Ligand Selectivity of the Peroxisome Proliferator-Activated Receptor α†

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ABSTRACT: Peroxisome proliferator-activated receptors (PPAR α, *β*, and *γ*) are nuclear hormone receptors that play critical roles in regulating lipid metabolism. It is well established that PPARs are the targets for the hypolipidemic synthetic compounds known as peroxisome proliferators, and it has been proposed that various long-chain fatty acids and metabolites of arachidonic acid serve as the physiological ligands that activate these receptors in vivo. However, a persistent problem is that reported values of the equilibrium dissociation constants (K_ds) of complexes of PPARs with these ligands are in the micromolar range, at least an order of magnitude higher than the physiological concentrations of the ligands. Thus, the identity of the endogenous ligands for PPAR remains unclear. Here we report on a fluorescence-based method for investigating the interactions of PPAR with ligands. It is shown that the synthetic fluorescent long-chain fatty acid *trans*-parinaric acid binds to PPARα with high affinity and can be used as a probe to monitor protein−ligand interactions by the receptor. Measurements of K_ds characterizing the interactions of PPARα with various ligands revealed that PPARα interacts with unsaturated C:18 fatty acids, with arachidonic acid, and with the leukotriene LTB4 with affinities in the nanomolar range. These data demonstrate the utility of the optical method in examining the ligand-selectivity of PPARs, and resolve a long-standing uncertainty in understanding how the activities of these receptors are regulated in vivo.

Peroxisome proliferator-activated receptors (PPAR α, *β*, and *γ*) are ligand-inducible transcription factors that belong to the superfamily of nuclear hormone receptors. PPARs seem to be involved in regulation of a variety of biological processes including glucose homeostasis, macrophage activation (*1*), and differentiation of keratinocytes (*2*) and macrophages (*3*). However, the best characterized function of these receptors is the regulation of expression of genes that are involved in lipid storage and catabolism and in adipocyte differentiation (*4*−*6*; for review, see *7*). PPARα up-regulates the expression of genes that encode for enzymes involved in *β*-oxidation of fatty acids, and in proliferation of peroxisomes (*8*−*10*), while PPAR*γ* plays an important role in lipid storage and in adipocyte differentiation (*4*−*6*). Like other members of the superfamily of nuclear hormone receptors, PPARs contain a DNA-binding domain that serves to recognize particular DNA response elements in the promoter region of their target genes (PPREs, *11*). Also like other members of this family such as the retinoic acid-, the thyroid hormone-, and the vitamin D-receptors, PPARs bind to DNA and regulate transcription as heterodimers with the retinoid X receptor (RXR, *12*).

Activation of most nuclear receptors, including PPARs, is initiated by lipophilic ligands that bind to a ligand-binding site in their carboxyl-terminal region, termed the ligand-binding domain (LBD). Initial efforts to delineate the mechanism of action of hypolipidemic drugs such as fibrates and Wy 14 653 on peroxisome proliferation demonstrated that these xenobiotics act by activating PPARα (*13*, *14*). A search aimed at identifying physiological ligands revealed that PPARα is activated by a variety of long-chain fatty acids, and in particular polyunsaturated fatty acids (*12*, *15*). However, because the concentrations of fatty acids required for efficient activation were super-physiological and it proved difficult to demonstrate that fatty acids directly bind to PPARs, it was repeatedly suggested that the physiological ligands for these receptors are metabolites of fatty acids rather than the fatty acids themselves (e.g., *12*). Indeed, it was subsequently reported that the arachidonic acid metabolite 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ2) can activate and directly bind to PPAR*γ* (*16*), and that the inflammation mediator leukotriene B_4 (LTB4) is a ligand for PPARα (*17*).

