Rescue of MODY-1 by Agonist Ligands of Hepatocyte Nuclear Factor-4α*

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Missense mutations of the ligand binding domain of hepatocyte nuclear factor (HNF)-4α result in maturity onset diabetes of the young (MODY)-1. We show here that MODY-1 as well as Gn-185 missense mutants of the ligand binding domain of HNF-4α fail to transactivate transcription of HNF-4α-responsive genes. Defective transactivation by these mutants is accounted for by their reduced binding affinities for fatty acyl agonist ligands of HNF-4α. These mutants may be rescued by exogenous fatty acid agonist ligands of HNF-4α, yielding transcriptional activities in the wild type range. The effect of added ligands is synergistic with that of transcriptional coactivators of HNF-4α. These findings may indicate the means for treating selected MODY-1 subjects with HNF-4α agonist nutrients and drugs.

HNF-4α† is a member of the superfamily of nuclear receptors (1, 2). Its DNA binding domain (DBD) (amino acids 50–116) consists of two folded zinc-finger motifs and binds as homodimer to DR-1 response elements/enhancers of HNF-4α-responsive gene promoters. Its ligand binding domain (LBD) (amino acids 132–370) is closely homologous with that of RXRα and harbors a ligand binding site, dimerization domain, and a hydrophobic AF-2 transactivation domain (amino acids 360–370). HNF-4α-responsive genes encode some transcription factors (e.g. HNF-1α, pregnane-X-receptor (PXR)), enzymes, and proteins involved in fatty acid, lipoproteins, and lipid metabolism (e.g. apoA-I, -A-II, -B, -C-II, -C-III, microsomal triglyceride transfer protein), carbohydrate metabolism (e.g. insulin, GLUT2, phosphoenolpyruvate carboxykinase, pyruvate kinase), amino acid and protein metabolism, hematopoiesis, blood coagulation, and others (2–8).

Mutations in HNF-4α result in maturity onset diabetes of the young (MODY)-1. These consist of nonsense or frameshift mutations yielding truncated functionless proteins (e.g. Q66X, R154X) as well as missense mutations in the hinge region (e.g. R127W), ligand binding domain (e.g. V255M, E276Q, R324H), or the F-domain (e.g. V393I) that result in variable loss of transcriptional activity (9–16) (for review, see Ref. 17). Failure to activate transcription by missense MODY-1 mutants is ascribed to impaired dimerization, DNA binding, recruitment of coactivators, or protein destabilization (11–14). MODY-1 is characterized by early onset, autosomal dominant inheritance of impaired pancreatic insulin secretion (4, 18), in line with the direct (HNF-4α/DR-1-mediated (19)) and indirect (HNF-1α-mediated (7)) modes of transcriptional activation of the insulin gene promoter by HNF-4α. Concomitantly, MODY-1 patients are hypolipidemic (6), in line with the profile of liver HNF-4α-responsive genes coding for apolipoproteins (e.g. apoB, C-III, A-II) and their assembling proteins (e.g. microsomal triglyceride transfer protein) (20).

Until recently, HNF-4α was considered to be an orphan receptor. Long chain fatty acyl-coenzyme A (CoA) as well as CoA thioesters of xenobiatic amphiphatic carboxylates (e.g. hypolipidemic fibrate drugs or substituted dioic acids) have now been reported to specifically bind to HNF-4α (21–23). Binding affinities of CoA thioesters, as verified by direct fluorescence methods, are in the 1.0–4.0 μM range, whereas the respective free acids bind with significantly lower affinities (23). Binding of amphiphatic carboxylates to HNF-4α has been recently complemented by the x-ray crystal structure of recombinant HNF-4α-LBD reported to consist of long chain fatty acids in the ligand binding pocket (24, 25). HNF-4α ligands modulate its secondary structure and its transcriptional activity as a function of their chain length, degree of unsaturation, or respective substitutions (21, 22). Thus, fatty acids of C14–C16 activate, whereas long chain (ω-3) polyunsaturated fatty acids (e.g. C20:5, C22:6) and hypolipidemic fibrate or Medica drugs suppress HNF-4α transcriptional activity in transfection assays. Activation/inhibition of HNF-4α transcriptional activity by its acyl-CoA ligands or by its free acid proligands is due to modulating its binding affinity to its DNA cognate enhancers (21, 26) and/or shifting the equilibrium between active HNF-4α dimers and inactive oligomers (21) and/or modulating its transactivation capacity (21, 22). In analogy with other nuclear receptors, transactivation of HNF-4α is mediated by protein coactivators (e.g. CBP (cAMP-response element-binding protein (CREB)-binding protein)/P300, PGC-1, and others) (27–30).

