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Fatty Acyl-CoA Binding Activity of the Nuclear Thyroid Hormone Receptor

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Abstract Long-chain fatty acids and their acyl-CoA esters are potent inhibitors of nuclear thyroid hormone (T_3) receptor in vitro. In the present study, we obtained evidence for acyl-CoA binding activity in the nuclear extract from rat liver. The activity sedimented at a position (3.5 S) identical with that of the T_3 receptor, and the two activities sedimented together. Similarly, they coeluted on DEAE-Sephadex. After partial purification of the receptor, it was again inhibited strongly by acyl-CoAs. Heat stability and a partial trypsin digestion of the receptor both suggested that the action site of oleoyl-CoA overlapped the T_3-binding domain of the receptor. In addition, thyroid hormone receptor β1, synthesized in vitro, bound oleoyl-CoA specifically and its T_3-binding activity was inhibited. The dissociation constant for oleoyl-CoA binding to the partially purified receptor was 1.2×10^{-7} M. This value as well as its molecular size distinguished the nuclear binding sites from the cytoplasmic fatty acid/acyl-CoA binding proteins. Oleoyl-CoA had no effect on the glucocorticoid receptor, another member of the nuclear hormone-receptor superfamily. From these results, we propose that thyroid hormone receptor is a specific acyl-CoA binding protein of the cell nucleus.

Key words: thyroid hormone, fatty acyl-CoAs, long-chain fatty acids, Erb A protein, nuclear fatty acyl-CoA-binding protein

Thyroid hormone has a fundamental role in the regulation of development, differentiation, and metabolism [Braverman and Utiger, 1991]. Its receptors are intrinsic chromatin-associated proteins, which bind to T_3 with a high affinity. They regulate the expression of target genes by binding to their thyroid hormone response elements. The cDNAs of variant Erb A proteins (α1, β1, and β2) were isolated and found to encode T_3 nuclear receptors [see references in Evans, 1988; Lazar and Chin, 1990]. Thyroid hormone receptor is a member of the nuclear receptor superfamily. The family consists of receptors that are activated by the specific ligands such as steroids, retinoids, vitamin D_3, and thyroid hormones [Evans, 1988].

We reported that unesterified long-chain fatty acids inhibited T_3-binding to the nuclear receptors in HTC rat hepatoma cells in culture as well as to receptors that were solubilized or in isolated nuclei [Inoue et al., 1989]. CoA-esters of the fatty acids (acyl-CoAs) were more potent in the inhibition [Li et al., 1990]: The receptor extracted from rat liver nuclei was inhibited by 50% with oleic acid and oleoyl-CoA at 2.8 and 1.3 μM, respectively. Kinetic and displacement studies demonstrated that they compete with T_3 for the hormone binding site on the receptor. They also increase dissociation of the preformed hormone-receptor complexes [Inoue et al., 1989; Li et al., 1990]. All of these studies suggested that the thyroid hormone receptor interacts with acyl-CoA esters or with unesterified fatty acids.

We report here that the thyroid hormone receptor is an acyl-CoA binding protein of the cell nucleus.

MATERIALS AND METHODS

Materials

[^{125}I]Thyroid hormone (T_3, sp. activity > 1,200 μCi/μg) and [1-^{14}C]oleoyl-CoA (sp. activity 40–60

Abbreviations used: DTT, dithiothreitol; oleoyl-CoA, oleoyl coenzyme A; EDTA, ethylenediaminetetra acetic acid; hTRβ1, human thyroid hormone receptor β1; palmitoyl-CoA, palmitoyl coenzyme A; SDG, sucrose density gradient; T_3, 3,5,3′-triiodothyronine.

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mCi/mmol) were purchased from NEN. Unlabelled T_3, malonyl-CoA, oleoyl-CoA, and palmitoyl-CoA were obtained from Sigma. Acetyl-CoA, sucrose, and an in vitro translation kit were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), *Hin*dIII and DEAE-Sephadex A-25 were from Pharmacia Fine Chemicals, membrane filters (type HAWP 02500, 0.45 μm) were from Millipore Corp., and analytical grade anion exchange resin AG 1X8, 200–400 mesh, chloride form, was from Bio-Rad Labs. Riboprobe Gemini II for in vitro transcription was purchased from Promega (Madison, WI). Human placenta *c-erbA* β cDNA (peA 101) was provided by Dr. R.M. Evans of the Salk Institute.