Examination of the ligand specificities of PPARs is complicated by the poor water solubilities of the ligands and by the need to physically separate between protein-bound and -free ligands for measurement of equilibrium dissociation constants (K_d). In traditional binding assays, such separation is achieved by size-exclusion chromatography or by chromatography using activated charcoal. As lipophilic ligands tend to adhere to such matrixes, these methods may lead to equilibrium shifts and to apparent K_ds that are deceivingly high. An additional technical difficulty is the limited availability of radioactively labeled compounds that are required for K_d measurements utilizing the classical binding assays. Recent attempts to overcome these limitations led to the development of indirect binding assays for examining the ligand selectivities of PPARs. One such assay, termed the coactivator-dependent receptor ligand assay (CARLA), took advantage of the observation that ligand binding induces interactions of PPARs with transcriptional coactivators such as SRC-1 (*18*). Thus, the ability of PPAR to associate with

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SRC-1 in the presence of a variety of potential PPAR ligands was used as a "read-out" for ligand binding by the receptors. Data obtained from this study indicated that all three isoforms of PPAR bind several fatty acids, and confirmed that 15d-PGJ2 and LTB4 are ligands for PPAR γ and α, respectively (*19*). These observations were further substantiated by another study in which ligand binding was monitored by an assay that relied on the ability of PPAR ligands to enhance dimer formation between PPARs and RXR and DNA binding by the resulting heterodimer (*20*).

Hence, available information suggests that PPARs are promiscuous nuclear receptors with binding sites that are able to accommodate a wide spectrum of compounds including long-chain fatty acids, metabolites of arachidonic acid, and derivatives of synthetic hypolipidemic drugs. However, a serious difficulty in accepting this interpretation is that the reported values of the K_ds characterizing the formation of ligand−protein complexes of PPARs are in the range of 10−50 μM, which is orders of magnitude higher than the likely in vivo concentrations of the ligands (see Discussion). Here we report on the development of a fluorescence-based method for studying the ligand specificity of PPAR. This method has the advantage that it does not require physical separation between free and bound ligand, nor the availability of radioactively labeled ligand. Unlike the indirect methods described above, this assay reports on the equilibrium situation without the need to perturb this equilibrium by chromatography or by electrophoresis steps. Fluorescence-based methods have been extensively used for monitoring the association of proteins with hydrophobic ligands, especially with retinoids (e.g., *21*−*23*). These studies were aided by the fact that retinoids display absorption spectra that overlap with the fluorescence emission spectra of proteins and, thus, that binding of a retinoid to a protein is often accompanied by a decrease in the protein's intrinsic fluorescence. Additionally, some retinoids are efficient fluorophores, and their fluorescence is enhanced upon movement into a hydrophobic environment within a protein binding site. Unlike retinoids, none of the putative PPAR ligands displays useful optical properties. To overcome this limitation, we utilized a synthetic fluorescent long-chain fatty acid, parinaric acid, as a probe. It was found that parinaric acid binds to PPARα with a high affinity, and that its fluorescence is significantly enhanced upon binding to the protein. The K_d for association of parinaric acid with the receptor could thus be measured by following the change in the fluorescence of the probe upon its addition to the protein. K_ds for binding of other ligands were obtained by monitoring their ability to compete with the probe for binding. Screening of potential ligands for PPARα by utilizing this assay revealed that the receptor possesses a much stronger binding affinity for these compounds than previously reported. This work thus provides a straightforward method for assessing the ligand-selectivity of PPARs, indicates that, indeed, physiologically occurring fatty acids can directly bind to these proteins at physiological concentrations, and helps to resolve a long-standing problem in understanding the regulatory features of these important nuclear receptors.

EXPERIMENTAL PROCEDURES

Materials. cDNA of mPPARα was provided by Steven Kliewer (Glaxo Wellcome). A mRXR$\alpha\Delta$AB bacterial expression vector was obtained from Hinrich Gronemeyer and Pierre Chambon (IGBMC, Strasbourg). Antibodies against PPARα were purchased from Affinity Bioreagent Inc. *trans*-Parinaric acid was obtained from Molecular Probes Inc. (Eugene, OR). Oligonucleotides were synthesized and DNA constructs were sequenced at the Cornell University Biotechnology facility.