Missense mutants of the ligand binding domain of HNF-4α are shown here to be defective in transactivation capacity due to their reduced binding affinities for HNF-4α agonist ligands. The mutants are rescued by excess agonist ligands, reaching activities in the wild type range. These findings may indicate the means for treating selected MODY-1 subjects with HNF-4α agonist nutrients or drugs.

EXPERIMENTAL PROCEDURES

Plasmids and Reagents—The E276Q, V255M, Q185A, Q185K, and C179W mutants of HNF-4α were prepared by the Kunkel method (31) or by the Stratagene mutagenesis kit (La Jolla, CA). The full-length

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§ The abbreviations used are: HNF, hepatocyte nuclear factor; rHNF, rat HNF; hHNF, human HNF; DBD, DNA binding domain; LBD, ligand binding domain; CAT, chloramphenicol acetyltransferase; WT, wild type; NBD-stearate, 12-(N-methyl)-N-((7-nitrobenz-2-oxa-1,3-diazol-4-ylamino)-octadecanoic acid.

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22578
HNF-4α wild type and its respective mutants were cloned in pSG5 vector. GAL4-DBD-HNF-4α-LBD chimera, consisting of the DBD of GAL4 and the LBD (amino acids 132–410) of HNF-4α wild type or respective mutants, were prepared by fusing respective rat or human HNF-4α-LBD to the C-terminal end of yeast GAL4-DBD. CAT reporter plasmids for the full-length HNF-4α consisted of the (~854+/−22) apoC-III gene promoter or 3 copies of the C3P enhancer of the apoC-III gene promoter upstream of the thymidine kinase promoter (32). CAT reporter plasmid for the GAL4-DBD-HNF-4α-LBD chimera (UAS)-CAT consisted of 5 copies of the GAL4 binding site upstream of the adenovirus E1b promoter. Expression plasmid for P300 was from R. Eckner (Zurich, Switzerland). PGC-1 was prepared by reverse transcription-PCR from liver total RNA of 24 h-starved mice (30) and cloned into an expression vector pEDNA3. Rat HNF-4α-LBD (132–455) recombinant wild type and mutant proteins consisting of amino acids 132–455 were prepared by expressing the respective rHNF-4α cDNA synthesized with PCR by the 5’-CCGCGTCGAGGATGGCTTCCT-3’ and 5’-GGCCCATATGAGGTCAAGCTACGAG-3’ primers. The respective DNA fragments were cloned into the NdeI/XhoI site of pET21b plasmid (Novagen, Milwaukee, WI) and sequenced. The recombinants were expressed in BL21(DE3)pLyS strain of Escherichia coli, and the His-tagged proteins were purified by affinity chromatography on a nickel nitrilotriacetic resin (Qiagen, Chatsworth, CA), desalted, lyophilized, and stored at −70 °C. Purity of recombinants was assessed by SDS-PAGE and Western blotting.

**Transient Transfection**—Cultured COS7, HeLa, Huh7, HepG2, and C. Purity of recombinants was assessed by SDS-PAGE and Western blotting.

**Ligand Binding**—Binding of fatty acyl-CoA to recombinant HNF-4α-LBD (132–455) wild type or missense mutants was measured by the extent of quenching of the intrinsic emission fluorescence of tryptophan. Methyl esters were identified by their respective retention time, molecular ion, and fragmentation pattern based on known standards.

