Acyl-CoA Esters Antagonize the Effects of Ligands on Peroxisome Proliferator-activated Receptor α Conformation, DNA Binding, and Interaction with Co-factors

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The peroxisome proliferator-activated receptor α (PPARα) is a ligand-activated transcription factor and a key regulator of lipid homeostasis. Numerous fatty acids and eicosanoids serve as ligands and activators for PPARα. Here we demonstrate that S-hexadecyl-CoA, a nonhydrolyzable palmitoyl-CoA analog, antagonizes the effects of agonists on PPARα conformation and function in vitro. In electrophoretic mobility shift assays, S-hexadecyl-CoA prevented agonist-induced binding of the PPARα-retinoid X receptor α heterodimer to the acyl-CoA oxidase peroxisome proliferator response element. PPARα bound specifically to immobilized palmitoyl-CoA and WY14,643, but not BRL49653, abolished binding. S-Hexadecyl-CoA increased in a dose-dependent and reversible manner the sensitivity of PPARα to chymotrypsin digestion, and the S-hexadecyl-CoA-induced sensitivity required a functional PPARα ligand-binding pocket. S-Hexadecyl-CoA prevented ligand-induced interaction between the co-activator SRC-1 and PPARα but increased recruitment of the nuclear receptor co-repressor NCoR. In cells, the concentration of free acyl-CoA esters is kept in the low nanomolar range due to the buffering effect of high affinity acyl-CoA-binding proteins, especially the acyl-CoA-binding protein. By using PPARα expressed in SF21 cells for electrophoretic mobility shift assays, we demonstrate that S-hexadecyl-CoA was able to increase the mobility of the PPARα-containing heterodimer even in the presence of a molar excess of acyl-CoA-binding protein, mimicking the conditions found in vivo.

Members of the nuclear receptor superfamily mediate ligand-dependent transactivation of genes controlling development, differentiation, and homeostasis in response to nutritional, metabolic, and hormonal signals (1). The peroxisome proliferator-activated receptor α (PPARα, NR1C1 (2)) belongs to the nuclear hormone receptor superfamily (3). Through heterodimerization with the retinoid X receptors (4) (NR2B1-3) and binding to DR-1 response elements, PPARα regulates transcription of several genes encoding enzymes involved in lipid metabolism (5, 6). Accordingly, PPARα is predominantly expressed in tissues with a high turnover of fatty acids (7).

Activation of nuclear receptor-mediated transcription involves an agonist-dependent release of co-repressors and recruitment of co-activators. Accumulating evidence obtained by x-ray crystallography has revealed a significant ligand-dependent conformational change involving repositioning of the conserved AF-2 helix in the ligand-binding domain of nuclear receptors (8–11). This ligand-induced conformational change has been demonstrated to be a determining event governing interactions with co-activators and co-repressors (see Refs. 12–14; reviewed in Ref. 15). The crystal structures of the PPARγ and PPARδ ligand-binding domains (16, 17) have revealed an overall folding pattern similar to that observed for other nuclear receptor ligand-binding domains (8–11). However, the PPAR ligand-binding pocket is substantially larger than those of other nuclear receptors, and this may in part explain the observed promiscuity in terms of ligand binding (16, 17). The interior of the ligand-binding pocket has been suggested to be solvent-accessible via a channel between helix 3 and the β-sheet. The entrance is lined by polar side chains, and its dimension indicates that ligands may enter the cavity without affecting the overall structure of the receptor (16–18). Crystalization of a ternary complex containing the PPARγ ligand-binding domain, the PPARγ agonist BRL49653, and the

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The abbreviations and trivial names used are: PPAR, peroxisome proliferator-activated receptor; ACBP, acyl-CoA-binding protein; ACO, acyl-CoA oxidase; BRL49653, (±)-5-(4-[2-methyl-2-(pyridylamino)-ethoxy]phenyl)methyl]2,4-thiazolidinedione; DR-1, direct repeat separated by one nucleotide; EMSA, electrophoretic mobility shift assay; GST, glutathione S-transferase; HNF-4α, hepatocyte nuclear factor-4α; NCoR, nuclear receptor co-repressor; PPARγ, peroxisome proliferator-activated receptor; mPPAR, mouse peroxisome proliferator-activated receptor; sPPAR, rat peroxisome proliferator-activated receptor; RXR, retinoid X receptor; rRXR, rat RXR; PAGE, polyacrylamide gel electrophoresis; SRC-1, steroid receptor co-activator-1; TFA, tetradecylthioacetic acid; WY14,643, 4-chloro-6-(2,3-xylidino)2-pyrimidinylthioacetic acid; PPRe, peroxisome proliferator-responsive element; Tricine, N[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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nuclear receptor-binding domain of the steroid receptor co-activator-1 (SRC-1) has revealed that association between liganded nuclear receptors and co-activators depends on conserved residues in helix 3 and the AF-2 helix forming a charge clamp and hydrophobic interactions involving helices 3, 4, and 5 and the AF-2 helix (16). Although the three-dimensional structure of PPARα has yet to be reported, it has been shown that the C terminus of the ligand-binding domain is essential for the ligand-induced co-activator interaction (19–22).

