Published in final edited form as:
FEBS Lett. 2013 November 29; 587(23): 3787–3791. doi:10.1016/j.febslet.2013.09.043.

Liver-type fatty acid binding protein interacts with hepatocyte nuclear factor 4α

Avery L. McIntosh[a], **Anca D. Petrescu**[a], **Heather A. Hostetler**[c], **Ann B. Kier**[b], and **Friedhelm Schroeder**[a,*]

[a]Department of Physiology and Pharmacology, Texas A&M University, TVMC, College Station, TX 77843-4466, United States

[b]Department of Pathobiology, Texas A&M University, TVMC, College Station, TX 77843-4467, United States

[c]Department of Biochemistry and Molecular Biology, Wright State University, Dayton, OH 45435, United States

Abstract

Hepatocyte nuclear factor 4α (HNF4α) regulates liver type fatty acid binding protein (L-FABP) gene expression. Conversely as shown herein, L-FABP structurally and functionally also interacts with HNF4α. Fluorescence resonance energy transfer (FRET) between Cy3-HNF4α (donor) and Cy5-L-FABP (acceptor) as well as FRET microscopy detected L-FABP in close proximity (~80 Å) to HNF4α, binding with high affinity K_d ~250–300 nM. Circular dichroism (CD) determined that the HNF4α/L-FABP interaction altered protein secondary structure. Finally, L-FABP potentiated transactivation of HNF4α in COS7 cells. Taken together, these data suggest that L-FABP provides a signaling path to HNF4α activation in the nucleus.

Keywords

Liver; Fatty acid binding protein; Hepatocyte nuclear factor 4α

1. Introduction

Hepatocyte nuclear factor 4α (HNF4α) is a very conserved nuclear receptor expressed at high level in liver hepatocytes but also in small intestine, colon, pancreatic beta cells, and kidney proximal tubules. HNF4α controls expression of multiple hepatic genes involved in lipid and glucose homeostasis, especially the cytosolic long chain fatty acid LCFA/LCFA-CoA binding proteins liver-type fatty acid binding protein (L-FABP) and acyl-CoA binding protein (ACBP) [1–4]. In a positive feed-back mechanism, ACBP directly binds and activates HNF4α transcriptional activity [5]. Unknown is whether L-FABP likewise interacts with HNF4α to impact transcription.

Resolving potential contributions of L-FABP to HNF4α regulation is important since mutations, whether in the HNF4α binding site or in the gene itself, are directly linked to human disease, especially monogenic non-insulin-dependent diabetes mellitus type II (MODY1) [6]. Indirectly, HNF4α has also been linked to human disease through regulation

*Corresponding author. Fax: +1 979 862 4929. fschroeder@cvm.tamu.edu (F. Schroeder).

of other genes contributing to diabetes (via PEPCK, L-PK, and HNF1), atherosclerosis, thrombosis, hypoxia, MCAD deficiency, OTC deficiency, and cancer [7–9].

The present study begins to address the question of whether L-FABP interacts with and activates HNF4α analogous to ACBP binding/activating HNF4α [5] and L-FABP binding/activating peroxisome proliferator activated receptor-α (PPARα) [10–12]. As shown by fluorescence resonance energy transfer (FRET), FRET binding assay, circular dichroism, and transactivation as well as laser scanning confocalmicroscopy (LSCM) FRET, L-FABP structurally and functionally bound HNF4α to stimulate HNF4α transcriptional activity in the presence of known ligands of each protein.

2. Materials and methods

2.1. Chemicals

Bezafibrate was from Santa Cruz Biotechnology (Dallas, TX) while 2-bromopalmitic, palmitic, and arachidonic acid as well as β-galactosidase were from Sigma–Aldrich (St. Louis, MO). Non-hydrolyzable C16:0-S-S-CoA was prepared as in [13].

2.2. Expression and purification of recombinant rat L-FABP and HNF4α

The full-length rat L-FABP and HNF4α recombinant proteins were obtained and purified as described [10,14,15]. Protein purity was determined by SDS–PAGE and concentrations were determined by BCA Protein Assay (Pierce, Rockford, IL).

2.3. Fluorescence resonance energy transfer (FRET) between Cy3- HNF4αCy5-L-FABP: intermolecular distance and binding affinity

Purified recombinant HNF4α and L-FABP were labeled with the fluorophores Cy3 and Cy5, respectively, to form a FRET donor–acceptor pair for determining intermolecular distance and binding affinities as described earlier [5,10,11].

