Dehydroepiandrosterone Sulfotransferase Gene Induction by Bile Acid Activated Farnesoid X Receptor*

Received for publication, August 7, 2001, and in revised form, August 27, 2001
Published, JBC Papers in Press, August 30, 2001, DOI 10.1074/jbc.M107557200

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Dehydroepiandrosterone sulfotransferase (STD) is a hydroxysteroid sulfo-conjugating enzyme with preferential substrate specificity for C-19 androgenic steroids and C-24 bile acids. STD is primarily expressed in the liver, intestine and adrenal cortex. Earlier studies have shown that androgens inhibit the rat Std promoter function through a negative androgen response region located between −235 and −310 base pair positions (Song, C. S., Jung, M. H., Kim, S. C., Hassan, T., Roy, A. K., and Chatterjee, B. (1998) J. Biol. Chem. 273, 21856–21866). Here we report that the primary bile acid chenodeoxycholic acid (CDCA) also acts as an important regulator of the Std gene promoter. CDCA is a potent inducer of the Std gene, and its inducing effect is mediated through the bile acid-activated farnesoid X receptor (FXR), a recently characterized member of the nuclear receptor superfamily. The ligand-activated FXR acts as a heterodimer with the 9-cis-retinoic acid receptor (RXR) and regulates the Std gene by binding to an upstream region at base pair positions −199 to −179. This specific binding region was initially identified by bile acid responsiveness of the progressively deleted forms of the Std promoter in transfected HepG2 hepatoma and enterocyte-like Caco-2 cells. Subsequently, the precise RXR/FXR binding position was established by protein-DNA interaction using in vitro footprinting and electrophoretic mobility shift analyses. Unlike all other previously characterized FXR target genes, which contain an inverted repeat (IR) of the consensus hexanucleotide half-site (A/G)G(G/T)TCA with a single nucleotide spacer (IR-1), the bile acid response element of the Std promoter does not contain any spacer between the two hexanucleotide repeats (IR-0). A promoter-reporter construct carrying three tandem copies of the IR-0 containing −169/−193 element, linked to a minimal thymidine kinase promoter, can be stimulated more than 70-fold in transfected Caco-2 cells upon CDCA treatment. Autoregulation of the Std gene by its bile acid substrate may provide an important contributing role in the enterohepatic bile acid metabolism and cholesterol homeostasis.

Bile acids modulate gene expression by serving as ligands for the farnesoid X receptor (FXR), which is a member of the nuclear receptor superfamily. They are produced in the liver as C-24 hydroxysteroid metabolites of cholesterol and serve as intestinal emulsifying agents for hydrophobic nutrients (1–3). These cholesterol derivatives also function as the autoregulated sensor to modulate the expression of the enzymes and proteins that are involved in bile acid and cholesterol metabolism through enterohepatic re-absorption and fecal excretion. For example, bile acids repress expression of the gene for CYP7A1, the rate-limiting enzyme for the neutral pathway to bile acid biosynthesis, and for CYP8B1, a 12α-hydroxylase enzyme that routes the neutral pathway to cholic acid synthesis (2–5). Bile acids also direct their own uptake by negatively regulating the hepatic expression of the sodium/taurocholate cotransporter polypeptide, a basolateral protein that imports bile acids into hepatocytes from the sinusoidal blood (6). On the other hand, bile acids induce the genes for the ileal bile acid-binding protein (IBABP), the bile salt efflux pump (BSEP), the phospholipid transfer protein (PLTP), and the inhibitory orphan receptor protein, known as the small heterodimer partner (SHP) (7–11).