A large variety of long-chain fatty acids, eicosanoids, and synthetic compounds have been shown to serve as PPARα ligands and activators (17, 23–25). Several natural and synthetic PPARα ligands are activated to the corresponding CoA esters (26–28), and these have been demonstrated to accumulate in tissues of treated rats (29, 30). Generally, the formation of CoA esters has been considered a process that merely reduces the availability of the activating PPARα ligands (31). In the present study, we present evidence that acyl-CoA esters directly affect PPARα conformation and function in a manner indicating that acyl-CoA esters may act as PPARα antagonists.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—pSG5-mPPARα is kindly supplied by J. D. Tugwood (32). The plasmid encoding SRC-1 (pBKCMV-hSRC1) was a kind gift from B. W. O’Malley (33). pGEX-N-CoR (2239–2453) for bacterial expression of GST-N-CoR (amino acids 2239–2453) was kindly provided by M. A. Lazar (34). Plasmids pTLL-mPPARαAB, and pTLL-mPPARαAβ/Δ425–468 were kindly provided by M. Leid (35). The following constructs have been described previously: rPPARα (p7zf-PPARBE) and rRXRα (pRXRRT7) (4, 23), pGEX-5X-1-mPPARαLBD (amino acids 166–469) (36) and pCA4-rRXRα (37). The plasmid pCA2-mPPARα was constructed by subcloning the murine PPARα cDNA into the BamHI/SalI site of pCA2, which is derived from pCA4 by insertion of the CUP1 promoter cassette into pSH14 (38). The murine PPARα (39) and RXRα (40) fragments for this study were obtained by polymerase chain reaction from pSG5-mPPARα using BamHI/SalI-tagged primers.

**Ligands**—Linoleic acid was purchased from Sigma-Aldrich. Wy14463 and 9-cis-retinoic acid were from Biomol. S-Hexadecyl-CoA was synthesized as described by Rosendal et al. (39), and tetracetylated-tissue acid (TTA) was synthesized as described by Barge et al. (40). BRL49653 was kindly provided by Novo Nordisk A/S.

**Sf21 Whole Cell Extracts**—Rat PPARα was expressed in Spodoptera frugiperda Sf21 insect cells as previously described (4). The Sf21 whole cell homogenate was prepared by disrupting 4 × 107 cells in 2.3 ml of buffer (25 mM KPO4, pH 7.4, 100 mM KCl, 1 mM EDTA, 2 mM dithioerythritol, 1 mM phenethylsulfonyl fluoride, 1 mg/ml leupeptin, 1 mg/ml aprotinin, 1 mg/ml pepstatin, 0.01 trypsin inhibitory units/ml antipain, 1 μg/ml aprotinin) with 50 strokes in an all glass Dounce homogenizer. The ionic strength was increased by slowly adding 1.2 ml of 1 M KCl, and the ionic strength was adjusted by the addition of 0.5–1.0 mM Wy14463. After which 0.5 ng of probe was added, and the reactions were incubated for 20 min at 25 °C in a total volume of 8 μl of binding buffer with the addition of ligand or vehicle. The final concentrations in the binding buffer were 22 mM Tris-HCl, pH 8.5, 75 mM KCl, 5% (v/v) glycerol, and 2 μM dithioerythritol.

**Linoleic acid and S-Hexadecyl-CoA were used in final concentrations of 5 μM and 5–15 μM, respectively. Chymotrypsin (Roche Molecular Biochemicals) was dissolved in 50 mM NH4HCO3, allowing the formation of 2 μl of chymotrypsin was added to final concentrations of 0–120 μM or 260 μg/ml. The digestions were carried out at 25 °C for 20 min and stopped by boiling in Tris-Tricine loading buffer (0.1 M Tris-HCl, pH 6.8, 24% (v/v) glycerol, 5% (v/v) SDS, 0.2 M dithiothreitol, 0.02% (v/v) G-250 Coomassie Brilliant Blue). The resulting peptide mixtures were separated by SDS-PAGE and visualized using a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA).