2.4. LSCM FRET imaging of L-FABP/HNF4α interaction in cultured T-7 rat hepatoma cells

T-7 cells were grown to near 80% confluency in 4-well LabTek chambered slides, fixed and labeled with primary antibodies to HNF4α and L-FABP followed by secondary antibodies conjugated with FITC, Cy3, and Cy5, respectively. Cells were imaged by LCSM using a Bio-Rad MRC-1024 (Zeiss, Inc, Thornwood, NY) equipped with an Ar+/Kr+ laser as described [5]. Detector gain and black levels were adjusted to minimize autofluorescence and excitation was at sufficient low powers prevent bleed through into the other channels.

2.5. Circular dichroism (CD) of L-FABP/HNF4α complex

Far UV CD spectra of recombinant L-FABP and full-length HNF4α, separately and in combination were obtained as described [5,10]. Percentage composition of helixes, β-strands, turns and random coil was calculated as described therein. Controls were run for buffer alone in the absence of protein and spectra subtracted from protein-containing sample spectra. Theoretical CD spectra (no interaction) were calculated from HNF4α and L-FABP experimental CD spectra as in [5,10].

2.6. Functional consequence of L-FABP/HNF4α interaction: transactivation assay

Transactivation assay was performed as described earlier except that COS-7 cells (ATCC, Manassas, VA) were transfected with pcDNA3-L-FABP instead of an ACBP expressing vector [5]. The ApoBLuc reporter plasmid and pLEN4S HNF4α expression vector were from Dr. F. Sladek (University of California, Riverside, CA). Cells were washed twice, incubated in serum free medium for 2 h, 10 μM of lipidic ligand/BSA complex added,

incubated overnight, and assayed as in [5]. Data was presented as firefly luciferase activity normalized to Renilla luciferase activity and corrected for effect of empty pLEN4 vector, with the L-FABP-bezafibrate sample arbitrarily set to 100%.

2.7. Statistical analysis

Statistical analysis on the data was performed using either a *t*-test or by one-way ANOVA using statistical software (GraphPad Prism, San Diego, CA). Values represent the mean ± S.E.M., with $P < 0.05$ considered statistically significant.

3. Results

3.1. FRET between purified Cy3-HNF4α and Cy5-L-FABP

Cy3- and Cy5-form effective FRET donor–acceptor pairs for determining intermolecular distance and binding affinity [5,10,11]. With increasing Cy5-L-FABP (acceptor), strong quenching (Fig. 1A) and saturable binding (Fig. 1A inset-solid circles) to Cy3-HNF4α were observed. Likewise, with increasing Cy5-L-FABP (acceptor), increasing sensitized emission of Cy5-L-FABP (Fig. 1B) and saturable binding (Fig. 1B inset-solid circles) to Cy3-HNF4α were observed. Nonlinear regression analysis of the binding curves (Fig. 1A and B solid lines) yielded K_ds of 250 ± 50 nM and 300 ± 150 nM, respectively (Table 1). The mean FRET interaction distances was $r = 70 \pm 10$ Å and 80 ± 20 Å, respectively (Table 1). Control Cy3-labeled β-galactosidase was similarly titrated with Cy5-L-FABP, but Cy3-emission was only weakly quenched (Fig. 1C), sensitized emission from Cy5 did not appear (Fig. 1C inset), and saturable binding curves were not obtained (not shown). Thus, Cy3-HNF4α/Cy5-L-FABP FRET was not due to random distribution of Cy3 and Cy5 fluorophores or diffusion-enhanced effects.

3.2. Circular dichroism (CD) detected altered secondary structure on L-FABP/ HNF4α binding

Coregulator induced conformational changes in nuclear receptors such as HNF4α and PPARα are crucial to receptor activation [5,10,11]. To determine if L-FABP/HNFα interaction altered conformation, CD spectra were acquired for HNF4α alone (Fig. 2A circles), L-FABP alone (Fig. 2A triangles), and HNF4α and L-FABP together (Fig. 2A squares). A theoretical CD spectrum (no interaction, Fig. 2A, diamonds) was calculated from HNF4α and L-FABP experimental CD spectra as described in Methods. L-FABP and HNF4α differed significantly in the percentage of α-helix, β-strand, turns, and unordered secondary structure (Fig. 2B, C and D). While L-FABP/HNFα interaction did not alter the proportions of α-helical structures from non-interacting (Fig. 2B, gray vs. dark gray bars), the proportions of regular β-sheet and total β-sheet (Fig. 2C) were significantly decreased while unordered structure (Fig. 2D) was increased as compared to non-interacting.