Extraction of Thyroid Hormone Receptor

The nuclear thyroid hormone receptor was prepared from rat liver by a method published before [Inoue et al., 1989]. The protein concentration of the extract was 2.4 mg/ml.

Sucrose Gradient Centrifugation

A portion (0.15 ml) of the receptor extract, previously labeled with [^{125}I]T_3 (0.1 nM) by incubation for 20 h at 0°C, was centrifuged at 4°C on 12 ml of a 5–20% (w/v) linear sucrose gradient in buffer T (50 mM NaCl, 2 mM EDTA, 10% (w/v) glycerol, 0.16 mM DTT, 20 mM Tris-HCl, pH 8.2) at 50,000 rpm for 4 h in a RP55VF vertical rotor (Hitachi). After centrifugation, the gradients were separated into 13 aliquots. The S-value was obtained with hemoglobin, bovine serum albumin, and yeast alcohol dehydrogenase, as references.

DEAE-Sephadex A-25 Chromatography

The receptor extract (7.2 mg protein), dialyzed against buffer T and centrifuged, was incubated with [^{125}I]T_3 (0.1 nM) for 12 h at 0°C, and put on a column of DEAE-Sephadex A-25 (8 mm in diameter, 4-ml bed) equilibrated with buffer T. After washing of the column, the receptor was eluted by a linear gradient of 0–0.4 M NaCl in buffer T (40 ml); 0.6-ml fractions were collected. All steps were done at 4°C.

Partial Digestion of Nuclear Thyroid Hormone Receptor With Trypsin

The receptor (1.2 mg of protein in 0.5 ml) that had been dialyzed, centrifuged, and incubated with [^{125}I]T_3 as above was digested under the conditions used by Ichikawa and DeGroot [1986], with 3 μg of trypsin at 10°C for 30 min. The digestion was stopped by the addition of 15 μg of trypsin inhibitor.

In Vitro Translation of Thyroid Hormone Receptor (hTRβ1)

In vitro transcription and translation were carried out basically by the method of Weinberger et al. [1986]: The plasmid hTRβ1 was linearized with *Hin*dIII (2 units/μg DNA), treated with proteinase K [Sambrook et al., 1989], and extracted with phenol-$CHCl_3$. TRβ1 mRNA was transcribed from the template by T7 RNA polymerase, and translated with a Wako translation kit, as suggested by the manufacturer.

Binding Assays for T_3, Dexamethasone, and Oleoyl-CoA

The nuclear receptor (usually 50 μl) or TRβ1 synthesized in vitro (usually 4–8 μl) and [^{125}I]T_3 (0.1 nM) in a total 0.5 ml of buffer T were incubated for 12 h at 0°C. [^{125}I]T_3 specifically bound to the receptor was assayed by the Dowex AG 1X8 method or the filter-binding method [Inoue et al., 1989, 1983a). Nonspecific binding was obtained in the presence of a 1,000-fold molar excess of unlabeled T_3 added in parallel in the incubation mixtures. Each assay was done in duplicate or triplicate. Dexamethasone-binding and fatty acyl-CoA binding activities were measured in the same way by incubation with [^{3}H]dexamethasone (0.2 μM) or [1-^{14}C]oleoyl-CoA (0.1 μM) for 12 h at 0°C. Specifically bound ligands were measured by the filter-binding method: The mixtures were mixed with chilled buffer T (3 ml) and filtered at the rate of 15–20 s in a cold room (4°C) on washed membrane filters of nitrocellulose. The filters were then washed 3 times with 6 ml each of cold buffer T and their radioactivity was counted.