The ligand-activated FXR forms a heterodimer with the 9-cis-retinoic acid receptor (RXR) and directly interacts with the bile acid-responsive DNA element of the target gene (12–14). The consensus DNA element for binding of the RXR/FXR heterodimer is an inverted repeat (IR) of the hexanucleotide (A/G)G(G/T)TCA, having the two half-sites separated by a single nucleotide (15). This IR-1 arrangement is present in the bile acid response elements of IBABP, PLTP, BSEP, and SHP genes (7–11). In contrast to its direct role in gene induction, the FXR signaling acts indirectly to cause feedback repression of CYP7A1 (3, 5, 10, 11). In this case, the activated FXR induces SHP expression that in turn inhibits CYP7A1 gene transcription through functional interference with the fetoprotein transcription factor (FTF), which acts as a competence factor in the basal and oxysterol-induced expression of the CYP7A1 gene. Unlike the ubiquitous RXR, FXR has a restricted tissue distribution (5, 16). FXR is expressed in the liver, intestine, and kidney, the same tissues that play critical roles in bile acid

* This work was supported in part by a merit review grant from the Department of Veterans Affairs. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: FXR, farnesoid X receptor; RXR, 9-cis-retinoic acid receptor; IR, inverted repeat; TLC, thin layer chromatography; oligo, oligonucleotide; TK, thymidine kinase; 5α-DHT, 5α-dihydrotestosterone; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; HNF, hepatocyte nuclear factor; STD, dehydroepiandrosterone sulfotransferase; SHP, small heterodimer partner; IBABP, ileal bile acid-binding protein; BSEP, bile salt efflux pump; PLTP, phospholipid transfer protein; FTI, fetoprotein transcription factor; DHEA, dehydroepiandrosterone; CDCA, chenodeoxycholic acid; LCA, lithocholic acid; CAT, chloramphenicol acetyltransferase; EMSA, electrophoretic mobility shift assay.
metabolism and thus need to respond to changes in the bile acid flux. Additionally, FXR is expressed in the adrenal cortex (5, 16), where it may also be involved in the regulation of steroid-metabolizing enzymes.

Dehydroepiandrosterone-sulfotransferase, known as STD (or SULT2A1, recommended by the International Sulfotransferase Nomenclature Workshop) is a cytosolic enzyme that mediates sulfate conjugation of a number of hydroxysteroid substrates such as bile acids, pregnenolone, androgens, and estrogens (17–21). Sulfo-conjugation of cholesterol itself appears to be mediated by two closely related members of the hydroxysteroid sulfotransferase family, bearing 48% amino acid sequence identity with STD (22, 23). STD has the highest substrate affinity for dehydroepiandrosterone (DHEA), which is a C-19 steroid and precursor for testosterone. The genes encoding STD in the rat and human have been extensively characterized (24–27). STD is selectively expressed in the liver, small intestine, colon, and adrenal cortex (17, 18, 28–31). Interestingly, the same tissues also express FXR. Because of its bile acid sulfo-conjugating activity, STD is expected to be an important component of the cholesterol removal mechanism through fecal loss.

In this article, we show that the primary bile acid chenodeoxycholic acid (CDCA), a known ligand for FXR, is a potent inducer of the rat Std promoter and this induction is mediated through a response element located between −169 and −193 positions. Unlike the IR-1 arrangement of the RXR/FXR binding sites identified in the IABP, BSEP, PLTP, and SHP genes, the −169/−193 site in the Std promoter contains an IR-0 motif as the FXR cognate element. Thus, the rat Std promoter provides the first example of an IR-0-containing FXR target gene. We speculate that increased solubility of sulfated bile acids facilitates their intracellular transport and clearance, thereby contributing to overall cholesterol homeostasis.