Proteins. The cDNA encoding mPPARα with a deletion of the amino-terminal A/B domain (amino acids 101−468) was cloned into pET28a vector, a $(His)_6$-tagged bacterial-expression vector. Bacteria containing pET-PPAR$\alpha\Delta$AB were cultured to OD_{600} 0.8−1.0 at 37 °C. The expression of the recombinant PPAR$\alpha\Delta$AB protein was induced by addition of 0.5 mM IPTG for 2−3 h. Purification of the recombinant protein was performed as described previously (*24*, *25*). The purity of the resulting protein was assessed by SDS−PAGE followed by Coomassie blue staining and by immunoblotting using an anti-PPARα antibody (Affinity Bioreagent Inc.). Expression and purification of recombinant mRXR$\alpha\Delta$AB were carried out as previously described (*24*, *25*). Purified proteins were dialyzed against a buffer containing 10 mM Hepes (pH 8.0), 0.1 mM EDTA, 0.4 mM DTT, 400 mM KCl, and 5% glycerol, and stored at −20 °C in 50% glycerol. Protein concentrations were determined by the Bradford method (BioRad).

Ligand-Binding Assays. Binding of parinaric acid to mPPAR$\alpha\Delta$AB was monitored by measuring the fluorescence of the probe upon titration of the protein. Protein (1 μM in a buffer containing 10 mM Hepes, pH 8.0, 0.1 mM EDTA, 0.4 mM DTT, 400 mM KCl, and 5% glycerol; final volume 1 mL) was placed in a cuvette and parinaric acid was added from a concentrated solution in ethanol. Fluorescence (excitation, 305 nm, emission, 413 nm) was measured after each addition using a SPEX Fluorolog-2 spectrofluorometer. The total ethanol concentration never exceeded 1%. Titration curves were analyzed to obtain the number of binding sites and apparent K_d by fitting the data to an equation derived from simple binding theory (*36*). Data analysis was performed using Origin software (MicroCal Inc.). To assess the K_ds for the different ligands, mPPAR$\alpha\Delta$AB (1 μM) was mixed with parinaric acid at a mole ratio corresponding to the number of binding sites obtained from the titrations described above. The holoprotein was titrated with the appropriate ligand from a concentrated solution in ethanol, and probe fluorescence was measured following each addition. Titrations were carried out until a plateau, indicating saturation with the ligand, was achieved. K_ds were estimated by the relationship $EC_{50ligand}/[\text{parinaric acid}] = K_{d,ligand}/K_{d,\text{parinaric acid}}$.

Electrophoretic mobility shift assays were carried out as previously described (*26*) using a ^{32}P-labeled double-strand oligonucleotide containing the PPRE 5′-TCGACGGA-CAAAGGTCACGTTGGAT-3′.

RESULTS

In this study, mPPARα lacking the N-terminal A/B domain (mPPAR$\alpha\Delta$AB) was used. This construct, which contains the complete ligand-binding domain of the protein and is expected to show ligand-binding properties identical to those of the full-length receptor (*16*, *31*), was obtained by

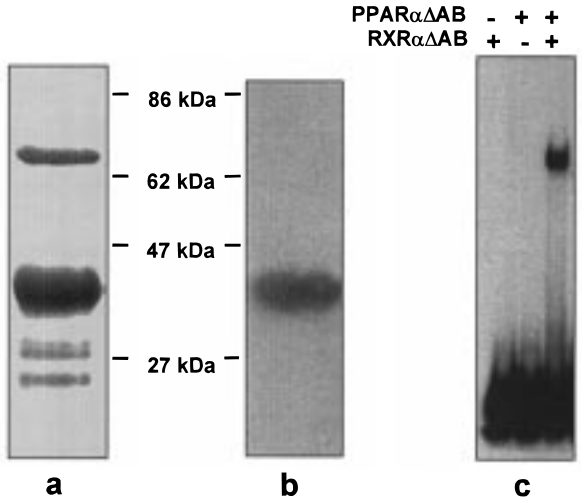

FIGURE 1: Bacterially expressed mPPARαΔAB. (a) Purified recombinant PPARαΔAB (10 μg) was resolved by 12% SDS−PAGE and visualized by Coomassie-blue staining. (b) Immunoblotting of the purified recombinant PPARαΔAB protein with an anti-PPARα antibody. (c) Gel mobility-shift assays were carried out using purified recombinant PPARαΔAB (100 nM) and ^{32}P-labeled double-strand PPRE probe 5′-TCGACGGACAAAGGT-CACGTTGGAT-3′ (125 nM), in the absence or in the presence of RXRαΔAB (5 nM) as denoted (reaction mixture volume: 20 μL).