RESULTS

Fatty Acyl-CoA Binding Activity in the Nuclear Extract

Table IA shows that the extract from rat liver nuclei had activity to bind [1-^{14}C]oleoyl-CoA at a tracer concentration (10^{-7} M). The binding of [^{14}C]oleoyl-CoA was blocked by the 1,000-fold molar excess of unlabeled oleoyl-CoA added in the assay mixture, suggesting that the binding was specific. The same results were obtained

TABLE I. Fatty Acyl-CoA Binding Activity in Rat Liver Nuclear Extract*

Excess unlabeled oleoyl-CoA	Bound [^{14}C]oleoyl-CoA (cpm/100 μg protein)	(pmol)
(A) −	1,100 ± 60	18
+	200 ± 30	3
(B) −	1,700 ± 90	27
+	520 ± 20	8

*Rat liver nuclei were purified by extensive washing with 0.5% Triton X-100 in SMT (0.25 M sucrose, 2 mM $MgCl_2$, and 20 mM TrisHCl, pH 7.6) [Inoue et al., 1989] (A) or centrifugation through 2.3 M sucrose, 3 mM $MgCl_2$, and 0.2 mM phenylmethylsulfonyl fluoride [Inoue et al., 1983] followed by one washing with 0.5% Triton X-100 in SMT (B). The nuclei were washed again with SMT, and the receptors were extracted. The receptor extract (50 μl) and [1 − ^{14}C]-oleoyl-CoA (0.1 μM) were incubated for 12 h at 0°C in the presence or absence of a large excess of unlabeled oleoyl-CoA (0.1 mM). Protein-bound radioactive oleoyl-CoA was determined by the filter-binding method and values were expressed as means with range of duplicate determinations.

with the receptor extracted from highly purified nuclei (Table IB).

Sedimentation on a Sucrose Density Gradient and Elution on DEAE-Sephadex

In the centrifugation of the rat liver nuclear extract on sucrose density gradients, the specific oleoyl-CoA binding activity sedimented as a single peak at 3.5 S, and superimposed on the T_3-binding activity peak of the receptor (Fig. 1).

Figure 2 shows elution profiles of the T_3 and fatty acyl-CoA binding activities on DEAE-Sephadex A-25. The acyl-CoA-binding activity was eluted at 0.13 M NaCl. Again, the binding activities for thyroid hormone and fatty acyl-CoA were eluted together.

In the fractionations, the oleoyl-CoA binding activity and the bulk proteins were fractionated separately (Figs. 1, 2), thereby indicating that the peaks of oleoyl-CoA binding did not result from nonspecific binding by the bulk proteins. The T_3 and oleoyl-CoA bindings corresponded well to each other, as seen in the similarity of their relative activity-ratios in the two fractionations.

Inhibition by Acyl-CoAs of T_3-Binding of the Purified Nuclear Receptor

Acyl-CoA esters have been shown to inhibit T_3-binding by the receptor in crude nuclear extracts [Li et al., 1990]. With the receptor partially purified on sucrose density gradients, the same inhibition was obtained (Fig. 3). Oleoyl-CoA and palmitoyl-CoA at 10 μM reduced the receptor's T_3-binding activity to 17% and 34%,

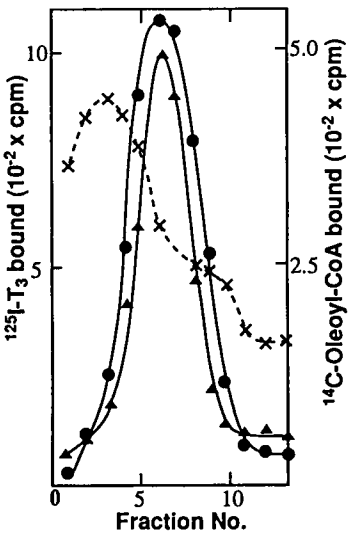

Fig. 1. Cosedimentation of acyl-CoA binding activity and thyroid hormone receptor. Hormone-receptor complex, preformed by incubating the nuclear receptor and [^{125}I]T_3 (0.1 nM) for 12 h at 0°C, was centrifuged on a sucrose density gradient. Aliquots of the fractions were filtered on nitrocellulose membranes to measure the receptor-bound [^{125}I]T_3 (●), or incubated with [1-^{14}C]oleoyl-CoA in the presence or absence of an excess of unlabeled oleoyl-CoA and filtered to count for specifically bound [1-^{14}C]oleoyl-CoA (▲). Broken line represents proteins. Direction of sedimentation was from right to left.

