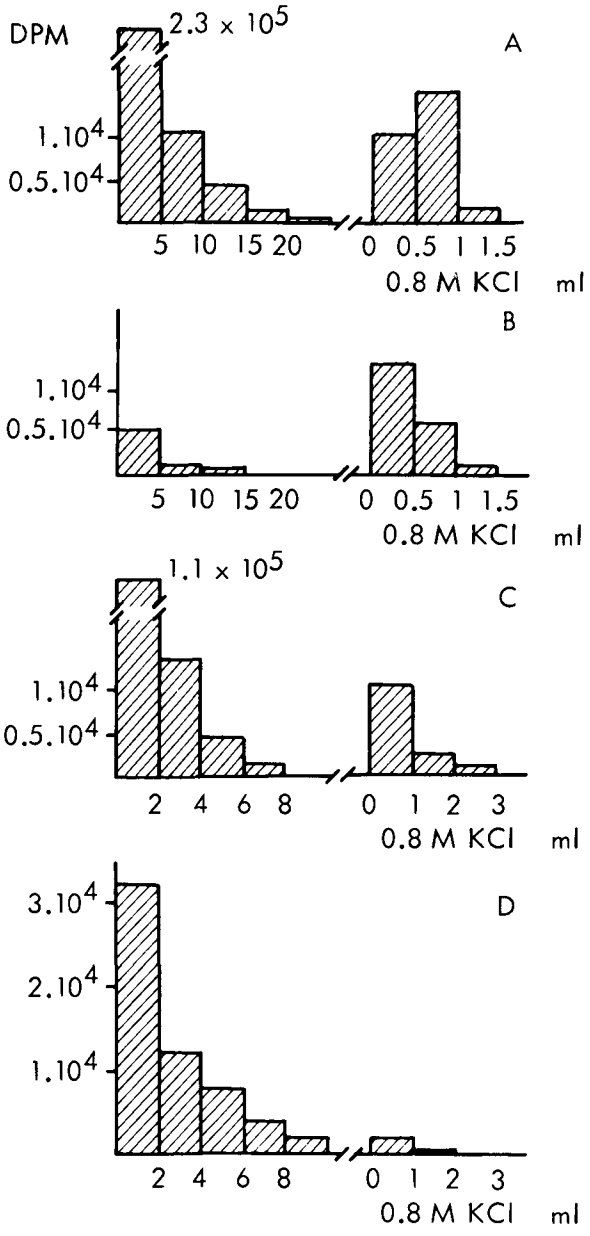

Fig.1. Radioactive steroid recovered from heparin agarose and DNA cellulose adsorbents. Labelled receptor preparations (5 ml) in 0.08 M KCl were incubated with 0.5 ml suspensions of the adsorbents. The adsorbents were washed with TEDG buffer followed by 0.6 M KCl. The radioactivity recovered in each fraction after washing are shown as vertical columns in the figures. (A) Cytoplasmic receptor on heparin agarose; (B) Nuclear receptor on heparin agarose; (C) Cytoplasmic receptor on DNA cellulose; (D) Nuclear receptor on DNA cellulose.

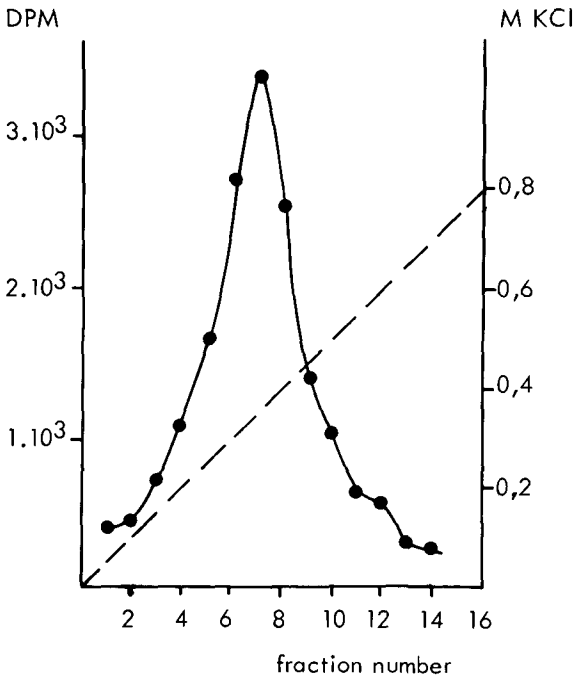

Fig.2. Elution of cytoplasmic androgen receptor from heparin agarose using a KCl gradient. Receptor concentration of preparation applied to the column: 17 fmol/mg protein; receptor concentration in fraction 5:55 fmol/mg protein; in fraction 7:156 fmol/mg protein.