3.3. L-FABP/HNF4α interaction in vivo: confocal immunofluorescence FRET imaging in rat T-7 hepatoma cells

T-7 cells were fixed, labeled with Cy3-anti-HNF4α and Cy5-anti-L-FABP, and imaged by LSCM. First, Cy3-anti-HNF4α was excited at 567 nm and emission detected through HQ598/40 nm filter (Fig. 3A) while Cy5-anti-L-FABP was excited at 647 nm and emission detected through D680/30 nm filter (Fig. 3B). Superposition revealed significant colocalization as shown by the yellow pixels in the merged image (Fig. 3C) and a fluorogram (Fig. 3D). Since resolution of LSCM (~200 nm) is not high enough to determine if the two proteins interacted at the molecular level (0–10 nm), FRET experiments were performed. First, the gain and black levels of the red channel photomultipliers were set so that there was no presence of Cy3-HNF4α emission in the D680/30 nm detection (red)

channel (Fig. 3E) using only Cy3-HNF4α immunolabeled cells. Subsequently, in cells double immunolabeled with both Cy3-HNF4α and Cy5-L-FABP, excitation at 568 nm yielded sensitized emission that was detected in the Cy5-L-FABP emission channel using the D680/30 nm filter (Fig. 3F)—indicating L-FABP in close proximity to HNFα for binding—consistent with data using purified proteins (Fig. 1, Table 1).

3.4. Effect of L-FABP expression on HNF4α transactivation

To determine if L-FABP/HNF4α interaction is functionally significant, a transactivation assay was performed. Control and L-FABP overexpressing COS-7 cells transfected with an apoB reporter plasmid and either HNF4α or empty vector were incubated overnight with BSA or BSA complexed with bezafibrate, palmitic acid, bromo-palmitate, C16:0-S-S-CoA, or arachidonic acid as in Methods. The luciferase activity of the apoB reporter plasmid resulting from HNF4α transactivation (not shown) was corrected by normalization to the internal control (*Renilla* luciferase) activity and corrected for the effect of the empty pLEN4 vector (Fig. 4B) with the L-FABP bezafibrate sample arbitrarily set to 100% (Fig. 4A). The control (transfection using empty pLEN4 vector) revealed only slight activity with no significant response to any of the lipid incubations and/or overexpression of L-FABP (Fig. 4B). However, in HNF4α transfected cells L-FABP overexpression significantly enhanced (corrected for the controls) HNF4α-mediated activation of ApoB transcription in the presence of each of the lipids (Fig. 4A); the largest response obtained with bromo-palmitate and non-metabolizable C16:0-S-S-CoA.

4. Discussion

HNF4α, a member of the steroid hormone receptor superfamily, is involved in regulating hepatocyte lipid and carbohydrate metabolic genes [4,16]. Significant evidence suggests HNF4α binds and may be activated by lipidic ligands such as long chain fatty acids LCFA/LCFA-CoA [14,17–22] and xenobiotics [18,20,21,23,24]. Importantly, linoleic acid has been observed natively bound to HNF4α from mouse liver [25]. Missense mutations in HNF4α, specifically the ligand binding domain, lead to a loss-of-function and result in the maturity onset diabetes of the young (MODY 1) [26]. In certain mutations, ligand binding affinities are decreased resulting in failure of transactivation [14]. Interestingly, HNF4α itself controls hepatic expression of the cytosolic LCFA/LCFA-CoA binding proteins L-FABP and ACBP [1–4,27]—both contributing to uptake, intracellular transport, and nuclear targeting of these ligands [28,29]. In a positive feed-back loop, ACBP has been shown to directly bind and activate HNF4α transcriptional activity [5]. The key question of whether L-FABP likewise binds and activates HNFα was the focus of the current investigation.

The data presented herein were consistent with the hypothesis that L-FABP also physically and functionally interacts with HNF4α. L-FABP/HNF4α interaction was shown by a FRET binding assay, CD, as well as immunofluorescence LSCM and FRET. Furthermore, L-FABP overexpression enhanced the HNF4α transcriptional activity of an apoB reporter plasmid in the absence of exogenous ligand and, even more so, in the presence of metabolizable and non-metabolizable LCFAs and LCFA-CoAs as well as peroxisome proliferators (i.e. bezafibrate) in COS-7 cells. All these ligands are bound by L-FABP. L-FABP may facilitate lipidic ligand mediated activation by enhancing ligand uptake and/or mediating bound ligand delivery to HNF4α for direct transfer upon L-FABP/HNF4α interaction. In the latter mechanism, L-FABP would provide a signaling path to HNF4α analogous to its ability to facilitate ligand activated transcriptional activity of PPARα [10–12,29]. Taken together, these findings suggest that L-FABP may effectively act as a coregulator of both HNF4α and PPARα targeted gene transcription, both of whose response elements are present in the L-FABP promoter [30].