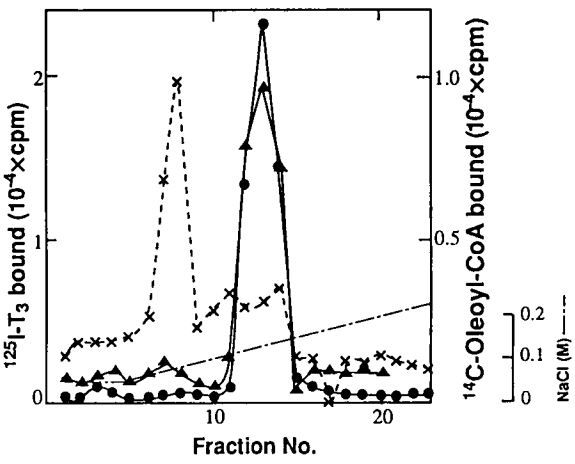

Fig. 2. Elutions of acyl-CoA binding activity and T_3-receptor on DEAE-Sephadex A-25. [^{125}I]T_3-receptor complex was prepared as described in Figure 1 and eluted on a DEAE-Sephadex column with a linear NaCl concentration gradient (0–0.4 M) in buffer T. The symbols represent specific binding activities for [^{125}I]T_3 (●) and [^{14}C]oleoyl-CoA (▲) measured as described in Figure 1. Broken line represents proteins.

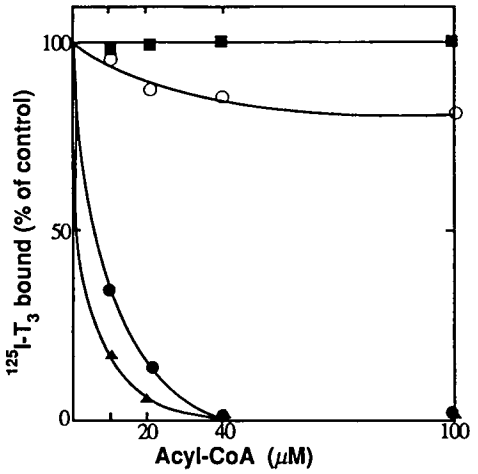

Fig. 3. Inhibition of purified T_3 receptor by long-chain acyl-CoA esters. The receptor, partially purified by sucrose density gradient centrifugation, was incubated at 0°C for 12 h with [^{125}I]T_3 (0.1 nM) together with fatty acyl-CoAs at various concentrations. [^{125}I]T_3 specifically bound was determined by the Dowex AG 1X8 method and expressed as a percentage of the control (minus acyl-CoA in the assay mixtures). The symbols represent acetyl-CoA (■), malonyl-CoA (○), oleoyl-CoA (C18:1) (●), and palmitoyl-CoA (C16:0) (▲). Values are the means of duplicate determinations.

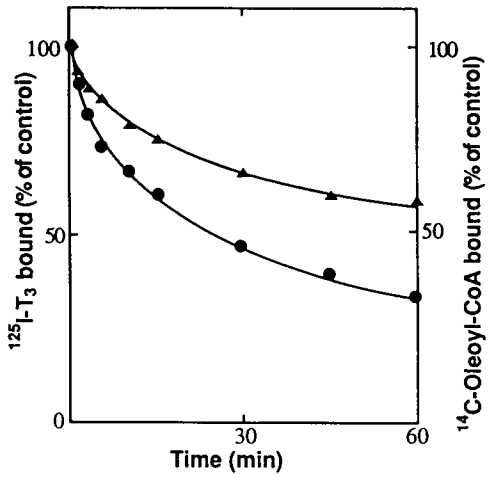

Fig. 4. Heat stabilities of acyl-CoA and T_3 binding activities. The receptor extract was heat treated at 37°C for various times, and then incubated with [^{125}I]T_3 (0.1 nM) or [^{14}C]oleoyl-CoA (0.1 μM) at 0°C for 12 h with or without a large excess of the respective unlabeled ligand. Specifically bound [^{125}I]T_3 (●) and [^{14}C]oleoyl-CoA (▲) were determined by the Dowex AG 1X8 method and the filter binding method, respectively. Duplicate determinations were averaged.

respectively, the control and the inhibitions were complete at 40 μM. The short-chain acyl-CoAs, acetyl-CoA and malonyl-CoA, had no inhibitory activity.