**EXPERIMENTAL PROCEDURES**

**Enzyme Assay for Sulfotransferase Activity—Chenodeoxycholic acid, lithocholic acid, dehydroepiandrosterone, and 5α-dihydrotestosterone, the substrates for the sulfotransferase enzyme, were purchased from Sigma. Recombinant, baculovirally produced DHEA-sulfotransferase was produced in Sf9 insect cells from the AcMNPV baculoviral vector after the viral polyhedrin was replaced by the full-length Std cDNA through gene of AcMNPV was replaced by the full-length Std cDNA through molecular cloning (27). The heterologous constructs (IR0)-TK-Luc and 3×(IR0)-TK-Luc were prepared by cloning, respectively, a single copy and three tandemly repeated copies of the −169 to −193 sequence of the rat Std promoter into the 5′ end of the TK promoter of the TK-Luc vector. We prepared TK-Luc by first removing the NF-κB consensus element from the NF-κB-TK-Luc plasmid (CLONTECH) using restriction digestion, and then an oligonucleotide sequence was ligated to the TK vector backbone to create multiple cloning sites. The −169/−193 oligonucleotide duplex as a single copy was cloned into the TK-Luc plasmid via KpnI and BglII sites. Three copies of the −169/−193 sequence in a tandem repeat were cloned into TK-Luc via MluI and BglII sites. Oligonucleotides with appropriate restriction sites at 5′ and 3′ ends were custom synthesized commercially. The mutant construct (IR0mut-TK-Luc was engineered by ligating one copy of an oligonucleotide containing 3-base mutations within the IR-0 of the −169/−193 element (CAT→ACC) to TK-Luc. The constructs were verified by DNA sequencing.

**Cell Transfection and Analysis of Reporter Expression—**HepG2 and Caco-2 cells (ATCC) were propagated using the minimum essential medium in the presence of 5% fetal bovine serum. Cells at −0.6 × 10^6/well (12-well flasks) were seeded overnight in Dulbecco’s modified Eagle’s medium containing 5% charcoal-stripped serum (Life Technologies, Inc.) and transfected with 1 μg of reporter construct and 100 ng each of FXR or the RXR-β-galactosidase reporter plasmid using either the calcium phosphate or lipofection method. The cells were treated with Dexamethasone (5, 16), where it may also be involved in the regulation of steroid-metabolizing enzymes.

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Recombinant DHEA-sulfotransferase was produced in SF9 insect cells from the AcMNPV baculoviral vector after the viral polyhedrin gene of AcMNPV was replaced by the full-length Std cDNA through homologous recombination (32). The transfer vector pVL1393 (Invitrogen, CA), containing the Std cDNA cloned into the PstI site, and the AcMNPV baculoviral DNA (Invitrogen), were transfected into SF9 cells. The cells were grown at 27 °C for 4 days, and the progeny viral particles released into the culture medium were used to infect new SF9 cells. The monolayer culture of infected SF9 cells was overlaid with agarose, and the plates were incubated at 27 °C in a humid chamber for 3 days to grow plaques. The occlusion body negative recombinant plaques were selected for the subsequent rounds of SF9 cell infection to produce a high titer recombinant stock. This stock was used to infect fresh SF9 cells, and the cells were cultured for 3 days to allow viral propagation. The cytosol of the harvested SF9 cells was the source for recombinant Std. Immunoblot of the recombinant SF9 cytosol and immunostaining of the recombinant SF9 cells showed specific expression of Std.

**Plasmid Constructs and, Site-directed Mutagenesis—**The natural Std promoter-containing reporter construct (366/38 Std-CAT) was described earlier (27). The heterologous constructs (IR0)-TK-Luc and 3×(IR0)-TK-Luc were prepared by cloning, respectively, a single copy and three tandemly repeated copies of the −169 to −193 sequence of the rat Std promoter into the 5′ end of the TK promoter of the TK-Luc vector. We prepared TK-Luc by first removing the NF-κB consensus element from the NF-κB-TK-Luc plasmid (CLONTECH) using restriction digestion, and then an oligonucleotide sequence was ligated to the TK vector backbone to create multiple cloning sites. The −169/−193 oligonucleotide duplex as a single copy was cloned into the TK-Luc plasmid via KpnI and BglII sites. Three copies of the −169/−193 sequence in a tandem repeat were cloned into TK-Luc via MluI and BglII sites. Oligonucleotides with appropriate restriction sites at 5′ and 3′ ends were custom synthesized commercially. The mutant construct (IR0mut-TK-Luc was engineered by ligating one copy of an oligonucleotide containing 3-base mutations within the IR-0 of the −169/−193 element (CAT→ACC) to TK-Luc. The constructs were verified by DNA sequencing.