**RESULTS**

Transcriptional activation by wild type HNF-4α in transfection assays in the absence of added exogenous ligands prompted us to search for putative ligand-dependent transactivation by making use of transactivation-defective mutants of HNF-4α. MODY-1 E276Q (11–13, 34) and V255M (12, 13, 35) mutations in helices 7 and 8 of HNF-4α-LBD, respectively, offered natural examples, whereas other mutations were designed by analyzing conserved HNF-4α sequences as well as the close sequence homology between the LBDs of HNF-4αs and RXRa. Gln-185 and Cys-179 of helix 3 were selected for site-directed mutagenesis of HNF-4α-LBD in light of the strictly conserved VC179FSMKEQ185 sequence of HNF-4α from Droso- phila to humans and in light of Gln-185 homology with Gln-280 of RXRs. Gln-280 of RXRa is intimately associated with the bound oleic acid in the ligand binding pocket of RXRa (36, 37, 39). To evaluate the transactivation capacity of HNF-4α wild type and its mutants, the respective HNF-4α-LBDs were fused with GAL4-DBD, and the transactivational activity of the expressed chimera was analyzed in COS7 cells transfected with a reporter plasmid consisting of GAL4-responsive promoter upstream of CAT. Transactivation was robustly increased by the HNF-4α-LBD(WT) chimera while being significantly decreased or essentially zero with the human or rat HNF-4α-LBD(E276Q) mutants compared with their respective wild types (Fig. 1a).

The pronounced loss of transactivation of the rat HNF-4α-LBD(E276Q) chimera as compared with the respective human mutant indicated that transactivation-defective MODY-1 mutations were better exemplified in the rat HNF-4α-LBD context. In line with previous reports, the extent of loss of transactivation of the HNF-4α-LBD(E276Q) mutant was dependent on the cell type used for transfection (11, 12). Thus, transactivation by the GAL4-HNF-4α-LBD(E276Q) mutant was similar to that of wild type HNF-4α-LBD in HepG2 (not shown) but robustly impaired in COS7 (Fig. 1, a and b), Huh7 (Fig. 1b), or HepG2 (not shown) cells. In line with the previous report (12), transactivation was less affected by the conservative V255M than the nonconservative E276Q MODY-1 mutation (Fig. 1a). Similarly to MODY-1 mutants, transactivation by the rHNF-4α-LBD(Q185A), rHNF-4α-LBD(Q185K), and rHNF-4α-LBD(C179W) chimera was robustly decreased or essentially zero as compared with rHNF-4α-LBD(WT) (Fig. 1a).

Loss of transactivation by the MODY-1 and other missense HNF-4α(LBD) mutants prompted us to attempt their rescue by HNF-4α agonist ligands. Myristic (C14:0) and palmitic (C16:0) acids were used in light of their reported efficacies in transactivating wild type HNF-4α (22). 9-cis-Hexadecenoic acid (C16:1) was selected in light of being the predominant fatty acid bound to recombinant HNF-4α-LBD(WT) expressed in E. coli and purified to homogeneity (24, 25). The transactivation-defective human HNF-4α-LBD(E276Q) mutant was indeed rescued by C14:0 or C16:1 added to the culture medium, resulting in extending its transactivation capacity to wild type levels both in COS7 and Huh7 cells (Fig. 1b). Also, the E276Q mutation expressed in the rat HNF-4α-LBD context was dose-dependently rescued by C14:0, C16:0, or C16:1, resulting in increasing its transactivation by 15–20-fold within the 0–150 μM concentration range of added fatty acid (Fig. 1c).

Similarly, the E276Q, V255M, Qu185K, and Qu185A (but not the C179W) mutations expressed in the rat HNF-4α-LBD context were effectively rescued by added C16:1 (Fig. 1d). The extent of
activation by added ligand (Fig. 1d) was inversely proportional to the basal transactivation of respective mutants (Fig. 1a), being more evident with the highly impaired E276Q as compared with the highly defective Q185K as compared with the Q185A mutant. Similarly, ligand activation of the wild type chimera by HNF-4α/9251 agonist ligands amounted to 2-fold only as compared with 5–25-fold activation of the MODY-1 and Gln-185 mutants (Fig. 1d).