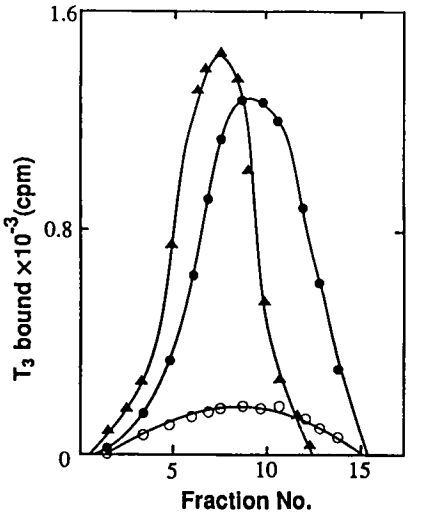

Fig. 5. Effects of oleoyl-CoA on the [^{125}I]T_3 fragments obtained by a limited trypsin digestion. The receptor labeled with [^{125}I]T_3 was incubated with 2.5 μg trypsin/mg protein in the absence (●) or presence (▲) of trypsin inhibitor (12.5 μg/mg of protein) at 10°C for 30 min. After the reaction, trypsin inhibitor was added to the former, and the samples were fractionated by sucrose density gradient sedimentation as described. Tryptic fragments in each fraction were incubated with (○) or without (●) 30 μM oleoyl-CoA for 12 h at 0°C, and T_3 remaining bound to the fragments was measured. Values are the means of duplicate determinations. Dissociation of the [^{125}I]T_3-intact receptor (▲) was similarly enhanced by oleoyl-CoA to the same extents, but is not shown here.

Effects of Heat Treatment on T_3 and Fatty Acyl-CoA Binding Activities

Heat treatment of the rat liver nuclear extract inactivated both T_3 and acyl-CoA binding activities. Inactivation curves of the two activities were very much alike as seen in Figure 4. At 60 min, they had lost 60 and 40% of the initial activities, respectively.

Partial Trypsin-Digestion of the Receptor

As seen in Figure 5, a partial digestion of preformed [^{125}I]T_3-thyroid hormone receptor complex produced a 2.8 S [^{125}I]fragment representing the hormone-binding domain of the receptor [Ichikawa and DeGroot, 1986; Lin et al., 1990]. Oleoyl-CoA did in fact act on this domain as shown by increased dissociation of T_3. This result is consistent with the previous kinetic and displacement data, which suggested that acyl-CoAs acted on the T_3-binding site of the receptor and brought about the inhibition [Li et al., 1990].

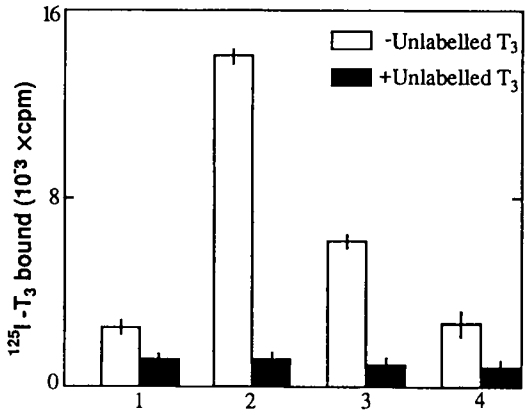

Fig. 6. Inhibition by oleoyl-CoA of T_3-binding to hTRβ1. hTRβ1 (4 μl) was synthesized by in vitro translation, and assayed for T_3-binding with 0.1 nM [^{125}I]T_3 in the presence or absence of various concentrations of oleoyl-CoA for 12 h at 0°C. [^{125}I]T_3 specifically bound was determined by the filter-binding method and values were expressed as the means with range of duplicate determinations. **1,** Mock translated mixture (rabbit reticulocyte lysate) without TRβ1 mRNA; **2,** complete, and assayed without oleoyl-CoA; **3,** complete, and assayed with 3 μM oleoyl-CoA; and **4,** complete, and assayed with 20 μM oleoyl-CoA. Open and closed columns represent [^{125}I]T_3-bindings in the absence and presence of unlabeled T_3, respectively.