Acknowledgments

This work was supported by the USPHS, National Institutes of Health Grants DK41402 (F.S. and A.K.) and NIH K99 award DK77573 (H.A.H).

Abbreviations

ACBP	acyl CoA binding protein
CD	circular dichroism
FRET	fluorescence resonance energy transfer
HNF4α	hepatocyte nuclear factor 4α
LCFA	long chain fatty acid
LCFA-CoA	long chain fatty acyl CoA
L-FABP	liver type fatty acid binding protein
LSCM	laser scanning confocal microscopy
PPARα	peroxisome proliferator activated receptor-α

References

1. Sladek FM, Zhong W, Lai E, Darnell JE. Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. Genes Dev. 1990; 4:2353–2365. [PubMed: 2279702]
2. Fraser JD, Keller D, Martinez V, Santiso-Mere D, Straney R, Briggs MR. Utilization of recombinant adenovirus and dominant negative mutants to characterize hepatocyte nuclear factor 4-regulated apolipoprotein ai and ciii expression. J Biol Chem. 1997; 272:13892–13898. [PubMed: 9153249]
3. Naiki T, Nagaki M, Shidoji Y, Kojima H, Imose M, Kato T, Ohnishi N, Yagi K, Moriwaki H. Analysis of gene expression profile induced by hepatocyte nuclear factor 4alpha in hepatoma cells using an oligonucleotide microarray. J Biol Chem. 2002; 277:14011–14019. [PubMed: 11834723]
4. Hayhurst GP, Lee YH, Lambert G, Ward JM, Gonzalez FJ. Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. Mol Cell Biol. 2001; 21:1393–1403. [PubMed: 11158324]
5. Petrescu AD, Payne HR, Boedeker AL, Chao H, Hertz R, Bar-Tana J, Schroeder F, Kier AB. Physical and functional interaction of acyl CoA binding protein (ACBP) with hepatocyte nuclear factor-4alpha (HNF4alpha). J Biol Chem. 2003; 278:51813–51824. [PubMed: 14530276]
6. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI. Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). Nature. 1996; 384:458–460. [PubMed: 8945471]
7. Martinez-Jimenez CP, Gomez-Lechon MJ, Castell JV, Jover R. Underexpressed coactivators PGC1 and SRC1 impair hepatocyte nuclear factor 4 function and promote dedifferentiation in human hepatoma cells. J Biol Chem. 2006; 281:29840–29849. [PubMed: 16891307]
8. Tsuchiya T, Kominato Y, Ueda M. Human hypoxic signal transduction through a signature motif in hepatocyte nuclear factor 4. J Biochem. 2002; 132:37–44. [PubMed: 12097158]
9. Rana R, Surapureddi S, Kam WK, Ferguson S, Goldstein JA. Med25 is required for RNA polymerase II recruitment to specific promoters, thus regulating xenobiotic and lipid metabolism in human liver. Mol Cell Biol. 2011; 31:466–481. [PubMed: 21135126]
10. Hostetler HA, McIntosh AL, Atshaves BP, Storey SM, Payne HR, Kier AB, Schroeder F. Liver type fatty acid binding protein (L-FABP) interacts with peroxisome proliferator activated receptor-a in cultured primary hepatocytes. J Lipid Res. 2009; 50:1663–1675. [PubMed: 19289416]
11. Hostetler HA, Balanarasimha M, Huang H, Kelzer MS, Kaliappan A, Kier AB, Schroeder F. Glucose regulates fatty acid binding protein interaction with lipids and PPARa. J Lipid Res. 2010; 51:3103–3116. [PubMed: 20628144]

12. Velkov, T. Interactions between human liver fatty acid binding protein and peroxisome proliferator activated receptor drugs. PPAR Res. http://dx.doi.org/10.1155/2013/938401

13. Huang H, Starodub O, McIntosh A, Atshaves BP, Woldegiorgis G, Kier AB, Schroeder F. Liver fatty acid binding protein colocalizes with peroxisome proliferator receptor alpha and enhances ligand distribution to nuclei of living cells. Biochemistry. 2004; 43:2484–2500. [PubMed: 14992586]