To further investigate the mode of ligand-dependent rescue of HNF-4α mutants, the combined effect of ligand and transcriptional coactivators of HNF-4α was analyzed in cells transfected with respective GAL4-DBD-HNF-4α-LBD chimera and cotransfected with P300. Activation of GAL4-HNF-4α-LBD chimera by P300 in the absence of added ligand was limited and proportional to their basal transactivation capacity, being maximal with wild type HNF-4α and essentially zero with the highly defective Q185K mutant. However, the addition of C16:1 resulted in robust activation of the MODY-1 and Gln-185 (but not the C179W) mutants, reaching 50–200-fold activation in the presence of both C16:1 and P300 (Fig. 1d). Hence, the full scope of ligand-dependent rescue of HNF-4α mutants requires the synergistic activation by ligand and coactivator. Transcriptional transactivation mediated by the constitutive N-terminal AF-1 domain of HNF-4α(1–22) (38) fused to GAL4-DBD was unaffected by C16:1 (not shown), thus indicating that the synergistic interaction between ligand and AF-2 coactivators was specific.

Rescue by ligand in the context of the full-length HNF-4α and an HNF-4α-responsive gene was verified in COS7 cells transfected with expression plasmids for the full-length wild type rHNF-4α variant and with a CAT reporter plasmid consisting of either the apoC-III gene promoter (Fig. 2a) or the HNF-4α cognate C3P enhancer of the apoC-III gene promoter upstream of the thymidine kinase promoter (32) (Fig. 2b). Transcriptional activation of the apoC-III promoter by the full-length wild type rHNF-4α was apparently constitutive and essentially independent of added C16:1 both in the absence or presence of transfected P300 (Fig. 2a). However, transcriptional activation of the apoC-III promoter by the full-length human HNF-4α(E276Q) MODY-1 mutant was essentially zero in the absence of C16:1 but increased to wild type levels in the presence of both C16:1 and P300 (Fig. 2a). Similarly, defective transcription of the apoC-III promoter by the full-length human HNF-4α(E276Q) mutant was increased to wild type levels by added C16:1 even in the absence of transfected P300 (Fig. 2a). Also, null transcriptional activation of the C3P-enhanced heterologous promoter by the transactivation-defective rHNF-4α(E276Q)
MODY-1 or rHNF-4α(H9251)(Q185K) mutants reached wild type levels in the presence of C16:1 and P300, acting in concert (Fig. 2b). These data indicated that missense mutants of HNF-4α/H9251 could be rescued by ligand acting synergistically with P300. The rescue specificity of agonist and antagonist ligands of HNF-4α/H9251 was verified in transfected COS7 cells incubated in the presence of the respective HNF-4α ligands (21–23). The rHNF-4α(E276Q) MODY-1 mutant was rescued by C16:1 and P300 (0.5 μg) and rHNF-4α(E276Q) (0.02 μg) transfected to COS7 cells with a CAT reporter plasmid comprising the apoC-III gene promoter (5.0 μg). Fold activation is relative to that of pSG5 presented as 1.0. *, significant as compared with the respective plasmids in the absence of added ligand. p < 0.05.

FIG. 2. Rescue of full-length HNF-4α missense mutants by agonist proligands of HNF-4α. Cells were transiently transfected as described under “Experimental Procedures” with the indicated reporter plasmids and with expression plasmids for full-length HNF-4α and transcriptional coactivators and were further incubated in the presence of the indicated HNF-4α proligands. a, activation by C16:1 and P300 (0.5 μg) of hHNF-4α(E276Q) (0.01 μg) and rHNF-4α(E276Q) (0.02 μg) transfected to COS7 cells with a CAT reporter plasmid comprising the apoC-III gene promoter (5.0 μg). Fold activation is relative to that of pSG5 presented as 1.0. b, activation by C16:1 and P300 (0.5 μg) of rHNF-4α(E276Q) (0.04 μg) and rHNF-4α(Q185K) (0.04 μg) mutants transfected to COS7 cells with a CAT reporter plasmid comprising the C3P enhancer of the apoC-III gene promoter upstream of the thymidine kinase promoter (3.0 μg). Fold activation is relative to that of pSG5 presented as 1.0. c, specificity of HNF-4α proligands in rescuing rHNF-4α(E276Q) (0.02 μg) transfected to COS7 cells with P300 (0.5 μg) and a reporter plasmid comprising the apoC-III gene promoter (5.0 μg). Fold activation is relative to that of HNF-4α(WT) (0.02 μg) in the absence of ligands, presented as 1.0. d, rescue by HNF-4α agonist proligands of HNF-4α(E276Q) (0.02 μg) transfected to COS7 cells with PGC-1 (0.1 μg) and the apoC-III/CAT reporter plasmid (5.0 μg). Fold activation is relative to that of pSG5 presented as 1.0.