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RESULTS

The Bile Acid Sulfating Activity of STD—Earlier studies with STD expressed in the liver cytosol have suggested that this sulfotransferase enzyme converts both DHEA and bile acids/salts to their sulfated derivatives (20, 34). To further demonstrate that STD is indeed a bile acid sulfotransferase, we have used baculovirally produced recombinant enzyme to examine its activity toward primary and secondary bile acids in an in vitro enzyme assay. The sulfated products were visualized on a TLC autoradiogram (Fig. 1). As test substrates, we used two C-24 bile acids, i.e. CDCA (a dihydroxy primary bile acid) and lithocholic acid (LCA, a monohydroxy secondary bile acid). Dehydroepiandrosterone (DHEA) and 5α-DHT, the two C-19 steroids, were used as the positive control substrates. The reaction utilized [35S]PAPS as the donor cofactor. The radiolabeled sulfated products were recovered as described under "Experimental Procedures" and resolved by TLC. The TLC autoradiogram shows that recombinant sulfotransferase can convert CDCA and LCA to the corresponding sulfo-conjugated forms. The migration rates of sulfated LCA and DHEA were similar, whereas sulfated CDCA migrated much more slowly with the TLC solvent system used in this experiment. Sulfated 5α-DHT showed an intermediate migration rate. We analyzed an aliquot of each reaction product by liquid scintillation counting for incorporated radioactivity into the sulfated products to quantitatively assess the efficiency of the substrates as sulfate acceptors. Sulfo-conjugation of CDCA and LCA was about 25% as efficient as DHEA under the in vitro assay conditions used in this study. These results demonstrate conclusively that DHEA-sulfotransferase can mediate sulfation of C-24 bile acids.

Bile Acid-mediated Induction of the Std Promoter and Mapping of a Bile Acid-responsive Region—Fig. 2A shows the effect of increasing concentrations of CDCA on reporter CAT expression from the (−366/+38) Std-CAT promoter-reporter construct in transiently transfected HepG2 hepatoma cells with or without co-transfection of the FXR expression plasmid. In the absence of FXR co-transfection, CDCA was only weakly effective in increasing the Std promoter function over that in vehicle-treated cells. On the other hand, supplementation with the FXR expression vector led to a 5-fold increase of the CDCA response with saturation at 25 μM CDCA. Upon co-expression of FXR and RXR-α, either CDCA (the FXR-specific ligand) or 9-cis-retinoic acid (RA) (the RXR-specific ligand) was almost equally effective in stimulating the promoter function (Fig. 2B). This result concurs with the known permissive role of the RXR/FXR heterodimer in bile acid and retinoid signaling (35).

When the cells were treated simultaneously with CDCA and 9-cis-RA, the extent of induction was only slightly higher than that treated with either one of these ligands alone. These results indicate that a functional bile acid-responsive region is present between −366 and +38 nucleotide positions of the Std promoter, and it can be activated by FXR in a ligand-dependent manner.
To identify this response element, we conducted functional promoter assay with progressively 5′ deleted Std promoter fragments. Upon analysis of different lengths of the Std promoter for CDCA-mediated induction in transfected HepG2 cells, we found that the -215 to +38 and -366 to +38 promoter fragments were induced by CDCA, whereas a shorter promoter deleted beyond -158 was unresponsive (Fig. 3). From these results we conclude that the bile acid response region resides between -215 and -158 bp of the Std promoter.

Characterization of a RXR/FXR Binding Site within the Bile Acid-responsive Region—The RXR/FXR binding site (bile acid response element) was further narrowed down by footprinting analysis of the -215 to -158 region. Fig. 4 shows a footprint with the liver nuclear extract covering -169 to -193 and includes a palindromic sequence (GGGTCA TGAACT) from -178 to -189. This palindrome is an IR-0 of the consensus (A/G)(G/T)TCA half-site. The specificity of the protein-DNA interaction at the footprinted sequence is evident from the oligonucleotide competition assay, which shows that the protection at the -169/-193 element was lost in the presence of the cold homologous oligo duplex (Fig. 4, lane 4), but not the heterologous oligo sequence that corresponds to the HNF-1 element between positions -32 and -55 of the Std promoter (lane 3).

