Ligand type-specific Interactions of Peroxisome Proliferator-activated Receptor γ with Transcriptional Coactivators*

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The nuclear peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear receptor superfamily and acts as a ligand-dependent transcription factor mediating adipocyte differentiation, cell proliferation and inflammatory processes, and modulation of insulin sensitivity. Members of the 160-kDa peroxisome proliferator-activated receptor γ (SRC-1/TIF2/AIB-1) family of coactivators, CBP/p300, and TRAP220/DRIP205, are shown to interact with PPARγ and potentiate nuclear receptor transactivation function in a ligand-dependent fashion. Three different classes of PPARγ ligands exert partially overlapping patterns of biological action through PPARγ to examine whether interaction of PPARγ with known coactivators is ligand specific. 

Peroxisome proliferator-activated receptor γ (PPARγ),† a member of the nuclear hormone receptor superfamily, acts as a ligand-inducible transcription factor (1, 2). PPARγ forms a heterodimer complex with one of the three retinoid X receptor (RXR) proteins, which then binds to PPAR-responsive elements (PPRE) within the promoters of PPARγ target genes (3, 4). It is thought that the ligand binding domain (LBD) mediates the ligand-dependent transactivation function of PPARγ, although two transactivation domains, at the N-terminal (AF-1) and C-terminal ends (AF-2), are present in most nuclear receptors. Ligand-induced transactivation is achieved by the nuclear receptor recruiting one of several types of nuclear receptor coactivator complex. One class of coactivator complex includes three SRC-1 family members (5), CBP/p300 (6), and SRA (7), as well as other proteins (8, 9). The SRC-1 family members (SRC-1 (p160/NCOr-1) (10), TIF2 (GRIP-2) (11), and AIB1 (p/CIP/ACTR) (12)) interact with the AF-2 nuclear receptors. This interaction is highly dependent through direct binding to the minimal nuclear receptor AF-2 (AF-2 AD), mapped to the C-terminal region of the LBD (13). CBP/p300 serves as a platform for nuclear receptors by recruiting multiple coactivators and coregulatory factor (14).

Many biological actions of PPARγ ligands have been reported on function of PPARγ, prostaglandin derivatives, such as 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) and 9-hydroxyoctadecadienoic acid (9-HODE or 13-HODE) are antidiabetic, allowing different coactivator associations. This conjecture of ligand type-specific structures on PPARγ, allowing different coactivator associations. This conjecture of ligand type-specific structures on PPARγ, allowing different coactivator associations. This conjecture of ligand type-specific structures on PPARγ, allowing different coactivator associations. This conjecture of ligand type-specific structures on PPARγ, allowing different coactivator associations.

The abbreviations used are: PPARγ, peroxisome proliferator-activated receptor γ; RXR, retinoid X receptor; LBD, ligand binding domain; AF-2, activation function-2; 15d-PGJ2, 15deoxy-Δ12,14-prostaglandin J2; 9-HODE, 9-hydroxyoctadienoic acid; SRC-1, steroid receptor coactivator-1; TIF2, transcriptional intermediate factor 2; AIB-1, amplified in breast cancer-1; CBP, CREB-binding protein; SRA, steroid receptor RNA coactivator; TRAP220, thyroid hormone-associated protein 220; PGC-1, PPAR γ coactivator-1; PPRE, peroxisome proliferator-activated response element; acyl-CoA, acyl-coenzyme A oxidase; PAGE, polyacrylamide gel electrophoresis; AD, activation domain; CAT, chloramphenicol acetyltransferase; GST, glutathione S-transferase; UAS, upstream activating sequence.

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ligand-specific way, resulting in distinct interactions between PPARγ and coactivators.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—cDNA encoding human PPARγ2 obtained from a human liver cDNA library (23) was subcloned into the pGEX (Amersham Pharmacia Biotech) and pSG5 (Stratagene) expression vectors. The pVP16(GAL4-AD)-PPARγ2(DEF) fusion plasmid was constructed by inserting human PPARγ2 ligand-binding regions (encoding amino acids 183–505) into the pVP16 expression vector (CLONTECH). Each coactivator cDNA was inserted into the pM vector (CLONTECH) that included a GAL4 transactivation domain. The following plasmids constructed in a mammalian expression vector (Invitrogen) have been described previously (24): pCDNA3-human SRC-1 (hSRC-1), pCDNA3-hTIF2, pCDNA3-hAIB-1, and pCDNA3-hp300. The pCDNA3-TRAP220 expression vector was created by isolating TRAP220 cDNA from a human brain cDNA library. Mouse RXRβ cDNA expression vector pGEX-mRXRβ, a gift from P. Chambon, was subcloned into the pSG5 vector (25).