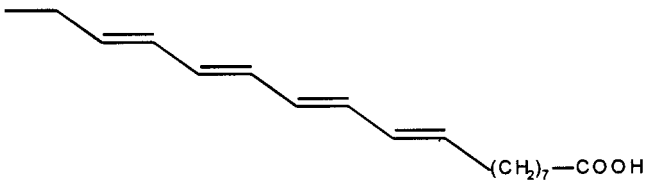

FIGURE 2: Structure of *trans*-parinaric acid.

overexpression in *E. coli* of a histidine-tagged protein and isolated from bacterial extracts by Ni^{2+} affinity chromatography. Examination of the SDS−PAGE profile of the resulting preparation, visualized by Coomassie-blue staining (Figure 1a), showed a predominant band with a molecular weight corresponding to that of the predicted protein. Minor bands of contaminating proteins could also be seen. Quantitation of the observed bands (Scion Image) indicated that PPAR comprised about 57% of the total proteins in the preparation. Immunoblots of this gel confirmed that the predominant protein band corresponded to mPPARα (Figure 1b). To examine the viability of the receptor, gel-mobility shift assays were carried out using an oligonucleotide containing a PPRE (see Experimental Procedures for sequence details) in the presence and in the absence of the PPAR heterodimerization partner RXR (Figure 1c). As expected (*12*), neither RXR nor PPAR bound to the response element on their own. In contrast, in the presence of both receptors, PPAR−RXR heterodimers bound to the oligonucleotide with a high affinity. These observations verify that the purified receptor retained its native DNA selectivity and its ability to form high-affinity heterodimers with RXR.

To examine the ligand-binding selectivity of mPPAR, the synthetic fluorescent fatty acid *trans*-parinaric acid was used. Parinaric acid contains 18 carbons and 4 unsaturated bonds in its acyl chain (Figure 2). Hence, it is a polyunsaturated fatty acid with close structural similarities to the reported PPAR ligands linoleic and linolenic acids (18:2 and 18:3, respectively). The conjugated double bond system of parinaric acid renders this compound highly fluorescent, and, like other fluorophores, this fluorescence is enhanced in a hydrophobic vs in a polar environment (*27*). We therefore reasoned that parinaric acid might serve as an efficient probe for examining the ligand-binding properties of PPAR. A fluorescence titration curve of PPARα with parinaric acid followed by monitoring the fluorescence of the ligand is shown in Figure 3a (circles). Also shown in Figure 3a is a control titration in which the fluorescence of increasing concentrations of parinaric acid was monitored in the presence of the retinoid X receptor α, a nuclear receptor with high homology to PPAR which does not bind fatty acids. A corrected fluorescence titration, depicting the difference between the signals in the presence vs in the absence of PPAR, is shown in Figure 3b. Analysis of this curve yielded 0.48 mol of binding sites/mol of protein and a K_d of 45.5 nM. The mean number of binding sites and apparent K_d obtained from multiple titrations of four independent preparations were 0.53 ± 0.15 mol of parinaric acid/mol of protein and 30 ± 6.5 nM, respectively ($n = 10$). The apparent number of binding sites thus corresponds well to the fraction of PPAR in the preparation (Figure 1). Hence, PPARα possesses a single binding site for parinaric acid and binds this ligand with a high affinity.

