DNA BINDING OF THYROID HORMONE RECEPTORS

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SUMMARY

Thyroid hormone receptors, isolated from rat liver nuclei, bind to purified DNA. By contrast, free triiodothyronine and plasma proteins which bind thyroid hormone do not associate with DNA. Thus, the nuclear localization of thyroid hormone in target tissues may be explained by the association of its receptors with DNA.

INTRODUCTION

The thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4), regulate metabolism, macromolecular synthesis and differentiation in a number of eukaryotic tissues (1-3). Recently, nuclear binding sites for T_3 and T_4 which appear to mediate some of the actions of thyroid hormone have been found in tissues which respond to these hormones (4-10). In contrast with the steroid hormones (11), thyroid hormone does not appear to influence the nuclear localization of its "receptors" (12). Thus, thyroid hormones may act in a fundamentally different way than the steroids.

The thyroid hormone receptors are located in the chromatin component of nuclei, and to date, receptors with similar properties have not been found in other cell fractions. The receptors can be solubilized by high salt concentrations (0.4 M KCl), and behave as non-histone proteins (5,13,14). A tight receptor association with chromatin is implied. First, the receptors remain bound to chromatin at 0.2 M KCl (15). Second, the receptors, like bacterial RNA polymerase and histones, but unlike most other proteins which have been examined, can be fixed to chromatin by formaldehyde treatment (17). Thus, these proteins represent one of the few examples of a tightly-associated chromatin protein whose function can be identified.

We have recently reported that the thyroid hormone receptors are non-randomly distributed in chromatin subfractions; centrifugation of sheared chromatin in sucrose gradients shows a preferential concentration of bound $[^{125}I]T_3$ in the more slowly sedimenting fractions (16). These fractions contain most of the endogenous RNA polymerase II (B) activity and appear to have more "open" DNA as based on template activity with exogenous polymerase (16). Because the receptors are closely associated with chromatin and are concentrated in chromatin subfractions which contain more "open" DNA, it seemed possible that the receptors are localized in chromatin in vivo because they bind to DNA. To explore this possibility we asked whether the solubilized thyroid hormone receptors bind to purified DNA.

MATERIALS AND METHODS

Preparation of solubilized $[^{125}I]T_3$-receptor complexes. The methods for preparation of nuclei and solubilization of $[^{125}I]T_3$-receptor complexes are based on those of Samuels and Tsai (9,10,14). Liver (3 g) obtained from a male Sprague-Dawley rat was minced in 9 ml of buffer A (0.25 M sucrose, 0.02 M Tricine, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, pH 7.4). All procedures were at 0^{o} C unless stated. The mince was homogenized in a Teflon-glass tissue grinder by five strokes at 1000 rpm, filtered through cheesecloth, brought to 30 ml, and centrifuged (800 x g, 10 min). The pellet was washed three times with buffer A---twice in the presence of 0.5% Triton X-100 and once without. These nuclei, stained with 0.1% azure A, appeared intact by light microscopy with little debris. Nuclei were incubated for 5 hr in 60 ml of buffer B (0.25 M sucrose, 0.02 M Tris, 1.1 mM $MgCl_2$, 5% glycerol, 0.1 mM dithiothreitol, pH 7.8), containing 2 mM EDTA, and 10^{-8} M $[^{125}I]T_3$ (S. A. 370 mCi/mg, Abbott Labs.). Nonspecific binding was determined by a parallel incubation which, in addition, contained nonradioactive T_3 (Calbiochem) at 10^{-6} M. Following centrifugation, the radiolabeled nuclei were washed with 6 ml of buffer B containing 0.5% Triton X-100, resuspended in 0.8 ml of 0.4 M KCl in buffer B, incubated for 20 min and centrifuged (17,000 x g, 20 min). Macro-

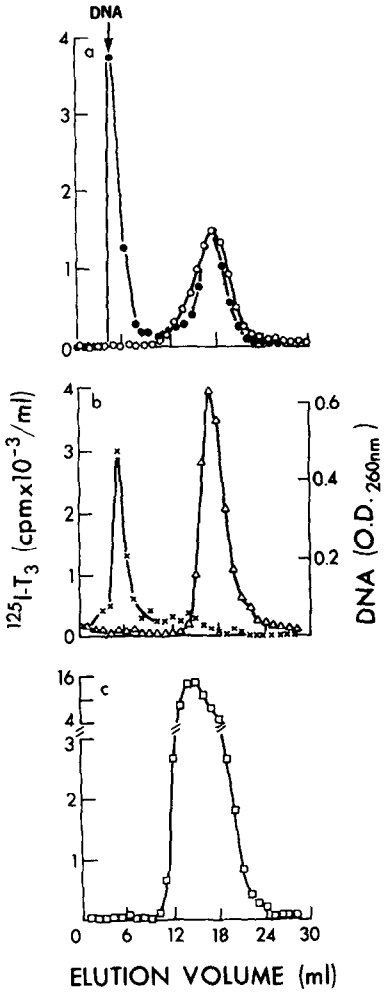

Figure 1. Binding of thyroid hormone receptors by DNA as measured by filtration on Sepharose 2B. a) Elution of solubilized [^{125}I]T_3-receptor complexes alone (o---o), or after incubation with DNA (●---●). b) Elution profile (O. D., 260 nm) of DNA on the column (x---x). Elution of [^{125}I]T_3 (3.7×10^{-10} M) after incubation with DNA (Δ---Δ). The buffer conditions used were similar to those for [^{125}I]T_3-receptor binding by DNA. c) The elution of [^{125}I]T_3-plasma complexes after incubation with DNA (□---□).