**GST Pull-downs**—GST fusion proteins were captured on glutathione-Sepharose beads as described (36). 8 μl of 35S-labeled in vitro translated mPPARα, or SRC-1 was incubated in buffer A (50 mM NaCl, 20 mM Tris-HCl, pH 7.9, 0.1% (v/v) Nonidet P-40, 10% (v/v) glycerol, and 1% (v/v) essentially fatty acid-free milk powder) with 500 μM Wy14463, 30 μM TTA, or vehicle. 10 μl of GST fusion protein on beads was added, and the reaction was allowed to proceed for 20 min. The reaction was washed three times in 150 μl of buffer A and finally once in 150 μl of buffer A without milk powder. The bound proteins were eluted by boiling in SDS-PAGE sample buffer, separated by SDS-PAGE, and evaluated by immunoblot analysis. The anti-PPARα antibody was raised against the mouse PPARα AB domain and affinity-purified, and anti-RXRα antibody was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Enhanced chemiluminescence detection was used for visualization.

**Gel Mobility Shift Assay**—The DNA probe was prepared by annealing 32-pair oligonucleotides covering the PPARα/RXR binding motif in the promoter of the acyl-CoA oxidase (ACO) gene (32). The probe was labeled by filling in the 3′-recessive ends with α-32PdCTP (10 μCi, 3000 Ci/mmol; Amersham Pharmacia Biotech) using the Klenow fragment of the *Escherichia coli* DNA polymerase. Binding reactions were performed in a total of 25 μl containing Sf21 extract, 2.4 μl of poly(dI-dC) (Amersham Pharmacia Biotech), 25 mM Hepes, pH 7.6, 40 mM KCl, 0.1 mM EDTA, 1 mM dithioerythritol, and 10% (v/v) glycerol. The reactions were preincubated for 20 min on ice, after which 0.5 ng of probe was added, and the reactions were incubated for 20 min at room temperature. Free DNA and DNA-protein complexes were resolved by electrophoresis (12% gel, 1.5 h at 4 °C) on a 4% (w/v) polyacrylamide/bisacrylamide gel (30:1) in a buffer containing 50 mM Tris-HCl, pH 8.5, 380 mM glycine, and 2 mM EDTA. Ligand-induced complex formation assays were performed as described above, except that in vitro translated PPARα and RXRα were used instead of Sf21 extract, the total reaction volume was 20 μl, and reactions contained 130 mM KCl and 5% (v/v) glycerol. Combined electrophoretic mobility assay (EMSA) and Western blotting was performed as described previously (42) using a polyclonal affinity-purified anti-rPPARα antibody (21).

**Protein Expression**—2 μl aliquots of the 35S-labeled in vitro translated protein was incubated for 20 min at 25 °C in a total volume of 8 μl of binding buffer with the addition of ligand or vehicle. The final concentrations in the binding buffer were 22 mM Tris-HCl, pH 8.5, 75 mM KCl, 5% (v/v) glycerol, and 2 μM dithioerythritol. Linoleic acid and S-hexadecyl-CoA were used in final concentrations of 5 μM and 5–15 μM, respectively. Chymotrypsin (Roche Molecular Biochemicals) was dissolved in 50 mM NH4HCO3, allowing the formation of 2 μl of chymotrypsin was added to final concentrations of 0–120 μM or 260 μg/ml. The digestions were carried out at 25 °C for 20 min and stopped by boiling in Tris-Tricine loading buffer (0.1 M Tris-HCl, pH 6.8, 24% (v/v) glycerol, 5% (v/v) SDS, 0.2 M dithiothreitol, 0.02% (v/v) G-250 Coomassie Brilliant Blue). The resulting peptide mixtures were separated by SDS-PAGE and visualized using a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA).
Fig. 1 demonstrates that the addition of TTA to limiting amounts of in vitro transcribed/translated PPARα and RXRα, as reported previously (24), induced a considerable increase in the binding of the PPARα-RXRα heterodimer to the ACO PPRE probe in an electrophoretic mobility shift assay (EMSA) (Fig. 1, lanes 2 and 3), whereas no binding of the RXRα homodimer was observed (lane 8). Interestingly, the presence of 5 μM S-hexadecyl-CoA abrogated TTA-induced PPARα-RXRα-ACO PPRE complex formation (Fig. 1, lane 4). Increasing the concentration of TTA restored PPARα-RXRα-ACO PPRE complex formation in the presence of S-hexadecyl-CoA (Fig. 1, lanes 5–7), demonstrating that the inhibitory effect of S-hexadecyl-CoA was reversible and that competition between fatty acid and acyl-CoA appears to regulate PPARα-RXRα-ACO PPRE complex formation.

