GLUCOCORTICOID AND THYROID HORMONE-BINDING TO NUCLEAR RIBONUCLEOPROTEIN PARTICLES

N. DEFER, M. M. SABATIER and J. KRUH
Institut de Pathologie Moléculaire,+ 24, Rue du Faubourg Saint-Jacques, 75014 Paris

and

M. C. DABAUVALLE, C. CREUSET and J. LOEB
Institut de Recherches Scientifiques sur le Cancer, BP No. 8, 94 800 Villejuif, France

Received 25 February 1977

1. Introduction

In previous works, we have shown that glucocorticoid and thyroid hormones are able to bind directly to rat liver chromatin non-histone proteins (NHP) [1,2]. Evidences have been given that NHP contain the translocated glucocorticoid cytoplasmic receptor [3] whereas triiodothyronine (T_3) binds to NHP in a free-form [4]. In addition, a lower affinity binding site of glucocorticoid hormone has been found in NHP, which migrates in electrophoretic gel with an R_F different from that of the hormone–receptor complex.

Since it is generally admitted that steroid and thyroid hormones play a role in the control of gene expression, we found it interesting to see whether ribonucleoprotein (RNP) particules which have been assumed to carry precursors of messenger RNAs and also play a role in gene expression [5,6] contain hormone binding proteins.

By comparison with total rat liver NHP and with NHP prepared from chromatin freed from most of the RNP-particles, which will be referred to as chromosomal-NHP (chr.-NHP), we observed that part of the T_3-receptor and part of the lower affinity glucocorticoid receptor are present in RNP-particles, but not the high affinity steroid receptor.

+Groupe U 137 INSERM, Laboratoire Associé No. 85 CNRS

2. Materials and methods

2.1. *Preparation of NHP, RNP-particles and chromosomal NHP*

Rats fasted overnight and killed by decapitation were used for all preparations. All experiments were performed at 4°C.

NHP were prepared by the method of Kamiyama and Wang [7] from liver nuclei obtained by the procedure of Chauveau et al. [8].

Rats used for the preparation of RNP-particles and chr.-NHP were injected with 50 μCi [^{14}C]orotic acid (spec. act. 40 mCi/mmol – CEA, France). Nuclei were prepared by the technique of Aaronson and Blobel [9] except that the following buffer was used: 10 mM Tris buffer, pH 7.2, containing 25 mM KCl, 2.5 mM $MgCl_2$. The purity was controlled by light and electron microscopy. RNP-Particles were prepared by the method of Stevenin et al. [10]. This method includes a sonication of the nuclei, a centrifugation at 40 000 rev/min for 5 min which removes nucleoli, chromatin and unbroken nuclei. The RNP-particles were obtained with the use of a 10–25% sucrose-gradient over a cushion of 30% sucrose. The gradient was analyzed with an ISCO gradient analyzer.

The radioactivity was measured on 50 μl aliquots transferred on Whatman 3 MM disks. The disks were washed with 10% trichloracetic acid, alcohol, acetone and dried. They were counted in 10 ml of Popop–

North-Holland Publishing Company – Amsterdam

Ppo–toluene mixture. The radioactivity was found in a peak in the 30–40 S region. The particles were collected from this region by centrifugation at 35 000 rev/min for 18 h and controlled by electron microscopy which showed apparently homogenous 248 ± 75 Å particles. The absence of ribosomal proteins and of histones was controlled by extraction with 0.4 N H_2SO_4 and by electrophoresis performed by the methods of Panyim and Chalkley [11]. Electrophoresis performed according to Laemmli [12] showed approximately 60 bands in the range of molecular weights of 20 000–150 000, with two major classes in the range of 30 000–34 000 and of 40 000–80 000. The electrophoretic pattern of NHP was different but molecular weights were also in the range of 20 000–150 000.