14. Hertz R, Ben-Haim M, Petrescu A, Kalderon B, Berman I, Eldad N, Schroeder F, Bar-Tana J. Rescue of MODY-1 by agonist ligands of HNF4alpha. J Biol Chem. 2003; 278:22578–22585. [PubMed: 12697772]

15. Bogan AA, Dallas-Yang Q, Ruse MD, Maeda Y, Jiang G, Nepomuceno L, Scanlan TS, Cohen FE, Sladek FM. Analysis of protein dimerization and ligand binding of orphan receptor HNF4a. J Mol Biol. 2000; 302:831–851. [PubMed: 10993727]

16. Stoffel M, Duncan SA. The maturity-onset diabetes of the young (MODY1) transcription factor HNF4alpha regulates expression of genes required for glucose transport and metabolism. Proc Natl Acad Sci USA. 1997; 94:13209–13214. [PubMed: 9371825]

17. Hertz R, Magenheim J, Berman I, Bar-Tana J. Fatty acyl-CoA thioesters are ligands of hepatic nuclear factor-4a. Nature. 1998; 392:512–516. [PubMed: 9548258]

18. Hertz R, Sheena V, Kalderon B, Berman I, Bar-Tana J. Suppression of hepatocyte nuclear factor 4alpha by acyl-CoA thioesters of hypolipidemic peroxisome proliferators. Biochem Pharmacol. 2001; 61:1057–1062. [PubMed: 11301038]

19. Hertz R, Kalderon B, Byk T, Berman I, Za'tara G, Mayer R, Bar-Tana J. Thioesterase activity and acyl-CoA/fatty acid cross talk of hepatocyte nuclear factor-4a. J Biol Chem. 2005; 280:24451–24461. [PubMed: 15870076]

20. Petrescu A, Huang H, Hertz R, Bar-Tana J, Schroeder F, Kier AB. Role of regulatory F-domain in hepatocyte nuclear factor-4alpha ligand specificity. J Biol Chem. 2005; 280:16714–16727. [PubMed: 15741159]

21. Petrescu AD, Hertz R, Bar-Tana J, Schroeder F, Kier AB. Ligand specificity and conformational dependence of the hepatic nuclear factor-4alpha (HNF-4a). J Biol Chem. 2002; 277:23988–23999. [PubMed: 11940586]

22. Schroeder F, Huang H, Hostetler HA, Petrescu AD, Hertz R, Bar-Tana J, Kier AB. Stability of fatty acyl CoA thioester ligands of hepatocyte nuclear factor -4alpha and peroxisome proliferator-activated receptor alpha. Lipids. 2005; 40:559–568. [PubMed: 16149734]

23. Bernlohr DA, Simpson MA, Hertzel AV, Banaszak L. Intracellular lipid binding proteins and their genes. Annu Rev Nutr. 1997; 17:277–303. [PubMed: 9240929]

24. Kiselyuk A, Lee SH, Farber-Katz S, Zhang M, Athavankar S, Cohen T, Pinkerton AB, Ye M, Bushway P, Richardson AD, Hostetler HA, Rodriguez-Lee M, Huan L, Spangler B, Smith L, Higginbotham J, Cashman J, Freeze H, Itkin-Ansari P, Dawson MI, Schroeder F, Cang Y, Levine F. HNF4a antagonists discovered by a high-throughput screen for modulators of the human insulin promoter. Chem Biol. 2012; 19:806– 818. [PubMed: 22840769]

25. Yuan X, Ta TC, Lin M, Evans JR, Dong Y, Bolotin E, Sherman MA, Forman BM, Sladek FM. Identification of an endogenous ligand bound to a native orphan nuclear receptor. PLoS ONE. 2009; 4:e5609. [PubMed: 19440305]

26. Kyitha MP, Bacon S, Pannu KK, Rizvi SR, Colclough K, Ellar S, Byrne MM. Identification of a HNF1A-MODY and HNF4A-MODY in Irish families: phenotypic characteristics and therapeutic implications. Diab Metab. 2011; 37:512–519.