MODY-1 or rHNF-4α(Q185K) mutants reached wild type levels in the presence of C16:1 and P300, acting in concert (Fig. 2b). These data indicated that missense mutants of HNF-4α could be rescued by ligand acting synergistically with P300.

The rescue specificity of agonist and antagonist ligands of HNF-4α was verified in transfected COS7 cells incubated in the presence of the respective HNF-4α ligands (21–23). The rHNF-4α(E276Q) MODY-1 mutant was rescued by C16:1 and C14:0 but not by C20:5(ω-3), C22:6(ω-3), or xenobiotic HNF-4α antagonists (e.g., Medica 16(22)) (Fig. 2c), thus indicating that ligand-dependent rescue was specific for agonist ligands of HNF-4α. The coactivator specificity was investigated by replacing P300 with PGC-1 (30). In contrast to wild type HNF-4α, the E276Q MODY-1 mutant was nonresponsive to PGC-1 in the absence of added ligand (Fig. 2d) but was rescued by an agonist ligand and PGC-1 acting in concert, thus indicating that ligand-dependent rescue of HNF-4α missense mutants was essentially not dependent on the specific transcriptional coactivator in use.

Activation of transcriptional missense mutants of HNF-4α by its agonist ligands, as contrasted with the apparent constitutive nature of wild type HNF-4α, has suggested that in contrast to wild type HNF-4α, having ligand binding affinities in the range of endogenous ligand concentrations (23), the mutants could be putatively defective in binding of endogenous ligands, thus becoming dependent on added exogenous ligand. The respective ligand binding affinities of wild type HNF-4α and its missene mutants were measured by quenching of the tryptophan intrinsic emission fluorescence of respective HNF-
4α-LBD recombinant proteins by increasing concentrations of C14:0-CoA (23). The highest binding affinity was observed with wild type HNF-4α, which has a $K_d$ for C14:0-CoA of 0.9 nM (Fig. 3, a and f). The binding affinities of the MODY-1 missense mutants E276Q and V255M as well as of the transcriptional-defective Q185K mutant were robustly decreased, with $K_d$ values for C14:0-CoA of 25.4, 15.5, and 16.9 nM, respectively (Fig. 3, b–f). The $K_d$ for C14:0-CoA of the non-rescued C179W mutant amounted to 132 nM, 150-fold higher as compared with wild type HNF-4α-LBD and 5–10-fold higher than missense HNF-4α mutants rescued by added ligand. The decreased ligand binding affinities of the MODY-1, Gln-185, and Cys-179 mutants were not correlated with their water accessibility, as deduced from their respective intrinsic fluorescence intensities ($F_0$) (Fig. 3f).

The ligand binding affinities of missense HNF-4α mutants were further evaluated by the increase in fluorescence intensity upon binding of NBD-stearate (23). This method, based on the marked increase in NBD-stearate fluorescence when moving from the aqueous buffer to the hydrophobic HNF-4α-LBD pocket, allows for measuring ligand binding independently of quenching of the aromatic amino acids of HNF-4α as well as yielding relative binding data for the low affinity free acid ligands of HNF-4α (23). Differences in ligand binding were quantified by reverse titration of a constant amount of NBD-stearate with increasing concentrations of HNF-4α-LBD recombinant proteins (Fig. 4). Reverse titration of wild type HNF-4α-LBD exhibited a sigmoid binding curve with $K_d$ of 0.13 μM for NBD-stearate (Fig. 4, a and f). Binding curve sigmoid convolution was lower but still maintained for the E276Q (Fig. 4b), V255M (Fig. 4c), and Q185K (Fig. 4d) mutants, with $K_d$ values of 1.3, 1.7, and 1.1 μM, respectively (Fig. 4f). The C179W mutant, which could not be rescued by exogenous ligand in transfection assays, yielded a $K_d$ of 11.7 μM and showed an essentially nonsaturable binding curve (Fig. 4, e and f). The strong sigmoid convolution of wild type HNF-4α-LBD in reverse titration as reflected by its n value of 1.5 (Fig. 4f) may indicate low ligand binding affinity of HNF-4α-LBD monomers, but an increased ligand affinity of dimers progressively generated at higher protein concentrations. The loss of sigmoid convolution of the MODY-1 and C179W mutants, with concomitant loss of ligand binding affinity may indicate that high affinity binding of ligand may require homodimerization.