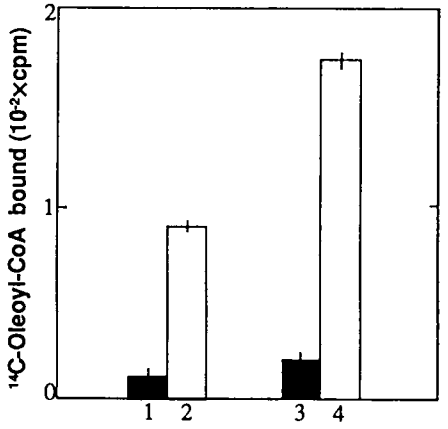

Fig. 7. Fatty acyl-CoA binding to hTRβ1. hTRβ1 synthesized by in vitro translation was assayed for oleoyl-CoA binding activity in a similar manner as described in Figure 1. Values were expressed as the means with range of duplicate determinations. **Lanes 1, 3:** 4 and 8 μl of the control rabbit reticulosyte lysate without TRβ1 mRNA, respectively. **Lanes 2, 4:** 4 and 8 μl of TRβ1 synthesized by in vitro translation using the lysate, respectively. Closed and open columns represent [^{14}C]oleoyl-CoA bindings in the presence or absence of unlabeled oleoyl-CoA, respectively.

Oleoyl-CoA Binding by TR β1 Synthesized by In Vitro Translation

Acyl-CoA binding activity of thyroid hormone receptor could be shown with the receptor synthesized by in vitro transcription of a plasmid containing human TRβ1 cDNA followed by translation in a rabbit reticulocyte lysate. The synthesized TRβ1 had a significant T_3 binding activity (Fig. 6), a 6.3-fold higher specific binding over the control reticulocyte lysate. And oleoyl-CoA added at 20 μM to the translation product inhibited the T_3-binding activity by 89%.

More importantly, the synthesized TRβ1 also bound [^{14}C]oleoyl-CoA specifically. The TRβ1 had a 14.0-fold or higher oleoyl-CoA binding activity than the control lysate. Figure 7 shows the dose dependency of oleoyl-CoA binding: TRβ1 at a 2-fold concentration had a 2-fold higher oleoyl-CoA binding activity.

K_d and MBC of Acyl-CoA Binding Activity of Partially Purified Thyroid Hormone Receptor

Scatchard plots of [^{14}C]oleoyl-CoA binding by the thyroid hormone receptor partially purified on sucrose density gradients demonstrated the existence of a binding site for oleoyl-CoA. It had a dissociation constant (Kd) of 1.2×10^{-7} M and

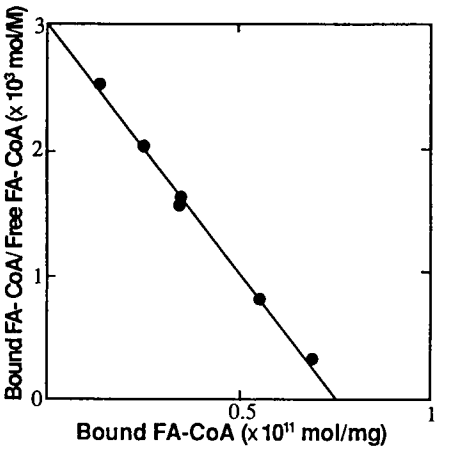

Fig. 8. Scatchard analysis of oleoyl-CoA binding. The receptor (40 μg protein) partially purified on sucrose density gradients was incubated at 0°C for 12 h with various concentrations of [1-^{14}C]oleoyl-CoA (4–400 nM) in the absence and presence of an excess of unlabeled oleoyl-CoA. Specifically bound [^{14}C]oleoyl-CoA was determined. Values are the means of duplicate determinations. Scatchard plots were obtained graphically.

maximum binding capacity (MBC) of 13×10^{-10} mole/mg protein (Fig. 8).

Effects of Oleoyl-CoA on the Thyroid Hormone Receptor and Glucocorticoid Receptors

The possibility that the effect of oleoyl-CoA might be common to the nuclear hormone receptors was examined with thyroid hormone recep-

tor (TR) and glucocorticoid receptor (GR). Rat liver nuclear extract, incubated with labeled T_3 or labeled dexamethasone (Dex), was further incubated with a large excess of corresponding unlabeled hormone in the presence or absence of oleoyl-CoA. Figure 9 demonstrates that oleoyl-CoA strongly promoted the dissociation of the T_3-TR complex but not that of the Dex-GR complex. This indicated that the effect of oleoyl-CoA on TR was specific and not a more general effect on the nuclear receptor proteins.