27. Bonzo JA, Ferry CH, Matsubara T, Kim JH, Gonzalez FJ. Gene regulation: suppression of hepatocyte proliferation by hepatocyte nuclear factor 4a in adult mice. J Biol Chem. 2012; 287:7345–7356. [PubMed: 22241473]

28. McArthur MJ, Atshaves BP, Frolov A, Foxworth WD, Kier AB, Schroeder F. Cellular uptake and intracellular trafficking of long chain fatty acids. J Lipid Res. 1999; 40:1371–1383. [PubMed: 10428973]

29. Schroeder F, Petrescu AD, Huang H, Atshaves BP, McIntosh AL, Martin GG, Hostetler HA, Vespa A, Landrock K, Landrock D, Payne HR, Kier AB. Role of fatty acid binding proteins and

long chain fatty acids in modulating nuclear receptors and gene transcription. Lipids. 2008; 43:1–17. [PubMed: 17882463]

30. Guzman C, Benet M, Pisonero-Vaquero S, Moya M, Garcia-Mediavilla MV, Martinez-Chantar ML, Gonzalez-Gallego J, Castell JV, Sanchez-Campos S, Jover R. The human liver fatty acid binding protein (FABP1) gene is activated by FOXA1 and PPARa; and repressed by C/EBPa: implication in FABP1 down-regulation in nonalcoholic liver disease. Biochim Biophys Acta. 2013; 1831:803–818. [PubMed: 23318274]

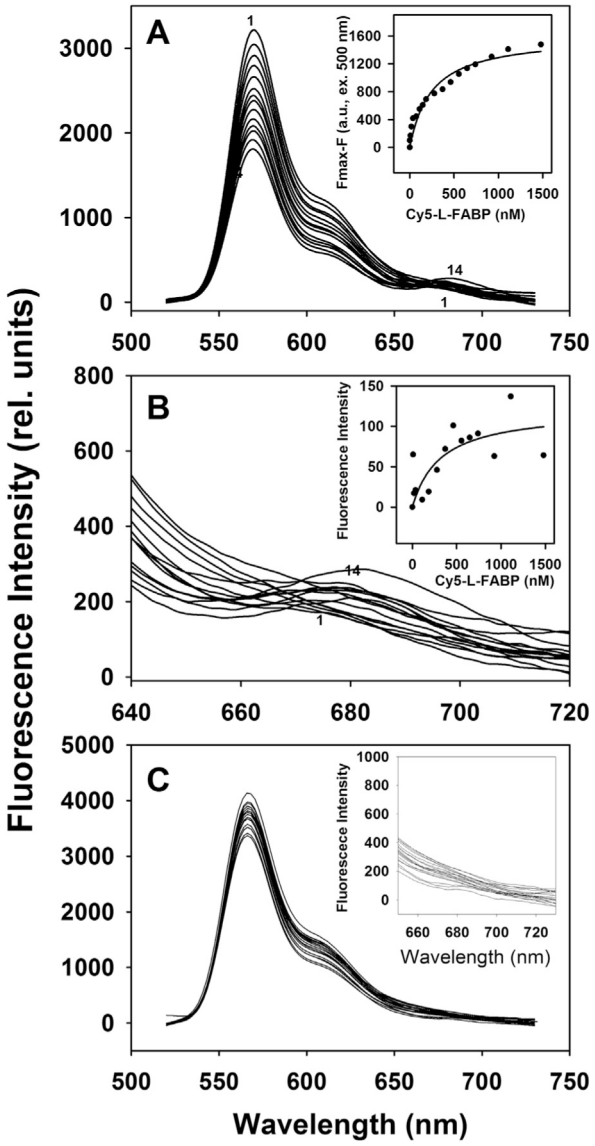

Fig. 1.

FRET detection of L-FABP/HNF4α interaction in vitro. HNF4α and L-FABP proteins were labeled with Cy3 and Cy5, respectively, spectra obtained, and binding curves calculated from FRET observed as Cy3 (donor) fluorophore quenching and Cy5 (acceptor) sensitized emission as in Methods. (A) Emission spectra of Cy3- HNF4α in absence (spectrum 1) and presence of increasing Cy5-L-FABP up to 1500 nM (spectrum 14). Inset: Plot of Cy3-HNF4α peak fluorescence at 570 nm (solid circles) and fitted ligand binding curve (solid line). (B) Scaled portion of the spectra near 680 nm in (A) showing Cy5-L-FABP sensitized emission resulted from FRET from donor Cy3-HNF4α. Inset: Plot of Cy5-L-FABP sensitized emission at 680 nm (solid circles) and fitted ligand binding curve (solid line). (C) Emission spectra of Cy3-β-galactosidase without or with increasing Cy5-L-FABP showing little to no quenching. Inset: enlarged spectra of the Cy5-L-FABP sensitized emission near 680 nm showing no sensitized emission.