**PPARα Binds Palmitoyl-CoA**—The ligand-induced complex formation assay described above indicated that fatty acid and acyl-CoA might compete for binding to PPARα or, alternatively, that simultaneous binding of an acyl-CoA ester and an agonistic ligand to the PPARα-RXRα complex antigenized the effect of the PPARα agonist. Due to the hydrophobic characteristics and micelle forming capacity of fatty acids and their CoA derivatives, we have been unsuccessful in performing reliable simple binding competition experiments between fatty acids and acyl-CoAs. To circumvent these problems, we reckoned that a possible direct binding of acyl-CoA to PPARα might be revealed by specific binding of PPARα to palmitoyl-CoA covalently coupled to agarose beads. Since the covalent bond between the palmitoyl-CoA and the agarose beads joins the amino group of the CoA moiety with the agarose matrix, this approach, furthermore, circumvented the inherent problem associated with assays involving acyl-CoA esters, namely the hydrolysis of the labile thioester bond. This is particularly important in the context of competition assays to determine ligand binding to PPARs, since hydrolysis of the acyl-CoA would generate a fatty acid, which would act as a regular agonist and thereby give false Kd values for the binding of acyl-CoA esters to the PPARs. In contrast, in a bead-based pull-down assay, hydrolysis of the covalently coupled palmitoyl-CoA would release the free fatty acid, and hence, if PPARα only interacted with the free fatty acid, no specific retention of PPARα would be observed.

Recombinant full-length PPARα and RXRα were expressed in yeast, and whole cell extracts were prepared as described (37). The extracts were incubated with palmitoyl-CoA agarose beads or protein A-agarose beads (control) in the absence or presence of PPAR-selective or RXR-selective ligands. After washing, bound material was recovered by boiling in SDS sample buffer, and PPARα and RXRα were detected by Western blotting. Fig. 2A shows that PPARα preferentially was retained on the palmitoyl-CoA-agarose beads compared with the protein A-agarose beads. The addition of the PPARα-selective ligand Wy14643 prevented PPARα binding to the palmitoyl-CoA beads in a dose-dependent manner, whereas the addition of the PPARγ-selective ligand BRL49653 was without effect on PPARα binding. In contrast, no specific interaction between RXRα and the palmitoyl-CoA agarose beads was detected irrespective of the presence of PPARα- or RXR-selective ligands (Fig. 2B). We conclude that the established PPARα ligand Wy14643 and palmitoyl-CoA compete for binding to PPARα.