The RXR/FXR binding site was further substantiated by EMSA. Fig. 5A shows that, upon incubation of the 32P-labeled -169/-193 element with the liver nuclear extract, a gel-retarded specific DNA-protein complex was formed (Fig. 5A, lane 1). The retarded band was competed out by 100-fold molar excess of the unlabeled homologous oligo duplex (lane 2), but not by the heterologous consensus element for NF-κB (lane 3), thus confirming the specificity of the protein-DNA complex. Antibody supershift assay showed that the retarded complex contains both FXR and RXR-α (Fig. 5A, lanes 5–8). The non-immune serum did not supershift the EMSA complex (lane 4). Furthermore, as shown in Fig. 5B, a mixture of recombinant FXR and RXR-α, produced by in vitro transcription and translation of the corresponding cDNAs, yielded an EMSA complex when incubated with the 32P-labeled -169/-193 oligo duplex (Fig. 5B, lane 4). This complex was specifically competed out by the cold -169/-193 element, designated as Std IR0 (lanes 5 and 6) but not by the mutant -169/-193 element (Std IR0mut) and the heterologous NF-κB element (lanes 7–9). The Std IR0mut oligo contains 3-base substitutions (CAT) instead of the cold IR-0 of the consensus element (ACC) within the IR-0 of the -169/-193 element. The results in Figs. 2–5, taken together, lead us to conclude that the -169/-193 footprint constitutes a bile acid response element harboring a cognate site for the heterodimeric RXR-α/FXR. Incomplete supershift of the retarded band by combined FXR and RXR-α antibodies (Fig. 5A, lane 8) suggests that other regulatory proteins are also possibly present as constituents of the multiple-protein-DNA complex at the -169/-193 footprinted element.

That the -169/-193 sequence functions as a bile acid response element is confirmed, based on the results that this sequence can confer CDCA dependent induction on the heterologous TK promoter in FXR-α and RXR-α co-transfected HepG2 cells (Fig. 6). When a single copy of the -169/-193 element was used in the reporter construct (IR0-TK-Luc), CDCA caused an ~2-fold increase in luciferase expression from the basal TK promoter, whereas point mutations of the IR-0 site led to a complete loss of the TK promoter induction in the mutant plasmid (IR0mut-TK-Luc (Fig. 6, A and B)). Furthermore, a
tandem repeat of three copies of this element in the reporter construct 3×(IR0)-TK-Luc caused a 15-fold increase in the bile acid response of the TK promoter in HepG2 cells (Fig. 6C). Thus, the −169/−193 element itself, without other interacting sites, can confer bile acid responsiveness to the minimal promoter.

From a physiological standpoint, both hepatocytes and intestinal epithelial cells play critical roles in bile acid metabolism, and, in addition to the liver, both FXR and STD are expressed in the small intestine and colon. We, therefore, examined whether bile acids can stimulate Std promoter function in an intestinal cell line. The Caco-2 cell line is derived from human colon adenocarcinoma and possesses certain enterocyte-like properties. Fig. 7 shows that the −366/+38 Std promoter was induced by more than 3-fold in FXR- and RXR-α-co-transfected Caco-2 cells upon treatment with CDCA (Fig. 7A). Furthermore, the −169/−193 element caused CDCA-dependent induction of the heterologous TK promoter in Caco-2 cells, and the induction was lost when the IR-0 within the −169/−193 element was altered by point mutations (Fig. 7B). CDCA-dependent induction of TK in the reporter construct 3×(IR0)-TK-Luc increased by as much as 70-fold. Thus, both liver and intestinal cells harbor the necessary transcription factors for the expression of the Std gene under the direct control by the ligand-activated FXR.