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molecular bound [^{125}I]T_3 (T_3-receptor complex) was collected in buffer B in the excluded volume of a G-25 Sephadex column (10 x 1 cm). For the representative experiment shown in Fig. 1, the eluate contained 2.3×10^{-10} M

$[^{125}I]T_3$-receptor complexes. Over 50% of the specifically-bound $[^{125}I]T_3$ in the nuclei had been extracted and more than 95% of the G-25-excluded radioactivity was due to that specifically bound by the receptor (see Methods (5)).

Plasma-bound $[^{125}I]T_3$. Plasma was obtained from the same animal. Some endogenous thyroid hormone was removed by incubation of plasma with 125 mg/ml of activated charcoal for 30 min at 37° C in a shaker bath. After centrifugation, the supernatant plasma was diluted into one volume of buffer B containing 2 mM EDTA, incubated with 10^{-8} M $[^{125}I]T_3$ at room temperature for 30 min, filtered over the Sephadex column, and incubated with DNA as described. The final concentration of plasma-bound $[^{125}I]T_3$ was 5.2×10^{-10} M.

DNA binding. Calf Thymus DNA (Calbiochem), at 1 mg/ml in H_2O, was sheared by 10 passes through a 27 gauge needle (18). The reaction mixture (0.6 ml) contained 0.12 mg/ml DNA, solubilized $[^{125}I]T_3$-receptor complexes (1.9×10^{-10} M for the experiment shown in Fig. 1) 2 mM EDTA and buffer B. After incubation for 90 min, the reaction mixture was filtered on a Sepharose 2B column (1 x 26 cm) and was eluted in buffer B containing 2 mM EDTA at 0.6 ml/min. Radioactivity was measured using 4 g/l Omnifluor (New England Nuclear Corp.), 25% Triton X-100 in toluene. Recovery of added DNA in the excluded fractions ranged from 85-95% in various experiments.

RESULTS AND DISCUSSION

The Sepharose gel assay for DNA binding is similar to that employed for DNA binding by steroid receptors (18). The gel separates DNA and associated molecules from free T_3-receptor complexes and T_3. $[^{125}I]T_3$-receptor complexes in the presence of DNA elute in the void volume with DNA (Fig. 1 (a)), whereas in the absence of DNA they do not. By contrast, after incubation of free $[^{125}I]T_3$ with DNA, no radioactivity elutes with DNA (b). These data suggest that T_3-receptor complexes, either alone or combined with other factors in the nuclear extract, bind to DNA. Although it seems unlikely, we cannot as yet exclude the possibility that the receptors or other factors in the extract are promoting a DNA-free $[^{125}I]T_3$ association.

A further indication that it is the DNA which determines whether the T_3-receptor complex will be excluded from the gel is obtained from experiments in which DNA was incubated with DNase I (Worthington, 0.1 mg/ml) prior to incubation with [^{125}I]T_3 and gel filtration. This resulted in at least a 75% reduction in the amount of [^{125}I]T_3 eluted in the void volume. This was associated with a corresponding shift of both the radioactivity and the optical density to the included volume. In occasional experiments (not shown) a small proportion of the T_3-receptor complexes which were not incubated with DNA eluted in the void volume and appeared as "DNA binding". This may have been caused by T_3-receptor-nucleoprotein complexes or by T_3-receptor aggregates present in some of the extracts. However, even when these extracts were incubated with DNA, there was a marked (more than five fold) DNA-promoted increase in radioactivity eluting in the void volume.

Since these experiments were performed at low salt concentrations, we tested whether DNA binding could also be observed at an ionic strength which more nearly approximates the physiological. Conditions used were similar to those which pertain to Fig. 1, except that 0.15 M KCl was included in the reaction mixture and columns. In two experiments, 0.26 $\pm$ 0.01 pmoles of [^{125}I]T_3 per mg DNA eluted with the DNA as compared with 0.4 pmoles per mg DNA observed in the experiment shown in Fig. 1.

At higher complex concentrations (ca. 10^{-9} M), we found up to 1.2 pmoles of T_3-receptor complex bound per mg DNA. Thus, the capacity of DNA for binding receptors equals or exceeds the quantity of receptors in the nucleus (1 pmole/mg DNA (10,12,16)). However, even at these complex concentrations, it has not been possible to determine the DNA capacity for binding receptors and if receptor-specific sequences are on DNA. In fact, the receptors may bind extensively to DNA, since we also detected roughly similar binding by rat, calf and bacteriophage lambda DNA.

We also investigated whether T_3-binding proteins other than the receptors bind to DNA. As shown in Fig. 1 (c), plasma-T_3 complexes, which have been

incubated with DNA, do not associate with the DNA.

These studies suggest that the T_3 receptor is a DNA-binding protein. This property can account for the localization of the receptors in the nuclear chromatin. The extent of T_3-receptor binding by DNA can account for the quantity of nuclear receptors. Of particular importance is the finding that DNA binding is observed at 0.15 M KCl. This result correlates with the retention of the receptors in nuclei at this salt concentration (15), and suggests that the DNA-receptor association can exist at physiological ionic strength. The lack of elution with DNA by T_3-plasma complexes indicates that DNA binding is not a general feature of proteins that interact with T_3. These findings are consistent with the idea that thyroid hormone controls genetic expression through nuclear events. This control could possibly be through influences on proteins which regulate at the level of DNA.

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