**Mammalian Two-hybrid Assay**—COS-1 cells were maintained in Dulbecco’s modified Eagle’s medium without phenol red, supplemented with 5% fetal calf serum stripped with dextran-coated charcoal. Cells were transfected with calcium phosphate coprecipitation as described previously (26). Reporter plasmid (1 μg) containing GAL4-UAS (17-mer × 2 (β-galactosidase) CAT) was cotransfected with 0.2 μg of pVP-PAR2(DEF) plus 0.2 μg of either pm-SRC-1, pm-TIF2, pm-AIB-1, pm-p300 or pm-TRAP220. As a reference plasmid for normalization, 2 μg of pCH110 plasmid was used (Amersham Pharmacia Biotech). Bluescribe M13” (Stratagene) was used as the carrier to adjust the total amount of DNA to 3 μg. 1 μM 15d-PGJ2 or troglitazone was added to the medium 12 h after transfection and every 8 h thereafter at each exchange of medium. After 48 h, β-galactosidase activity (from pGAL4-UAS) was used to measure transfection efficiency by Renilla luciferase activity (from pRL-CMV) as described previously (27).

**RESULTS AND DISCUSSION**

**Ligand Type-specific Interactions of PPARγ with Coactivators in the Mammalian Two-hybrid System and GST Pull-down Assay**—We first tested for ligand-induced and dose-dependent interactions of PPARγ using two distinct coactivator classes in a mammalian two-hybrid system. For this assay, the LBD of PPARγ containing AF-2 was fused to the VP16 domain in the pVP vector (pVP-PPARγ11-12), and several coactivators (SRC-1, TIF2, AIB-1, p300, and TRAP220) were transfected into COS-1 cells (Fig. 1A); however, no ligand-dependent interaction of PPARγ with SRC-1, TIF2, AIB-1, p300, and TRAP220 was detected (Fig. 1A). This ligand-dependent interaction was also not detected using a synthetic PPRE with any of the coactivator classes (data not shown).

**Transactivation Assays**—COS-1 cells were maintained as described above for the mammalian two-hybrid system. The following plasmids were used for transfection: respective reporter plasmid (1 μg) containing the pGL-GAL4-UAS (17-mer × 2 (β-galactosidase)-luciferase) co-transfected with 0.1 μg of pMGL4-DBD-PPARγ(DEF) or pm-PPARγ(DEF-ΔAF-2) with or without 1 μg of SRC-1, TIF2, or TRAP220 expression vector. As a reference plasmid for normalization, 10 ng of pRL-CMV plasmid (Promega) was used. Bluescribe M13” (Stratagene) was used as the carrier to adjust the total amount of DNA to 3 μg. 1 μM 15d-PGJ2 or troglitazone was added to the medium 12 h after transfection and every 8 h thereafter at each exchange of medium. After 48 h, β-galactosidase activity (from pGAL4-UAS) was used to measure transfection efficiency by Renilla luciferase activity (from pRL-CMV) as described previously (27).

**Troglitazone Binding Is Unable to Recruit TIF2 to the PPARγ-RXR Heterodimer upon PPRE Binding**—We next tested whether troglitazone binding induced coactivator interactions with a DNA-bound PPARγ/RXRβ heterodimer. An electrophoretic mobility shift assay with a well characterized consensus PPRE from the acyl-coenzyme A oxidase gene (acyl-CoA) promoter (3) was used. As shown in Fig. 2, despite the absence of 15d-PGJ2 or the RXR-specific ligand (LG268), PPARγ/RXRβ heterodimer DNA binding was observed, whereas binding of the single receptors was not observed (lane 4). TIF2 recruitment induced the formation of a larger complex, observed as a slow migrating band produced by the binding of 15d-PGJ2 or LG268 to PPARγ/RXRβ (lanes 9 and 11). However, TIF2 recruitment was not induced by troglitazone binding (lane 10).