DISCUSSION

We noted a specific oleoyl-CoA binding activity in the nuclear extract, and the activity exactly corresponded to the nuclear T_3 receptor in sedimentation (3.5 S) on sucrose density gradients and chromatography on DEAE-Sephadex A-25. After partial purification, T_3-binding of the receptor was again inhibited by acyl-CoAs. That acyl-CoA acts on the hormone-binding site of the receptor has been suggested by previous kinetic and displacement studies [Inoue et al., 1989; Li et al., 1990]. Similar results were also obtained in this study using a partial trypsin digestion of the receptor.[1] Furthermore, TRβ1 synthesized by in vitro transcription of cDNA followed by translation bound oleoyl-CoA specifically, and the receptor's T_3-binding activity was inhibited. The receptor could occur as a heterodimer with an acyl-CoA binding protein. If this were the case, it would be expected that the receptor alone or the acyl-CoA binding protein alone should be observed in addition to the heterodimer in the fractionations on sucrose density gradients or on DEAE-Sephadex. However, only a single peak with both T_3- and acyl-CoA binding activities was detected. This exact stoichiometry of the two populations in the cell nucleus may indicate that the acyl-CoA binding protein constitutes a subunit of the thyroid hormone receptor. It is, however, less likely because the previous kinetic and displacement studies indicated that acyl-CoA acts directly on the hormone binding domain of the receptor [Inoue et al., 1989; Li et al., 1990]. Thus, these observations suggest that the acyl-CoA binding activity is an intrinsic property of the thyroid hormone receptor, and that the thyroid hormone receptor is an acyl-CoA binding protein of the cell nucleus.

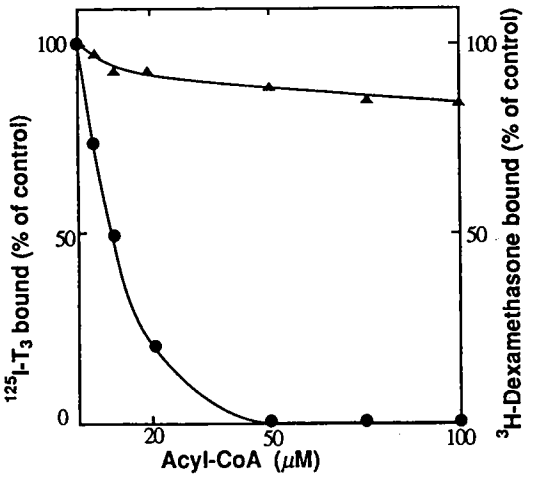

Fig. 9. Effects of oleoyl-CoA on thyroid hormone and glucocorticoid receptors. The nuclear extract from rat liver was incubated with [^{124}I]T_3 (0.1 nM) or [^{3}H]dexamethasone (0.2 μM) in 0.5 ml Buffer T at 0°C for 12 h; then oleoyl-CoA at various concentrations and a large excess of unlabeled T_3 or dexamethasone were added. Labeled hormones remaining bound to the receptors after further incubation at 0°C for 20 h were measured as described, and expressed as percentages of the controls (-oleoyl-CoA). Duplicate determinations were averaged.

Unesterified long-chain fatty acids similarly inhibited the thyroid hormone receptor [Inoue et al., 1989]. However, the free fatty acids were less potent: 50% inhibitions were obtained with oleic acid at 2.8 μM and with oleoyl-CoA at 1.3 μM, respectively [Li et al., 1990]. On sucrose density gradients [^{14}C]oleic acid-binding similarly gave a distinct peak corresponding to the receptor, as seen in Figure 1, indicating that the receptor also bound unesterified fatty acid. Its binding was, however, several-fold less than that of oleoyl-CoA (not shown).