Chromosomal-NHP were prepared from the pellets obtained by centrifugation after sonication of the nuclei. The pellets were layered over 30% sucrose according to Bhovjee and Pederson [13]. Nucleoli and unbroken nuclei were discarded by a centrifugation at 2000 rev/min for 15 min. The supernatant was centrifuged at 25 000 rev/min for 4 h. The pellets contained less than 10% of the radioactivity found in RNP-particles. Chromosomal-NHP were extracted from the pellet with 1 M NaCl by the procedure of Kamiyama and Wang [7].

2.2. *Hormone-binding assays*

The following radiochemicals were used: [1–2, 6–7-^{3}H]corticosterone (spec. act. 91 Ci/mmol – Radiochemical Centre, Amersham) and L-triiodothyronine (spec. act. 100 Ci/mmol – CEA, France). Non-radioactive corticosterone was from Visman (Italy), L-triiodothyronine from Sigma.

Nuclear RNP were dialyzed against the following buffers: 50 mM Tris buffer, pH 8.0, containing 0.4 M NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, 5 mM $MgCl_2$ for glucocorticoid binding; 10 mM Tris buffer, pH 7.5, containing 0.4 M NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol for triiodothyronine binding.

Incubations were performed in the appropriate buffer at 4°C for 24 h. Parallel experiments were always performed in the presence of a 500-fold excess of non-labelled homologous hormone for the correction for non-specific binding. Free hormone was removed by the charcoal method [14].

2.3. *Electrophoreses of hormone-binding protein complexes*

NHP- and RNP-particles were incubated with each hormone. RNP particles were digested with 50 μg of RNAase A (Sigma) for 30 min at 37°C. Samples containing 500 μg protein were layered on 6% polyacrylamide gels in the Rodbard and Chrambach system [15] modified by Sherman et al. [16]. The gels were cut into 2 mm wide slices and the radioactivity measured in 10 ml Popop–Ppo–toluene mixture in an SL Intertechnique liquid scintillation counter.

3. Results

3.1. *Glucocorticoid-binding to RNP-particles*

When total NHP-, RNP-particles and chr.-NHP were incubated with [^{3}H]corticosterone, a specific binding was observed, resistant to RNAase digestion. The Scatchard plots of the binding to the various fractions are shown in fig.1 A, and the binding characteristics summarized in table 1. The affinity of the RNP-particles towards the hormone was $5–6 \cdot 10^{-8}$ M^{-1}. Whatever the protein concentration, a single-slope was found corresponding to an homogenous class of binding sites. As previously described [1], in total

Table 1
Binding characteristics of corticosterone to RNP-particles and to NHP

Nuclear fraction	Affinity constant (K_a M^{-1})	Specific activity (hormone-bound mol/mg protein)
RNP	$5–6 \cdot 10^{8}$	$1 \cdot 10^{-13}$
Chromosomal-NHP	$2 \cdot 10^{10}$	$0.6 \cdot 10^{-14}$
	$1.3 \cdot 10^{9}$	$0.3 \cdot 10^{-13}$
Total-NHP	$5 \cdot 10^{9}$	$0.8–1 \cdot 10^{-14}$
	$9 \cdot 10^{8}$	$0.8 \cdot 10^{-13}$