**S-Hexadecyl-CoA Increases the Sensitivity of PPARα to Chymotrypsin**—Differential protease sensitivity assays have been widely used to examine the effect of ligand binding on receptor conformation. We applied this technique to compare the effects of a known PPARα ligand, linoleic acid, and S-hexadecyl-CoA on PPARα conformation. 35S-Labeled PPARα or RXRα was incubated with S-hexadecyl-CoA, linoleic acid, or vehicle (0.5% ν/ν Me2SO), digested with increasing concentrations of chymotrypsin for 20 min at 25 °C, and the digestion products were analyzed by SDS-PAGE. Binding of synthetic agonists to PPARα has been shown to decrease the sensitivity to chymotrypsin digestion, resulting in preservation of protease-resistant fragments (35). In contrast, we found that S-hexadecyl-CoA in a dose-dependent manner increased the sensitivity of PPARα to chymotrypsin, as indicated by the rapid disappearance of the diagnostic 26-kDa protease-resistant fragment (Fig. 3A). In comparison, incubation with 15 μM S-hexadecyl-CoA did not influence the sensitivity of RXRα to chymotrypsin, indicating that the effect of S-hexadecyl-CoA was receptor-dependent (Fig. 3B). To further corroborate the notion that the effects of S-hexadecyl-CoA on PPARα conformation were reversible and did not result from an irreversible denaturing action, the following experiment was performed. The 32S-labeled PPARα was incubated for 20 min with either 10 μM S-hexadecyl-CoA or water, and then each mixture received...
either 120 μM linoleic acid or vehicle (0.5% Me₂SO). Incubation was continued for 20 min, and each of the four incubations was subjected to digestion with increasing amounts of chymotrypsin. Fig. 4 shows that incubation with linoleic acid as expected decreased the sensitivity of PPARα to chymotrypsin, whereas incubation with S-hexadecyl-CoA increased the sensitivity to chymotrypsin. It is noteworthy that the addition of linoleic acid to PPARα preincubated with S-hexadecyl-CoA partially restored resistance to chymotrypsin digestion. Thus, S-hexadecyl-CoA interacted reversibly with PPARα and appeared to compete with the agonist linoleic acid for binding to PPARα. It has previously been shown that deletion of the putative helices H10–12 of the C-terminal region in the PPARα ligand-binding domain abolishes agonist-induced protease protection and transactivation (35). To examine whether the presence of this region was required for S-hexadecyl-CoA-induced protease sensitivity, the truncated forms mPPARαΔAB and mPPARαΔABΔ425 (35) were digested in the presence of either linoleic acid or S-hexadecyl-CoA. mPPARαΔAB contains the entire ligand-binding domain, whereas the putative helices 10–12 are deleted in mPPARαΔABΔ425 (35). As shown in Fig. 5, in the presence of linoleic acid, digestion of mPPARαΔAB was decreased, whereas S-hexadecyl-CoA increased the sensitivity to
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Chromotropin. However, neither linoleic acid nor S-hexadecyl-CoA affected the sensitivity of mPPARαΔAB/Δ255 (Fig. 5). Thus, it appears that helices 10–12 are required for interaction of S-hexadecyl-CoA with PPARα as well as for interaction of linoleic acid with PPARα.

S-Hexadecyl-CoA Abolishes Ligand-induced Interaction between SR-1 and PPARα but Enhances Interaction of NCoR with PPARα—Nuclear receptor-mediated transactivation is controlled by a complex interplay between co-activators and co-repressors. Agonist binding enhances recruitment of co-activators, whereas the hallmarks of antagonists are decreased or abolished interaction with co-activators coupled with induced or enhanced interaction with co-repressors (44–50). The experiments presented above indicate that S-hexadecyl-CoA has the characteristics of a PPARα antagonist. To further address this possibility, the effects of S-hexadecyl-CoA on the interaction between PPARα and the co-activator SR-1 or the co-repressor NCoR were determined using GST pull-down assays. Fig. 6A demonstrates that S-hexadecyl-CoA abolished agonist-induced recruitment of SR-1 to PPARα in a dose-dependent manner and even decreased interaction below that observed with the unliganded receptor. In contrast, S-hexadecyl-CoA enhanced almost 3-fold the interaction between NCoR and PPARα. Thus, by these criteria, S-hexadecyl-CoA behaves like a PPARα antagonist.