DISCUSSION

Sulfo-conjugation of hydroxyl and amino groups by sulfotransferases is a commonly utilized metabolic pathway for detoxification of hormones, neurotransmitters, drugs, and xeno-biotics (17, 36, 37). Sulfotransferases in mammalian cells constitute a large family of at least 44 similar enzymes, each containing two active sites: one for the sulfate donor PAPS and the other for the acceptor substrates (17, 38, 39). Individual members of this enzyme family display distinct, albeit somewhat broad, substrate specificity. STD, which was originally thought to be involved in the metabolism of C-19 hydroxysteroids and in the inactivation of androgenic hormones (40), is now realized to be a multifunctional enzyme with substrate affinity for both bile acids and androgens. In the rodent liver, the high expression of Std during the androgen-insensitive state of the hepatic tissue in senescent males is thought to be a result of the efficient conversion of androgens to androgen sulfates, which are receptor-inactive (19, 40). Although the exact role of bile acid sulfation is presently unknown, it is reasonable to speculate that sulfo-conjugation of bile acids will reduce their detergent properties, and at the same time enhance their aqueous solubility, thereby facilitating intracellular transport of these cholesterol catabolites. Sulfation may also aid in the fecal clearance of bile acids/salts and, consequently, cholesterol elimination from the body.

In the present study, we have shown that the recombinantly produced STD catalyzes sulfation conjugation of the dihydroxylated primary bile acid CDCA and the monohydroxy secondary bile acid LCA. Most importantly, we show that the promoter for the rat DHEA-sulfotransferase is induced by the primary bile acid CDCA in transfected liver and intestinal cell lines. Additionally, we observed that endogenous STD gene expression was stimulated ~2-fold in HepG2 and Caco-2 cells after CDCA treatment (data not shown). These results favor the speculation that sulfation may be a protective mechanism to help reduce the toxicity of high levels of bile acids and facilitate their intracellular transport and urinary and fecal clearance. The CDCA-dependent induction of the Std gene promoter is reminiscent of the self-regulatory mechanisms that are operational for a number of genes concerned with the synthesis, transport, and elimination of bile acids, thereby ensuring that the bile acid flux remains within an optimum range. FXR-mediated induction of the genes for IBABP and BSEP, the two proteins involved, respectively, in the intracellular trafficking of intestinal bile acids and bile acid/salt efflux out of the hepatocytes (4, 7, 41), are anticipated responses to increased enterohepatic pools of these cholesterol catabolites. Stimulation of the PLTP gene by bile acids is thought to be important in the regulation of reverse cholesterol transport (9, 42). Likewise, FXR-mediated repression of the genes for the bile acid uptake protein sodium/taurocholate cotransporter polypeptide and the bile acid biosynthetic enzymes serves to maintain optimal bile acid pools in the enterohepatic circulation (2, 3, 6). The rat Std promoter, besides its positive regulation by the bile acid-activated FXR, is also negatively regulated by the androgen-activated androgen receptor (27, 43). Thus, in a unique regulatory scheme, two principal substrates of this sulfotransferase, i.e. androgens and bile acids, are also the activating ligands for two members of the nuclear receptor superfamily (i.e. androgen receptor and FXR) that control the expression of this enzyme. Although such auto-regulatory loops have been extensively characterized for homeostatic schemes in prokaryotes (e.g. the classical lac operon), similar pathways to self-regulation in the mammalian system have not been clearly deciphered.