The functional relevance of the fatty acid entrapped in the ligand binding pocket of recombinant HNF-4α-LBD (24, 25) to transcriptional transactivation by HNF-4α variants was eval-

![Figure 3](http://www.jbc.org/)
uated by analyzing the fatty acid content and composition of recombinant HNF-4α-LBD wild type and respective missense mutants. The total entrapped fatty acids amounted to 0.63 ± 0.05, 0.58 ± 0.14, 0.58 ± 0.06, 0.61 ± 0.04, and 0.64 ± 0.05 (n = 3) mol of fatty acid/mol of HNF-4α-LBD protein for the wild type, E276Q, V255M, Q185K, and C179W HNF-4α variants, respectively, thus indicating that the entrapped fatty acid content is similar for all HNF-4α variants and may, therefore, not account for the robust differences in the transcriptional activity between wild type HNF-4α and its missense mutants. However, the fatty acid composition of HNF-4α missense mutants distinctly differed from that of wild type HNF-4α (Fig. 5). Thus, despite being hardly detectable in bacterial extracts, C16:1 consisted of 60% of the total entrapped fatty acids of wild type HNF-4α-LBD, thus reflecting the specific avidity of the wild type protein for this fatty acid. In line with the robust decrease in ligand binding affinities of HNF-4α missense mutants (Figs. 3 and 4), their entrapped fatty acids consisted of decreased content of C16:1, replaced by the core long chain fatty acids prevailing in bacterial extracts.

**DISCUSSION**

MODY-1 missense mutants of the ligand binding domain of HNF-4α are shown here to be pronouncedly defective in their binding affinities for the high affinity acyl-CoA and the low affinity free acid ligands of HNF-4α (Figs. 3 and 4). In line with their reduced binding affinities for HNF-4α agonist ligands, MODY-1 missense mutants of the ligand binding domain of HNF-4α fail to transactivate transcription of a GAL4 reporter.
plasmid (Fig. 1) or of HNF-4α-responsive genes (e.g. apoC-III) (Fig. 2). The MODY-1 mutants may be rescued by exogenous agonist fatty acids, yielding transcriptional transactivation activities in the wild type range (Figs. 1 and 2). Rescue by agonist fatty acids is synergized by HNF-4α coactivators (e.g. P300, PGC-1), thus indicating that agonist acyl-CoA ligands of HNF-4α may modulate its affinity for cognate transcriptional coactivator/corepressor proteins. Given the variable availability of endogenous fatty acyl-CoAs and transcriptional coactivators/corepressors in different cell types, the strict dependence of the MODY-1 mutants on added ligands may account for the previously inconsistent evidence for defective transactivation by MODY-1 mutants in transfection studies (12, 13).

MODY-1 missense mutants were complemented here by mutants generated by site-directed mutagenesis of Gln-185 of HNF-4α-LBD. In light of Gln-185 homology with Gln-280 of RXRα and because the latter stabilizes the carbonyl function of the oleic acid ligand of RXRα (36, 37), Gln-185 was expected to be intimately associated with ligand in the ligand binding pocket of HNF-4α, and Gln-185 mutants were expected to yield HNF-4α mutants defective in ligand binding. This prediction has indeed been confirmed by the recently reported x-ray crystal structure of HNF-4α showing pairwise of HNF-4α(Gln-185) with the carbonyl head group of the fatty acid bound in the LBD of the recombinant protein (25). Indeed, similarly to MODY-1 missense mutants, the Gln-185 mutants were shown here to have decreased affinities for HNF-4α ligands, leading to defective transactivation. Also, similar to MODY-1 mutants, the Gln-185 mutants were rescued by exogenous agonist ligands of HNF-4α. Hence, Gln-185 mutants of HNF-4α may represent artificial MODY-1-like mutants.