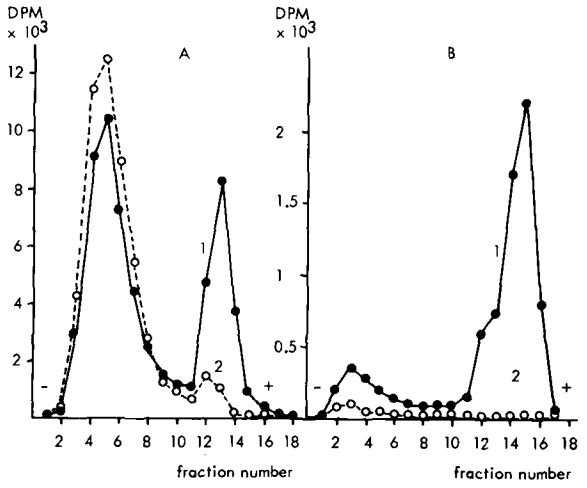

Fig.3. Agar gel electrophoresis of [³H]DHT-labelled receptor preparations. The samples were applied between fraction 10 and 11. After electrophoresis free steroid was recovered in fractions 3–7 and receptor-bound steroid in fractions 12–16. Aggregated steroid–receptor complexes remained in fractions 10–11. (A) Curve 1: Cytosol labelled with [³H]DHT. Curve 2: Cytosol labelled with [³H]DHT in the presence of an 100-fold excess of non-radioactive DHT. (B) Curve 1: Cytosol receptor labelled with [³H]DHT and eluted from heparin agarose. Curve 2: The same experiment with cytosol containing in addition an 100-fold excess of non-radioactive DHT.

preparations eluted with 0.6 M KCl and with heparin. The results from agar gel electrophoresis showed, that after elution and storage at –20°C the nuclear receptor preparation eluted with 0.6 M KCl contained mainly aggregated receptor (fig.4, curve 1).

In contrast the binding proteins in the heparin-containing eluate did not aggregate and moved for the major part to the anodic region of the gel (fig.4, curve 2). Prevention of aggregation by heparin has been generally observed also for other androgen receptor preparations.

For a quantitative comparison of the strengths of the interaction of both the cytoplasmic and nuclear receptor complexes with the adsorbents, diluted

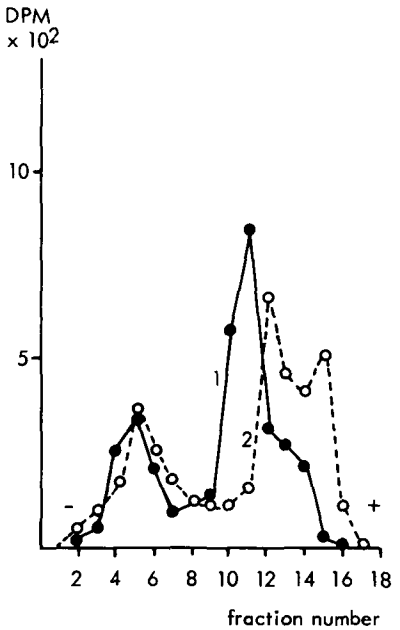

Fig.4. Nuclear receptor eluted from heparin agarose with 0.6 M KCl (curve 1) or heparin solution (curve 2) and subjected to agar gel electrophoresis as in fig.3. The samples were analyzed after storage at –20°C.

Table 1
Elution of prostatic cytoplasmic and nuclear androgen receptors from heparin agarose and DNA cellulose

Receptor preparation	Elution with	Receptor added		% adsorbed	Receptor recovered from adsorbents		% Recovery of receptor from adsorbents
		fmol	fmol/mg protein		fmol	fmol/mg protein	
Heparin agarose							
Cytoplasmic	Heparin	233	28	83	106	218	55
	0.6 M KCl	233	28	85	154	310	78
Nuclear	Heparin	143	89	71	83	85	85
	0.6 M KCl	143	89	71	89	80	90
DNA cellulose							
Cytoplasmic	0.6 M KCl	247	28	48	105	478	88
Nuclear	0.6 M KCl	295	112	< 15	[a]	[a]	