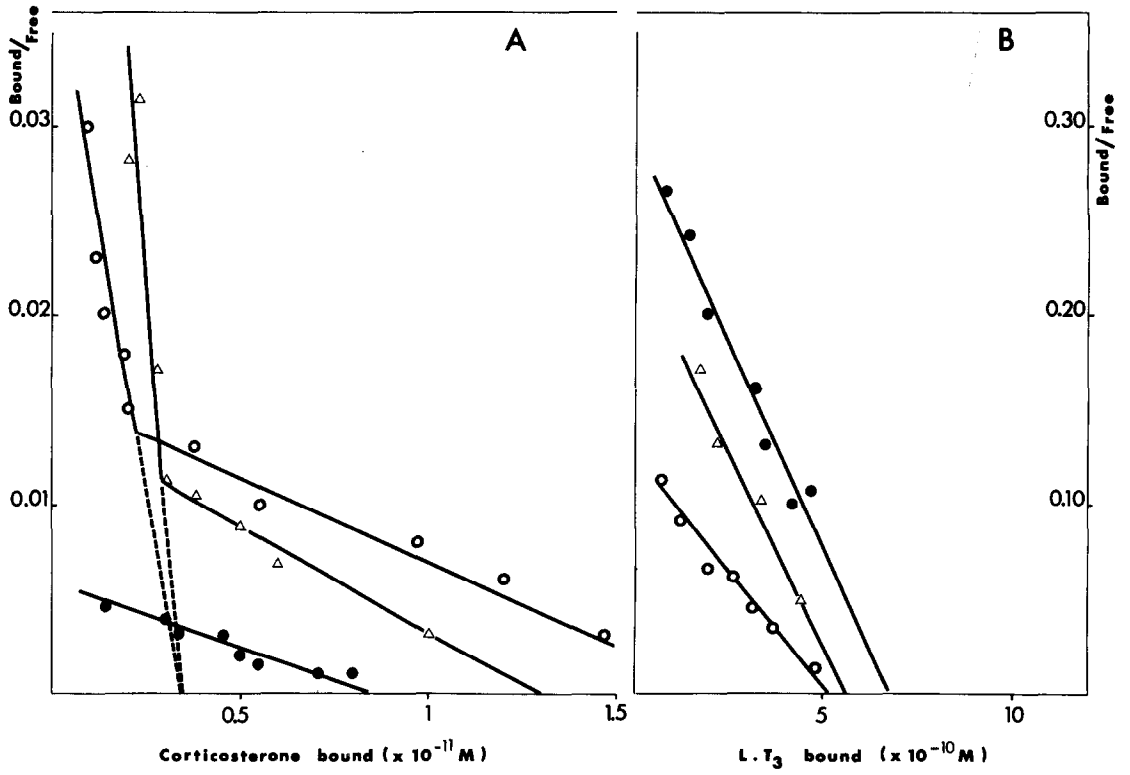

Fig.1. Hormone-binding to nuclear fractions – Scatchard plots. (A) Corticosterone-binding: RNP-Particles (•——•) 100 μg, chromosomal-NHP (△——△) 300 μg and total-NHP (○——○) 150 μg, were incubated with increasing concentrations of [^{3}H]corticosterone. (B) Triiodothyronine binding: RNP-Particles (•——•) 300 μg, chromosomal-NHP (△——△) 500 μg and total-NHP (○——○) 500 μg, were incubated with increasing concentrations of [^{125}I]tri-iodothyronine. Results are corrected for non-specific binding determined by binding in the presence of a 500-fold excess of non-labelled hormone.

NHP we found 2 corticosterone-binding sites with the following affinities: $5 \cdot 10^9$ M^{-1} and $9 \cdot 10^8$ M^{-1}. The same result was obtained with chr.-NHP.

Figure 2 A shows the electrophoretic pattern of protein–corticosterone complexes. Chromosomal-NHP complexes migrate in one main peak with an R_F of 0.25 and 2 lower peaks with R_F of 0.36 and 0.55. With RNP-particles only the 2 peaks with R_F 0.36 and 0.55 with higher magnitude were observed. In the same experimental conditions, total NHP showed 2 binding sites, the higher affinity (arrow a) with an R_F of 0.25 and the lower affinity (arrow b) with an R_F of 0.36. All these peaks represent specific binding since they were completely abolished in the presence of high concentrations of non-labelled corticosterone.

3.2. *Triiodothyronine-binding to RNP-particles*

Figure 1 B shows the Scatchard plot of T_3 binding to the various nuclear fractions. In the three sets of experiments, the binding sites appeared to be homogenous with no significantly different affinity constants. Both affinity constants and specific activities suggest an identity of the hormone-binding proteins in these fractions.