S-Hexadecyl-CoA Exerts Its Action on PPARα Even When Complexed to Its Natural Carrier Protein, the Acyl-CoA-binding Protein—In the cell, the concentrations of free acyl-CoA esters and fatty acids are kept in the low or medium nanomolar range by the buffering action of the acyl-CoA-binding protein (ACBP) and fatty acid-binding proteins, respectively (for a review, see Ref. 51). Thus, in order to evaluate the possible biological significance of acyl-CoA esters or for that matter fatty acids in PPAR-mediated signaling, it is imperative to establish assay conditions that mimic or at least approach in vivo conditions. These requirements have only been met partially in one report analyzing the interaction between fatty acids and PPARα using a fluorescence-based assay (52). During our initial EMSA experiments using saturating or nearly saturating amounts of in vitro translated PPARα and RXRs, we noted that the mobility of the PPARα-RXR-ACO PPRE complex in the presence of S-hexadecyl-CoA or TTA-CoA was marginally increased (data not shown). Subsequently, we discovered that this effect was much more pronounced in whole cell extracts of Sf21 insect cells expressing recombinant rat PPARα, suggesting that this might form the basis for a highly sensitive assay for S-hexadecyl-CoA interaction with PPARα. No binding was observed in extract prepared from mock-infected cells (Fig. 7A). The increase in electrophoretic mobility was not observed using millimolar concentrations of the detergent lauryl-sarcosine, underscoring the specific action of S-hexadecyl-CoA (results not shown). Sf21 cells contain an RXR analog, in this work referred to as Sf“RXR” (related to ultraspiracle (NR2B4) (2) in Drosophila (53)), with which PPARα is able to heterodimerize and subsequently bind to the ACO PPRE (4, 54). Accordingly, combining in vitro translated PPAR with extract of mock-infected cells allowed efficient binding to the ACO PPRE in EMSA experiments (results not shown). Using this assay, we next asked whether a preformed complex between S-hexadecyl-CoA and ACBP was able to modulate the mobility of the rPPARα-Sf“RXR”-ACO PPRE complex. Fig. 7B demonstrates that even in the presence of a molar surplus of ACBP, S-hexadecyl-CoA was able to increase the mobility of the rPPARα-Sf“RXR”-ACO PPRE complex. By combining EMSA with Western blotting (42) we demonstrated that the shifted band indeed contained PPARα (Fig. 7C). Using the established Kd for binding of long-chain acyl-CoA to ACBP, the concentration of free S-hexadecyl-CoA in a solution containing 0.3 μM S-hexadecyl-CoA and 15 μM ACBP was calculated as 0.7 nM. Thus, using conditions that mimic the in vitro conditions with respect to acyl-CoA availability, S-hexadecyl-CoA imparted an increase in the electrophoretic mobility of the rPPARα-Sf“RXR”-ACO PPRE complex.

As shown above, the presence of S-hexadecyl-CoA differentially altered the ability of PPARα to interact with co-activators and co-repressors. Formally, it was therefore possible that the increased electrophoretic mobility reflected an altered molecular mass of the rPPARα-Sf“RXR”-ACO PPRE complex. However, a Ferguson analysis (55) revealed that the presence of 10 μM S-hexadecyl-CoA, 10 μM ACBP, or a 10 μM ACBP plus 10 μM S-hexadecyl-CoA did not alter the molecular mass of the rPPARα-Sf“RXR”-ACO PPRE complex (results not shown). Thus, the increased electrophoretic mobility of the rPPARα-Sf“RXR”-ACO PPRE complex in the presence of S-hexadecyl-CoA is not due to an altered molecular mass of the bound heterodimer but rather reflects an altered conformation or change in charge of the heterodimer.

DISCUSSION

In the present work, we present evidence suggesting that acyl-CoA esters directly affect the conformation and function of PPARα. Using a variety of in vitro approaches, we show that the nonhydrolyzable acyl-CoA analogue, S-hexadecyl-CoA, antagonizes ligand-induced formation of a PPARα-RXR-ACO PPRE complex. We were able to demonstrate specific binding of PPARα to immobilized palmityl-CoA, and furthermore, we show that S-hexadecyl-CoA increases the sensitivity of PPARα to chymotrypsin digestion in a manner that depended on the integrity of the ligand-binding pocket. We show that S-hexadecyl-CoA, like well established antagonists for other receptors,
abolishes ligand-induced interaction with a co-activator, SRC-1, and conversely increases recruitment of a co-repressor, NCoR. Importantly, we show that S-hexadecyl-CoA is able to affect a PPARα-containing complex in the presence of a molar excess of the natural cellular acyl-CoA carrier, ACBP. These observations, taken together with our recent finding that ACBP and acyl-CoA esters are present in the nuclei of rat hepatocytes (58), are compatible with the notion that acyl-CoA esters also in vivo might be involved in the regulation of PPARα activity. Our results are furthermore supported by recent data showing interaction between acyl-CoA esters and PPARα and PPARγ in competition binding experiments with the labeled synthetic dual agonist, KR-297 (57).

Long-chain acyl-CoA esters have been estimated to have a van der Waals volume of not less than 850 Å³ (58). This size would exclude acyl-CoA esters from the ligand-binding pocket of most nuclear receptors except for the PPARs with ligand-binding pockets of ~1300 Å³ (16, 17). It was recently reported that docosahexaenoic acid is a ligand for RXRα, raising the question of whether acyl-CoA esters might also influence the PPARα-RXRα heterodimer via RXRα. However, as mentioned above, the size of the ligand-binding pocket of RXRα is not compatible with specific binding of acyl-CoA esters, and accordingly, we detected no alteration in the sensitivity to chymotrypsin digestion when RXRα was incubated with S-hexadecyl-CoA, and similarly, we observed no binding of RXRα to palmitoyl-CoA.