**Ligand-specific Potentiation of PPARγ Transactivation Function by Coactivators**—The observations that PPARγ interactions with SRC-1 family members, p300, and TRAP220 proteins were ligand type-specific, suggesting that the transactivation function of ligand-bound PPARγ was differentially potentiated by these coactivators. A transient assay was performed in COS-1 cells using pm-(GAL4-DBD)-PPARγ.
Troglitazone does not induce interactions between coactivators and PPARγ. Different interactions between coactivators were induced by natural ligands and were examined by coactivator recruitment, troglitazone-bound LBD may be modulated in some other way.

PPARγ-mediated signaling is involved in a variety of biological events, such as adipocyte differentiation, cell proliferation, and inflammatory processes. To modulate particular PPARγ-mediated events, synthetic PPARγ ligands have been developed in addition to the identification of endogenous ligands 15d-PGJ2 and 9-HODE. Interestingly, the biological actions mediated by PPARγ were reported to differ according to the ligand used (28). These observations led us to examine the molecular mechanism underlying the ligand-specific actions of the PPARγ ligands. Structural analyses by crystallography revealed that the LBD structures of many nuclear receptors were altered in a ligand-type-specific way, particularly at helix 12 (29, 30). As the alteration of the helix 12 angle upon ligand binding is unable to recruit TIF2 to the PPARγ-RXR heterodimer bound to PPRE. Purified PPARγ, RXRα, and the receptor-interaction domain of TIF2 fused to GST were incubated with 32P-labeled PPRE in a binding mixture in the presence or absence of 1 μM 15d-PGJ2, troglitazone, and LG268 for an electrophoretic mobility shift assay as described under "Experimental Procedures." The PPARγ-RXRα-bound and PPARγ/RXRα-TIF2 complex are indicated.

**Fig. 1.** Ligand type-specific interactions of PPARγ with coactivators in the mammalian two-hybrid assay. Different interactions between coactivators were induced by natural ligands (DEF), pM-PPARγ(DEF-ΔAF-2), and a reporter plasmid (GAL4-UCAS-luciferase) containing the luciferase gene along with consensus GAL4 upstream activating sequence. As shown in Fig. 3, the transactivation function of PPARγ by troglitazone (lane 4) was comparable with that induced by 15d-PGJ2 (lane 2). 9-HODE was unable to induce significant transactivation even with wild-type PPARγ (lane 3). Moreover, the transactivation function of PPARγ induced by the two ligands was disrupted in the same way when the AF-2 AD core (H12) sequence was deleted (PPARγ(DEF-ΔAF-2)) (lanes 6 and 8). SRC-1 and TIF2 significantly enhanced the transactivation function of PPARγ induced by 15d-PGJ2 (lanes 10, 13), and a potentiation by TRAP220 was also seen but was not statistically significant (lane16). However, the troglitazone-induced transactivation function of PPARγ was not potentiated by these coactivators (lanes 11, 14, and 17). Thus, although 15d-PGJ2 appears to alter PPARγ LBD structure to allow coactivator recruitment, troglitazone-bound LBD may be modulated in some other way.
classes of PPARγ ligands. Both 15d-PGJ2 and troglitazone at the same concentration (1 μM) were equally potent in the induction of PPARγ transactivation function, whereas the other endogenous ligand tested, 9-HODE, was unable to activate PPARγ transactivation. Ligand-dependent interactions of PPARγ with the tested coactivators were observed using 15d-PGJ2 by both in vivo and in vitro assays. However, troglitazone binding to PPARγ failed to induce coactivator interactions in these assays, indicating that the mode of coactivator interaction with PPARγ was ligand-type-specific. These findings imply that troglitazone-bound PPARγ may recruit components other than TRAP220/DRIP205 in the DRIP/TRAP coactivator complex, or proteins other than the 160-kDa family proteins and CBP/p300 in the SRC-1 family-type coactivator complex to form transcription initiation complexes. An alternative possibility is that an unknown coactivator complex may be recruited to troglitazone-bound PPARγ. In this respect, it would be interesting to examine whether troglitazone could induce PPARγ interaction with PGC-1 and PGC-2, which are also reported to act as PPARγ coactivators (31, 32). Nevertheless, as ligand-induced coactivator interactions with PPARγ appear to be distinct between 15d-PGJ2 and troglitazone, the overall structure of PPARγ and coactivator complexes may be different according to the ligands involved, resulting in the activation of a particular set of target gene promoters that exert different biological actions.

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