Table 2 summarizes the binding parameters and gives an estimation of the number of binding sites. Assuming that 1 g liver contains 10^8 cells, 570–840 binding-sites were found in RNP-particles and 5800 in chr.-NHP.

When submitted to polyacrylamide gel electrophoresis, RNP–T_3 complexes migrated in a broad peak centered at R_F 0.60. Chromosomal-NHP–T_3 complex

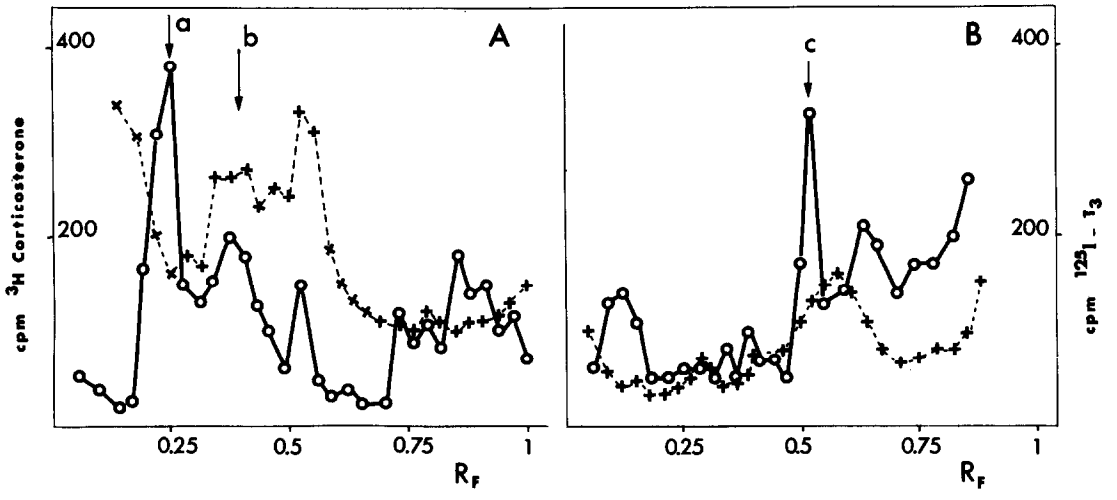

Fig.2. Electrophoretic patterns of hormone–RNP-binding proteins. Samples containing 500 μg RNP (+- - -+) or 500 μg chromosomal-NHP (o——o) were incubated in the presence of $2 \cdot 10^{-8}$ M [^{3}H]corticosterone (fig.2A) or $^{125}IT_3$ (fig.2B) in the presence or in the absence of 500-fold excess of unlabelled hormones. The figures show the specific binding. Arrows a and b represent respectively the higher and lower affinity corticosterone-binding sites in total-NHP. Arrow c shows the migration of NHP–T_3 complex. Free-T_3 migrates next to the front.

migrated in 2 peaks with R_F 0.55 and 0.63 (fig.2 B). Total NHP-complex migrated in a single peak with an R_F of 0.55 (arrow c). Dilution experiments show that these peaks represent specific binding sites.

4. Discussion

It has been clearly established that glucocorticoid and thyroid hormones enter the nuclei of the target-cells and modify gene expression. We have previously shown that these hormones bind to NHP [1,2]. In this work, we show that both hormones also bind to RNP-particles, but only the lower affinity glucocorticoid binding sites are present in these particles.

The presence of androgen and estradiol binding sites in RNP-particles was reported by Liang and Liao [17] using a method of preparation of RNP-particles which does not discard pre-ribosomal particles.

In this study we have prepared RNP-particles by the method of Stevenin et al. [10] which leads to particles free from histones and from ribosomal proteins as shown by the absence of proteins extractable by 0.4 N H_2SO_4 and by electrophoreses performed in the presence of urea or in the presence of SDS (manuscript in preparation).