The thyroid hormone receptor has a molecular weight of 50–60 kDa [Ichikawa and DeGroot, 1986]. In cytoplasm, there are fatty acid/acyl-CoA binding proteins (FABPs). The liver FABP has a molecular mass of 14 kDa [Gordon et al., 1983; Takahashi et al., 1983]. In contrast to thyroid hormone receptor, FABPs have higher affinities for unesterified fatty acids than for acyl-CoAs [Bass, 1985; Knudsen, 1990; Sweeter et al., 1987]. There is also a 10 kDa cytosolic acyl-CoA binding protein (ACBP) found in rat and bovine liver. ACBP does not bind fatty acids

[1]The broad 2.8 S peak in Figure 5 may represent a mixture of fragments of heterogenous sizes. Note that the smallest fragment that could still have bound T_3 and thus represent the smallest isolatable ligand-binding domain with bound T_3 (e.g., a 26 kDa peptide from rat liver nuclear TR [Ichikawa and DeGroot, 1986] and 25 kDa peptide from TRβ1 [Lin et al., 1990]) was affected by oleoyl-CoA in the same way as the larger fragments.

and is identical with a putative neurotransmitter diazepam-binding inhibitor [Knudsen, 1990]. The Kd for oleoyl-CoA binding to rat liver FABP is 1.2×10^{-6} M [Bass, 1985], and that to ACBP is 1.3×10^{-7} M [Knudsen, 1990]. The receptor had a Kd of 1.2×10^{-7} M. Thus, the thyroid hormone receptor was comparable to or even higher than the cytoplasmic sites in the affinity for oleoyl-CoA. The nuclear and cytoplasmic binding proteins can thus be distinguished with regard to the preference for esterified and unesterified fatty acids, affinity strength (Kd) for oleoyl-CoA, molecular size, and T_3-binding activity (the nuclear thyroid hormone receptor preferred acyl-CoAs to free fatty acids, and bound oleoyl-CoA with a Kd close to that of cytoplasmic acyl-CoA binding protein (ACBP), which does not, however, bind fatty acids). There are other fatty acid-binding proteins in the cell nucleus. They are a 71 kDa and its cognate 73 kDa stress proteins, and contain tightly bound unesterified palmitic and stearic acids [Guidon and Hightower, 1986a,b]

Heat treatment study showed very similar but slightly different inactivation curves of the T_3- and oleoyl-CoA-binding activities. Therefore it is thought that binding sites for these ligands are very similar in structure, or more likely that they overlap but not perfectly. The same conclusion has been drawn from the finding that the dissociation of [^{125}I]T_3-receptor complex is enhanced by oleic acid, but not by T_3 [Inoue et al., 1989].

TRβ1 synthesized in vitro was inhibited by oleoyl-CoA. The rat liver contains two isoforms of thyroid hormone receptor, TRα1 and TRβ1 [Murray et al., 1988; Macchia et al., 1990]. Their presence in the liver nuclear extract was shown by affinity labeling with a bromoacetylated derivative [Horiuchi et al., 1982] of [^{125}I]T_3 (followed by a DEAE-column chromatography). Since the T_3-binding of the liver nuclear extract was completely inhibited by oleoyl-CoA, both α1 and β1 are thought to be inhibited by the acyl-CoA. The single Kd obtained for oleoyl-CoA-binding (Fig. 6) suggests that α1 and β1 receptors have closely similar Kd values for oleoyl-CoA.

Binding of acyl-CoA by the thyroid hormone receptor was specific in two aspects. First, the specific [^{14}C]oleoyl-CoA binding was demonstrated by its saturability: It was blocked by an excess of unlabeled oleoyl-CoA. Also, the Kd for the binding was as small as 1.2×10^{-7} M. This is near that of the most specific oleoyl-CoA binding protein (e.g., 1.3×10^{-7} M of the ACBP found in the cytosol [Knudsen, 1990]). Second, oleoyl-CoA did not have any effect on the glucocorticoid receptor. This indicates that oleoyl-CoA does not have a general effect on the members of the nuclear hormone receptor superfamily, to which the thyroid hormone receptor belongs.

In conclusion, we suggest that the thyroid hormone receptor is a specific fatty acyl-CoA binding protein of the cell nucleus. The long-chain acyl-CoAs and their unesterified fatty acids may act as regulatory factors of the nuclear thyroid hormone receptors in vivo.

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