Apparent K_ds for several potential ligands for PPAR were estimated by monitoring their ability to compete with parinaric acid for binding to the receptor. PPAR was complexed with parinaric acid. To ensure that the entire population of viable PPAR is complexed but that no excess probe is present, parinaric acid was added at a concentration corresponding to the number of binding sites obtained from the fluorescence titrations. The protein−parinaric acid complex was then titrated with each putative ligand. In this system, an added ligand that is able to compete with parinaric acid for binding to the protein would displace the probe from the binding pocket, leading to dissociation of parinaric acid from the protein. As the fluorescence of parinaric acid is significantly quenched upon movement from the binding site to the aqueous phase, competition would be reflected by a decrease in fluorescence upon titration with a competing ligand. The fluorescence would reach a constant value once all of the parinaric acid dissociates from the protein. Representative competition titrations with various ligands are shown in Figure 4. Several of the tested ligands, markedly docosahexaenoic acid (panel f), 15d-PGJ2 (panel g), and palmitic acid (not shown), were ineffective in displacing parinaric acid from the receptor. Stearic acid (18:0, panel a) appeared to associate with the receptor very weakly, and it was not possible to obtain a complete competition curve. However, titrations with other ligands resulted in a saturable decrease in the fluorescence of the probe, indicating efficient competition. The apparent K_d for each competing ligand was calculated using the measured K_d for parinaric acid (Figure 3 and Table 1) and the concentration of the ligand that was required for displacing half of the probe ($EC_{50ligand}$) by the relationship: $EC_{50ligand}/[\text{parinaric acid}]_{total} = K_{d,ligand}/K_{d,parinaric\ acid}$. K_ds thus obtained are listed in Table 1. These data indicate that PPARα associates with the synthetic peroxisome proliferator WY14643, with several physiological fatty acids, and with the leukotriene LTB4 with an affinity in the nanomolar range (see Discussion).

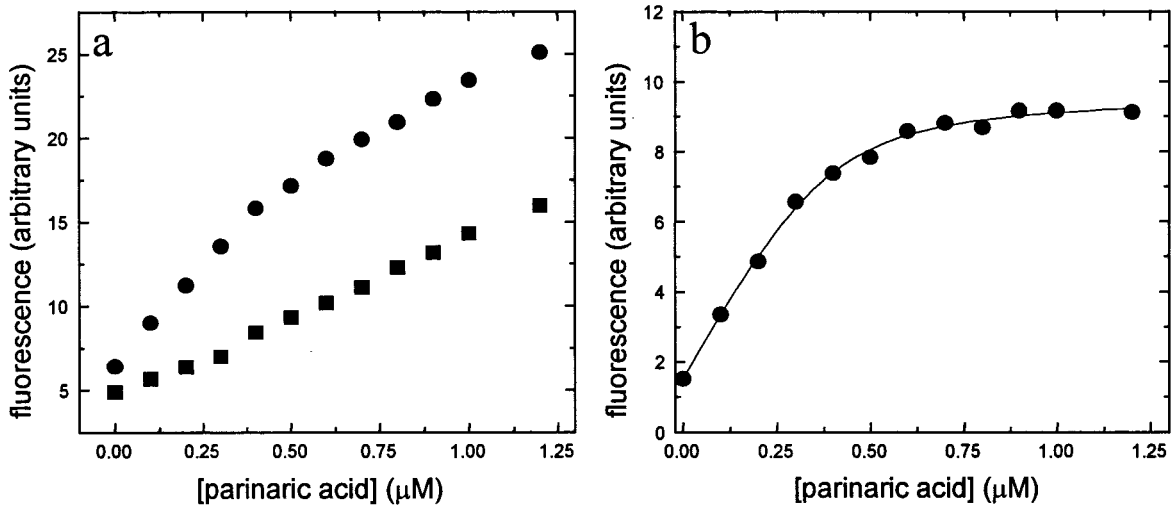

FIGURE 3: Fluorescence titration of mPPARα with *trans*-parinaric acid. Protein (1 μM in 10 mM Hepes, pH 8.0, 0.1 mM EDTA, 0.4 mM DTT, 400 mM KCl, and 5% glycerol; final volume 1 mL) was titrated with parinaric acid from a concentrated solution in ethanol. Fluorescence (excitation, 303 nm; emission, 413 nm) was measured after each addition. A representative titration curve is shown (panel a, circles). Titration curves were corrected for nonspecific increases in fluorescence observed when the concentration of parinaric acid was raised in the presence of 1 μM RXR, a protein that is homologous to PPAR which does not bind parinaric acid (panel a, squares). The corrected data (panel b) were analyzed to obtain the number of binding sites and apparent K_d by fitting the data to an equation derived from simple binding theory (panel b, solid line). Data analysis was performed using Origin software (MicroCal Inc.).