The presence of RNP-contaminants in chr.-NHP preparations has been estimated by injecting [^{14}C] orotic acid to rats. The radioactivity found in chromatin was less than 10% that found in RNP-particles/

Table 2
Binding characteristics of tri-iodothyronine to RNP-particles and to NHP

Nuclear fraction	Affinity constant (K_a M^{-1})	Specific activity (hormone-bound mol/mg protein)	mg protein/g liver	Binding sites/liver cell
RNP	$4.3 \cdot 10^8$	$1.35–2 \cdot 10^{-12}$	0.07	570–840
Chromosomal-NHP	$4.1 \cdot 10^8$	$0.97 \cdot 10^{-12}$	1	5800
Total-NHP	$2.5 \cdot 10^8$	$1 \cdot 10^{-12}$	1	6000

mg. Even if we assume that it represents 50% of the total RNP, no more than 15% of the total number of T_3-binding sites would be present in RNP-particles. It is excluded that the T_3-receptors found in chr.-NHP could be present only in contaminant-RNP. It would mean that 85% of chromatin proteins are actually RNP-packaging proteins.

The physiological signification of hormone-binding sites in RNP is not understood at the present time, in particular we do not know the meaning of the corticosterone lower affinity binding sites.

Two main hypotheses can be drawn:

(i) The hormones bind either to chromatin or to RNP-particles and their mechanism of action would be different according to their binding sites;

(ii) The hormones bind to chromatin and then move in RNP-particles where they control the availability of messenger RNAs [18].

Acknowledgements

We thank Dr G. Moyne from the Department of Electron Microscopy of IRSC at Villejuif (France) for having performed the control electron microscopy. This work was supported by grants from the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, the Délégation Générale à la Recherche Scientifique et Technique and the Commissariat à l'Energie Atomique.

References

[1] Defer, N., Dastugue, B. and Kruh, J. (1974) Biochimie 56, 559–566.
[2] Thomopoulos, P., Dastugue, B. and Defer, N. (1974) Biochem. Biophys. Res. Commun. 58, 499–506.
[3] Defer, N., Dastugue, B. and Kruh, J. (1974) Biochimie 56, 1549–1557.
[4] Defer, N., Dastugue, B., Sabatier, M. M. and Kruh, J. (1976) Biochimie 58, 881–883.
[5] Matringe, H. and Jacob, M. (1972) Biochimie 54, 1169–1178.
[6] Pederson, T. (1974) J. Mol. Biol. 83, 163–183.
[7] Kamiyama, M. and Wang, T. Y. (1971) Biochim. Biophys. Acta 228, 3213–3218.
[8] Chauveau, J., Moulé, Y. and Rouiller, (1956) Exptl. Cell Res. 11, 317–321.
[9] Aaronson, R. P. and Blobel, G. (1974) J. Cell Biol. 746–754.
[10] Stevenin, J., Gallinaro-Matringe, H. and Jacob, M. (1975) Biochimie 57, 1099–1102.
[11] Panyim, S. and Chalkley, R. (1969) Biochemistry 8, 3972–3979.
[12] Laemmli, U. K. (1970) Nature 227, 680–685.
[13] Bhorjee, J. S. and Pederson, T. (1973) Biochemistry 12, 2766–2771.
[14] Milgrom, E. and Baulieu, E. E. (1969) Biochim. Biophys. Acta 194, 602–605.
[15] Rodbard, D. and Chrambach, A. (1971) Anal. Biochem. 40, 95–134.
[16] Sherman, M. R., Corvol, P. L. and O'Malley, B. W. (1970) J. Biol. Chem. 245, 6085–6096.
[17] Liang, T. and Liao, S. (1974) J. Biol. Chem. 249, 4671–4678.
[18] Tomkins, G. M., Gelehrter, T. D., Granner, D., Martin, D., Samuels, H. H. and Thompson, B. E. (1969) Science 166, 1474–1480.