In line with our previous findings (21–23), both the wild type and missense mutants of HNF-4α have significantly higher affinities for acyl-CoAs as compared with the respective free acids (Figs. 3 and 4), thus indicating that acyl-CoAs, generated by ATP-dependent CoA-thioesterification of added agonist fatty acids, serve as the preferred ligands of HNF-4α. The 100-fold higher affinity for acyl-CoAs does not exclude, however, binding of the respective free acids if available in μM concentrations. However, the functional relevance of the bound free fatty acids as compared with respective acyl-CoA ligands of HNF-4α still remains to be investigated. Their functional role may not be deduced by the content, composition, and apparent inextractability of the fatty acids reported to occupy the ligand binding pocket of recombinant HNF-4α (Refs. 24 and 25; Fig. 5) in light of the following. (a) The content of the entrapped fatty acids of the inactive missense HNF-4α mutants is similar to that of wild type HNF-4α (Fig. 5), thus indicating that fatty acids occupancy per se is not causal in generating an active conformation and may, therefore, not account for the basal activity of wild type HNF-4α in transfection assays. HNF-4α offers a mirror image example to RXRα where both the wild type RXRα, which depends on 9-cis-retinoic acid for its activity as well as the constitutive RXRα(R318A) mutant, copurify with strongly bound fatty acid in their ligand binding pockets (36, 37, 39). Hence, the entrapped fatty acid is not the key to constitutive nature of HNF-4α or RXRα. (b) The composition of the entrapped fatty acids of transcriptionally active and inactive HNF-4α variants is not correlated with the rescue capacity of respective fatty acids. Indeed, missense mutants of HNF-4α are similarly rescued by C14:0, C16:0, or C16:1 (Fig. 1c), thus indicating that the C16:1 prevalence in recombinant wild type HNF-4α reflects the avidity of the wild type protein for this fatty acid rather than C16:1 being causal to the basal activity of wild type HNF-4α. Also, the entrapped fatty acid composition of the nonrescuable C179W mutant is similar to that of resceivable MODY-1 or Gln-185 HNF-4α mutants, thus indicating that rescue of HNF-4α missense variants in transfection assays does not correlate with their fatty acid composition but reflects their binding affinities for an agonist HNF-4α ligand. The binding affinity for acetyl-CoA of the nonrescuable HNF-4α(C179W) mutant is indeed 150- and 10-fold lower as compared with wild type HNF-4α or rescevable missense mutants, respectively. (c) The apparent inextractability of the entrapped fatty acids of recombinant wild type HNF-4α (24) does not preclude its displacement by high affinity acyl-CoA ligands of HNF-4α. Indeed, displacement of the copurified fatty acid was attempted by exchanging it with free fatty acid (24) but not with respective acyl-CoAs, having 100-fold higher affinity as compared with free acids (23). The HNF-4α case is similar in this respect to its homologous RXRα receptor, where its strongly bound oleate, which resists being dialyzed out throughout the extensive purification steps prior to crystallization (36, 37, 39), may be readily exchanged with the high affinity ligands of RXRα like 9-cis-retinoic acid, but not with oleate itself (40). That is in line with the 1000-fold higher affinity of 9-cis-retinoic acid in transactivating RXRα as compared with oleate (40, 41).

The high affinity of wild type HNF-4α for ligand as contrasted with the MODY-1 and Gln-185 mutants may indicate that the apparent constitutive nature of wild type HNF-4α in transfection studies may reflect its saturation with an endogenous agonist ligand in transfected cells. Saturation of wild type HNF-4α by an endogenous ligand may also explain the low activation of wild type HNF-4α in transfection assays by added exogenous agonist fatty acids as contrasted with robust activation of the binding-defective missense HNF-4α mutants. This view implies that transcriptional modulation by an HNF-4α agonist ligand may reflect its capacity as compared with that of the displaced endogenously bound ligand. Similarly, suppression by HNF-4α natural or xenobiotic antagonists (Refs. 21 and 22; Fig. 2c) may result from both displacement of an endogenously bound agonist complemented by direct suppression of transactivation by bound antagonist ligand. This view may further indicate the means for treating selected MODY-1 subjects by excess HNF-4α agonist ligands or by selective drugs designed to restore transcriptional activity by protein-ligand complementation (42, 43).

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