Biochemical and structural studies have revealed a unifying principle determining the interaction of nuclear receptors with co-activators and co-repressors involving an at least partially overlapping binding site (13–15, 59). The hydrophobic face of helical regions in the receptor interacting domains of co-activators or co-repressors harboring an LXXLL core motif or a related CoRNR motif, respectively, interacts with a hydrophobic pocket formed by helices 3–5 and the AF-2 helix in PPARγ (13–16, 59). Ligand-dependent positioning of the AF-2 helix and differences in the regions flanking the LXXLL and CoRNR motifs are critically involved in the differential interaction of co-activators and co-repressors with liganded and unliganded nuclear receptors, respectively (13, 15, 59). Interestingly, the crystal structure of PPARγ shows that the AF-2 helix even in the unliganded receptor may fold back against the body of the receptor, assuming a conformation similar to the conformation stabilized by interactions between the polar head group of ligands and the AF-2 helix (16, 18, 60), and as a consequence, interaction with co-activators and co-repressors may be less stringently regulated by ligands in the PPAR subfamily in comparison with other nuclear receptor subfamilies.

From the analysis of the structure of the estrogen receptor bound to agonists or antagonists, it is evident that subtle distortions in the placement of the AF-2 helix may have a profound effect on the interaction with co-activators or co-repressors (10). Our finding that S-hexadecyl-CoA decreases interaction with SRC-1 and increases recruitment of NCoR indicates that the bulky CoA head influences directly or indirectly the positioning of the AF-2 helix. Thus, the bulky CoA head group of S-hexadecyl-CoA may prevent the AF-2 helix from folding back, forcing the AF-2 helix to adopt an extended conformation contrasting with the unliganded conformation that allows the AF-2 helix to fold back. The increased sensitivity of PPARα to chymotrypsin digestion upon binding of S-hexadecyl-CoA is also indicative of a less compact conformation.

Examination of the crystal structure of PPARγ and PPARδ (16, 17) led to the suggestion that ligands might enter the ligand-binding pocket via a channel between helix 3 and the β-sheet. In addition, the crystal structure of liganded PPARγ and PPARδ revealed prominent interactions between the polar head group of the different agonists and the AF-2 helix (16–18). In contrast, co-crystalization of the partial agonist GW0072 with the ligand-binding domain of PPARγ revealed a mode of binding in which the carboxylic group of GW0072 was oriented toward the loop region between helices 2’ and 3 with no contacts to the AF-2 helix (61). In this context, it is intriguing that we observe specific binding of PPARα to palmitoyl-CoA immobilized via the CoA head group. If the palmitoyl-CoA entered the ligand-binding pocket via the channel between helix 3 and the β-sheet, this suggests that the orientation of palmitoyl-CoA mimicked that of GW0072. Alternatively, positioning of the palmitoyl-CoA molecule with the acyl chain in the characteristic tail-down configuration would imply that the acyl-CoA ligand entered the ligand-binding pocket via the AF-2 side. Interaction of PPARα with immobilized PPARα agonists would clearly be of interest to examine this possibility.

Several genes are transcriptionally regulated by antagonistic cross-talk between PPAR and HNF-4α through a shared DNA binding motif (62–64). It is well established that PPARα...
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is activated by polyunsaturated fatty acids (23, 24), and recently myristoyl-CoA and palmitoyl-CoA were reported to bind to HNF-4α and activate HNF-4α-mediated transactivation, whereas o-9 and o-6 polyunsaturated acyl-CoA esters and stearoyl-CoA were shown to antagonize HNF-4α-mediated transactivation (65). Based on this finding, it was proposed that the ratio of fatty acids to acyl-CoA esters and the composition of acyl-CoA esters might regulate cross-talk between PPARα and HNF-4α (65). However, it should be noted that recent data based on molecular modeling of HNF-4α and protease protection experiments have questioned the role of acyl-CoA esters in the regulation of HNF-4α activity (58). Thus, it remains to be established conclusively whether HNF-4α is a target for acyl-CoA-dependent regulation. If so, our findings add another level to the interplay between PPARα and HNF-4α, indicating that acyl-CoA esters, apart from activating HNF-4α, down-regulate PPARα-mediated transactivation via direct binding to PPARα, thereby imparting a conformation that reduces co-activator interaction and enhances recruitment of co-repressors (Fig. 8).

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