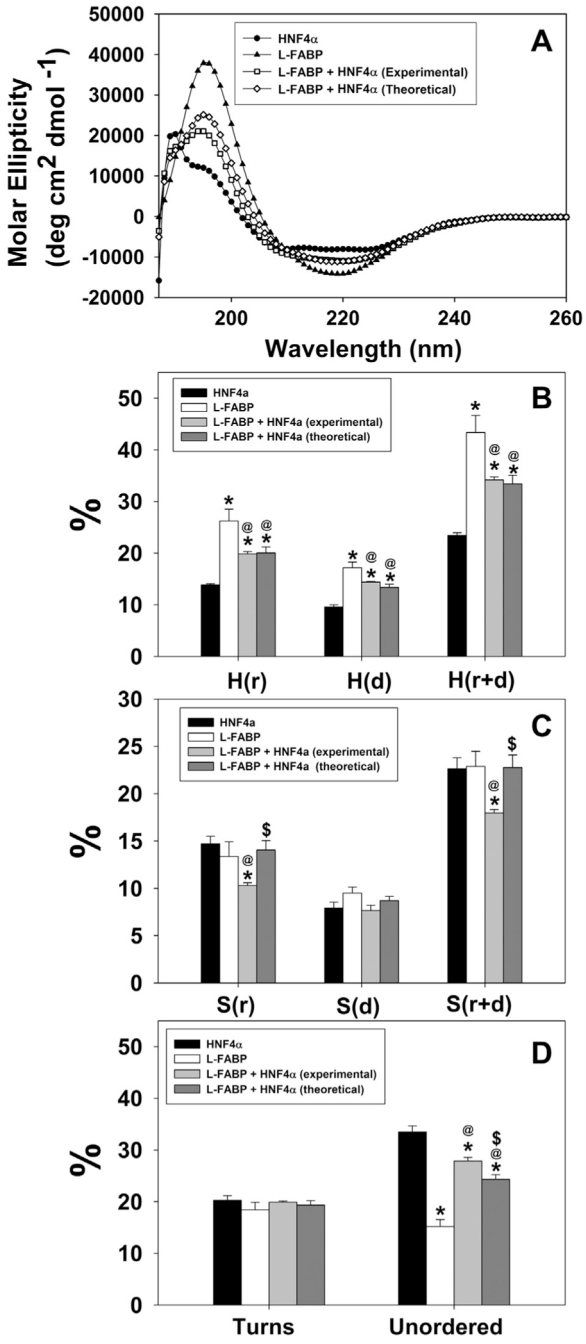

Fig. 2.

CD detection of conformational alterations in L-FABP/HNF4α complex. (A) CD spectra, converted to molar ellipticity, of recombinant rat L-FABP (triangles) and HNF4α (circles) were measured separately and together (squares). Data were compared to a theoretical molar ellipticity spectrum with no conformational change (diamonds) calculated for the mixture by averaging the individual spectra of the two proteins. CD analysis was performed to determine secondary structure parameters using CDPro (see Section 2). (B) Percent regular, distorted and total αhelix. (C) Percent regular, distorted, and total β-strand. (D) Percent turns

and unordered secondary structure. *$P < 0.05$ vs. HNF4α, $^{@}P < 0.05$ vs. L-FABP, $^{\$}P < 0.05$ vs. [HNF4α + L-FABP] experimental.