FXR functions as an obligate heterodimer with the 9-cis-retinoic acid receptor (RXR) and regulates target gene expression by binding to an inverted repeat of a hexanucleotide sequence motif. The optimal DNA binding element for the RXR/FXR heterodimer is an IR-1 sequence of the (A/G)G/G/T/TCA half-site, as ascertained from the analysis of the oligonucleotides that can be selectively amplified from a pool of random
FIG. 5. A, identification of a binding element for the FXR/RXR-α heterodimer within the −169/−193 footprinted sequence. Upper panel, an IR-0 motif within the −169/−193 sequence. Consensus half-site (A/G)G(G/T)TCA for nonsteroid nuclear receptors is shown. Lower panel, lanes 1–3: competition with homologous (lane 2) but not heterologous (NF-κB; lane 3) unlabeled oligo duplex. Lanes 4–8, antibody supershift assay. The up-shifted bands are shown as open triangle (lane 5), asterisks (lanes 6 and 7), and closed triangle (lane 8). 1 μl of anti-RXR-α (lane 5) and 1 μl (lane 6) or 2 μl (lane 7) of anti-FXR were used. Lane 4, the nonimmune serum control for antibody supershift. Due to long electrophoretic run, the free probe migrated out of the gel.

B, specific binding of recombinant FXR and RXR-α to the IR-0 sequence of the −169/−193 element. Recombinant FXR and RXR-α produced by in vitro translation were used in the EMSA analysis. FXR and RXR-α either individually (lanes 2 and 3) or together (lanes 4–11) were incubated with 32P-labeled −169/−193 oligo duplex. Competition with the unlabeled wild type −169/−193 sequence (Std IR0; lanes 5 and 6) and mutant −169/−193 sequence (Std IR0mut; lanes 7 and 8) was performed using 50- and 200-fold molar excess of the competitor. The NF-κB oligo (lane 9) was used at 200-fold molar excess.
sequence after multiple rounds of binding to a mixture of recombinant RXR-α and FXR (15). Initial reports on four natural target genes for FXR (IBABP, PLTP, SHP, and BSEP) revealed that all of them contain the functional FXR site with a single nucleotide spacer between the inverted hexanucleotide repeat (IR-1). However, our finding with the Std gene promoter extends this view to include an IR-0 palindrome (GGGTCA TGAACCT) as an alternative binding modality for the RXR/FXR heterodimer. Two lines of evidence further established the role of this IR-0 motif in the bile acid response. 1) the −169/−193 element can confer CDCA-dependent induction of a heterologous promoter (thymidine kinase) in HepG2 and Caco-2 cells; 2) disruption of the IR-0 sequence by point mutations (GGGTCA TGAACCT → GGGTACCGAACT) completely abrogated the CDCA-induced activity of the TK promoter. Because only part of the EMSA complex at the −169/−193 element is recognized by the anti-FXR or anti-RXR-α antibody, it is likely that other transcription factors are also present in this complex. In preliminary antibody supershift analysis, we have found evidence for the presence of the orphan nuclear receptor HNF-4 in this EMSA complex.

In conclusion, our results show that STD, a bile acid sulfotransferase, is induced by the primary bile acid CDCA in liver and intestinal cell lines via an IR-0 palindrome (GGGTCA TGAACCT) as an alternative binding modality for the RXR/FXR heterodimer. The physiological role of this regulatory pathway remains to be clearly delineated. The Cyp27 null mice that are deficient in sterol 27α-hydroxylase, the rate-limiting enzyme in the acidic pathway to CDCA synthesis, are reported to be severely deficient in bile acid pools (44). These animals display hypercholesterolemia and hyperlipidemia similar to the human CYP27 deficiency syndrome. It will be of interest to determine the impact of Cyp27 deficiency on Std gene expression. Because the enterohepatic metabolic loop plays a critical role in bile acid/cholesterol homeostasis, further characterization of the role of FXR signaling in Std gene expression may be of considerable physiological significance.

Acknowledgments—We are grateful to Dr. David Mangelsdorf (Southwestern Medical Center, Dallas, TX) for providing the FXR expression plasmid and to Dr. Ronald Evans (Salk Institute, San Diego, CA) for the RRX-α expression plasmid. We thank Dr. Dongbum Shin for interest in this study and acknowledge assistance in graphics from Gilbert Torralva.
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doi: 10.1074/jbc.M107557200 originally published online August 30, 2001

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