[a] Nuclear receptor was only minimally retained by DNA cellulose, cf. fig.1

Cytoplasmic and nuclear receptor preparations were as in section 2.2. Both preparations were brought into a TEDG buffer with 0.08 M KCl and 2.5 ml of these preparations were incubated for 2 h at 4°C with 0.5 ml suspensions of heparin agarose or DNA cellulose suspensions. The adsorbents were washed with TEDG buffer as shown in fig.1 and eluted either with heparin (3 mg/ml) in TEDG buffer of with 0.6 M KCl in TEDG buffer

receptor preparations were incubated with a small amount of adsorbent (table 1). Androgen receptor content was estimated in separate aliquots of all preparations by agar gel electrophoresis. Under the conditions of this experiment 55–78% of the amount of adsorbed cytoplasmic receptor was recovered after elution from heparin agarose. The elution efficiency with 0.6 M KCl was higher than with heparin. The cytoplasmic receptor was bound by DNA cellulose in a comparable way. There was no significant difference in the binding of cytoplasmic and nuclear DHT-labelled receptors by heparin agarose but a selective removal of non-steroid bound proteins was not achieved for the nuclear extract under the conditions used in table 1.

For preparative purposes a 20-fold purification of cytosol receptor could be reached by stepwise elution with diluted salt solutions, followed by elution with a small volume of 0.6 M KCl. (Initial receptor concentration 28 fmol/mg protein, after purification 570 fmol/mg protein.)

Cytoplasmic receptor can be transformed by warming at 30°C [12] into a form which is more readily bound by nuclear acceptor sites. Apart from a 20–50% loss of binding sites, warming for 20 min at 30°C did not affect the binding to heparin agarose.

4. Discussion

The results of this study show that heparin agarose can be used for purification of cytoplasmic androgen receptors without aggregation of the receptor. Also the nuclear form of the receptor was bound. A specific interaction of calf uterine oestradiol receptor was observed [3] only for the native cytoplasmic form. In this respect differences might exist between the oestradiol and androgen receptor. Warming of the cytoplasmic androgen receptor decreases the sedimentation value form 8 to 4.5 S [1], which may reflect transformation of the receptor into a form which is more readily bound by nuclear chromatin. This treatment did not influence the binding of the androgen receptor to heparin agarose or DNA cellulose. We could confirm, however, the observations in [2] that the androgen receptor extracted from prostate nuclei was bound by DNA cellulose only to a very limited extent. It is tempting to speculate that the differences

in interaction of the cytoplasmic and nuclear forms to heparin agarose and DNA cellulose are related to interactions with different parts of a receptor subunit structure. For the cytoplasmic receptor mol. wt 88 000 was proposed and for the extracted nuclear receptor 33 000 [13]. The part of the structure interacting with heparin agarose remains possibly intact after extraction of the nuclei, in contrast to the structure interacting with DNA. It is clear that detailed studies of the different forms of the androgen receptor should wait until purified receptor preparations are available.

References

[1] Mainwaring, W. I. P. (1977) The mechanism of action of androgens, Springer-Verlag, New York.
[2] Rennie, P. S., Van Doorn, E. and Bruchovsky, N. (1977) Mol. Cell. Endocrinol. 9, 145–157.
[3] Molinari, A., Medici, N., Moncharmont, B. and Puca, G. A. (1977) Proc. Natl. Acad. Sci. USA 74, 4886–4890.
[4] Høisaeter, P. A. (1973) Biochim. Biophys. Acta 317, 492–499.
[5] Mulder, E., Peters, M. J., De Vries, J., Van der Molen, H. J., Østgaard, K., Eik-Nes, K. B. and Oftebro, R. (1978) Mol. Cell. Endocrinol. 11, 309–323.
[6] Mulder, E., Peters, M. J., Van Beurden, W. M. O. and Van der Molen, H. J. (1974) FEBS Lett. 47, 209–211.
[7] Peterson, G. L. (1977) Anal. Biochem. 83, 346–356.
[8] Cuatrecasas, P. (1970) J. Biol. Chem. 245, 3059–3065.
[9] Bitter, T. and Muir, H. M. (1962) Anal. Biochem. 4, 330–334.
[10] Alberts, B. M. and Herrick, G. (1971) Methods Enzymol. 21, 198–217.
[11] Giles, K. W. and Myers, A. (1965) Nature 206, 93.
[12] Mainwaring, W. I. P. and Irving, R. (1973) Biochem. J. 134, 113.
[13] Bruchovsky, N., Rennie, P. S. and Vanson, A. (1975) Biochim. Biophys. Acta 394, 248–266.