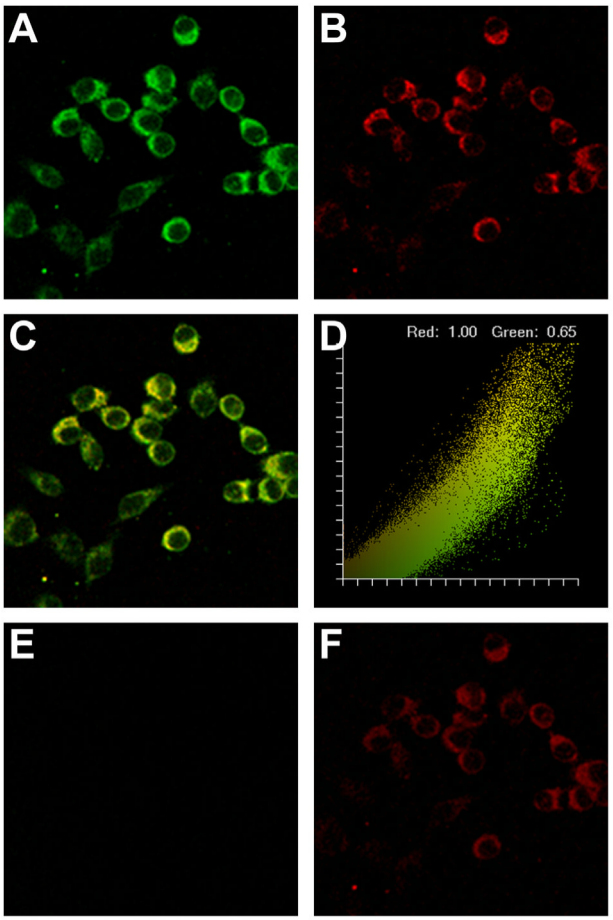

Fig. 3.

Immunofluorescence LSCM and FRET between double immunolabeled L-FABP/HNF4α in T-7 rat hepatoma cells. T-7 cells were labeled with Cy3-anti-HNF4α and Cy5-anti-L-FABP for LCSM imaging as in Methods. (A) Cy3-HNF4α, excited at 567 nm, was detected with a HQ598/40 nm filter and pseudo-colored green (green). (B) Cy5-L-FABP, excited at 647 nm, was detected using a D680/30 nm filter and pseudo-colored red. (C) Merged imaged of (A) and (B) showing the colocalization (yellow) of Cy3-HNF4α and Cy5-L-FABP. (D) Fluorogram of (C) with colocalization coefficients C_{red} = 1.00 and C_{green} = 0.65. **(E)** The absence of Cy3-HNF4α emission through the D680/30 nm filter (red) is shown as a control. (F) Cy5-L-FABP sensitized emission (Cy3-HNF4α excited at 567 nm) was detected with D680/30 nm filter (red).

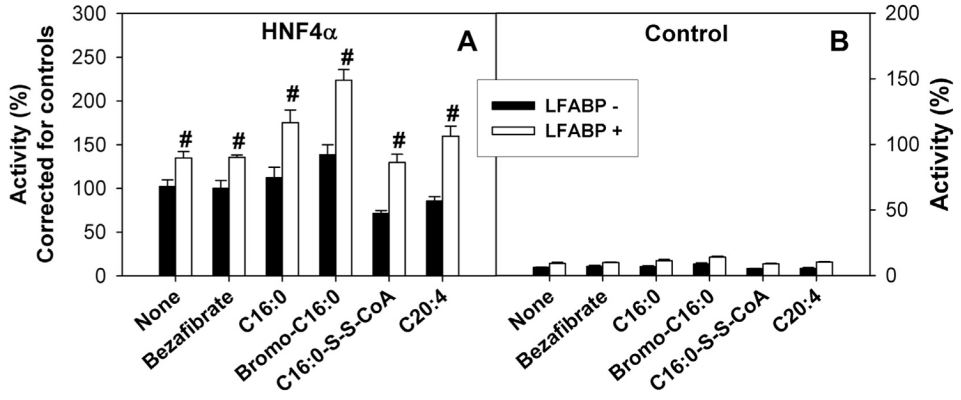

Fig. 4.

Effect of L-FABP expression on HNF4α transactivation activity of ApoB. Control and L-FABP overexpressing COS-7 cells were transfected with HNF4α or empty vector, and reporter plasmid as in Methods. Cells were incubated with serum free medium for 2 h. prior to adding 10 μM of bezafibrate, palmitic acid, bromo-palmitate, C16:0-S-S-CoA, or arachidonic acid overnight and then assayed. The resulting transactivation data (A) were presented as firefly luciferase activity normalized to Renilla luciferase activity and corrected for the effect of the empty pLEN4 vector (B), with the L-FABP bezafibrate sample arbitrarily set to 100%. # refer to $P < 0.05$ for L-FABP – vs. L-FABP+, respectively.

Table 1

FRET determination of intermolecular distance and binding affinity between L-FABP/HNF4α. Intermolecular distance and binding affinity (K_d, dissociation constant) were calculated from Cy3-HNF4α quenching and Cy5-L-FABP sensitized emission spectra in Fig. 1.

Interacting proteins	Cy3 quenching			Cy5 sensitized emission		
	K_d (nM)	*E* (%)	*r* (Å)	K_d (nM)	*E* (%)	*r* (Å)
Cy3-HNF4α + Cy5-L-FABP	250 ± 50	16 ± 2	70 ± 10	300 ± 150	6 ± 2	80 ± 20
Cy3-β-galactosidase + Cy5-L-FABP	>10000	<1	≫100	>10000	<1	≫100