Table 1: Apparent K_ds Characterizing Complexes of mPPARα with Various Ligands[a]

ligand	K_d (nM)
parinaric acid	30.0 ± 6.2 ($n = 11$)
palmitic acid (16:0)	not determined
stearic acid (18:0)	weak
oleic acid (18:1)	5.9 ± 0.5 ($n = 5$)
linoleic acid (18:2)	4.8 ± 0.9 ($n = 5$)
linolenic acid (18:3)	7.9 ± 1.2 ($n = 3$)
arachidonic acid (20:4)	17.3 ± 3.8 ($n = 3$)
docosahexaenoic acid (22:6, $n-3$)	not determined
15d-PGJ2	not determined
WY14643	28.8 ± 5.2 ($n = 5$)
LTB4	60.8 ± 2.3 ($n = 3$)

[a] Fluorescence competition titrations were carried out as described under Experimental Procedures. The K_d for parinaric acid was extracted from fluorescence titrations of PPAR with the probe (Figure 3). Apparent K_ds for the ligands were calculated for each individual titration using the relationship: $EC_{50,ligand}/[\text{parinaric acid}] = K_{d,ligand}/K_{d,parinaric\ acid}$ (see text).

DISCUSSION

PPARα was initially identified as a nuclear receptor that binds and responds to synthetic compounds collectively termed peroxisome proliferators. Subsequent research efforts that focused on identifying compounds that may serve to modulate the activity of PPARs in vivo resulted in the reports that several naturally occurring fatty acids and some arachidonic acid metabolites can bind to PPARs and activate them in the context of transactivation assays. However, a persistent problem in accepting these conclusions has been that the reported binding affinities between PPARs and these putative ligands were too weak to account for efficient interactions in vivo. These compounds are poorly soluble in water and distribute in vivo mainly between biological membranes and various binding proteins. In blood, long-chain fatty acids are bound to serum albumin (*28*), and in cells they are tightly associated with cellular fatty acid binding protein (*29*). The concentrations of free fatty acids dissolved in cytosol are difficult to assess, but available estimates indicate that the upper limit for the concentration of the abundant fatty acid, palmitate, in intracellular aqueous phases is on the order of 50 nM (*30*). To the best of our knowledge, aqueous concentrations of unsaturated fatty acids such as linoleic and linolenic acids have not been reported. These fatty acids are less abundant than palmitate but are also somewhat more soluble in water. Thus, it is reasonable to assume that their aqueous concentrations will be similar to that of palmitate. In comparison, previously reported EC_{50}s for binding of fatty acids to PPARs are on the order of 10 μM (*19*, *20*). We are not aware of measurements of the concentration of 15d-PGJ2 in cells, but the EC_{50} for binding of this ligand to PPARγ was reported to be about 5 μM (*31*), clearly orders of magnitude higher than the likely in vivo concentration.

The present work demonstrates the utility of optical methods for examining the ligand-binding characteristics of proteins, such as PPAR, that associate with hydrophobic ligands. The main advantage of the fluorescence-based assay is that it allows for quantitative assessment of equilibrium binding constants without perturbation of the equilibrium position by physical separation between free and bound species, or by nonequilibrium steps such as electrophoresis. The data obtained by utilizing this assay revealed that the apparent K_ds of PPAR−ligand complexes are in the low nanomolar rather than in the 5−10 μM range as previously proposed (*19*, *20*). Interestingly, despite the dramatic differences in apparent binding affinities, the ligand-selectivity of the receptor observed is in general agreement with previously reported profiles. Thus, similarly to the data of Krey et al. and of Forman et al. (*19*, *20*), mPPARα displayed a preference for oleic, linoleic, and linolenic acids which it binds with about 5 nM affinities. These findings, together with the observation that the C:18 polyunsaturated fluorophore parinaric acid also tightly associates with PPARα, further emphasize the preference of the receptor for C:18 fatty acids. The observation that stearic acid, a saturated C:18 fatty acid, associated with the receptor very weakly further verifies that efficient association of fatty acids with PPARα

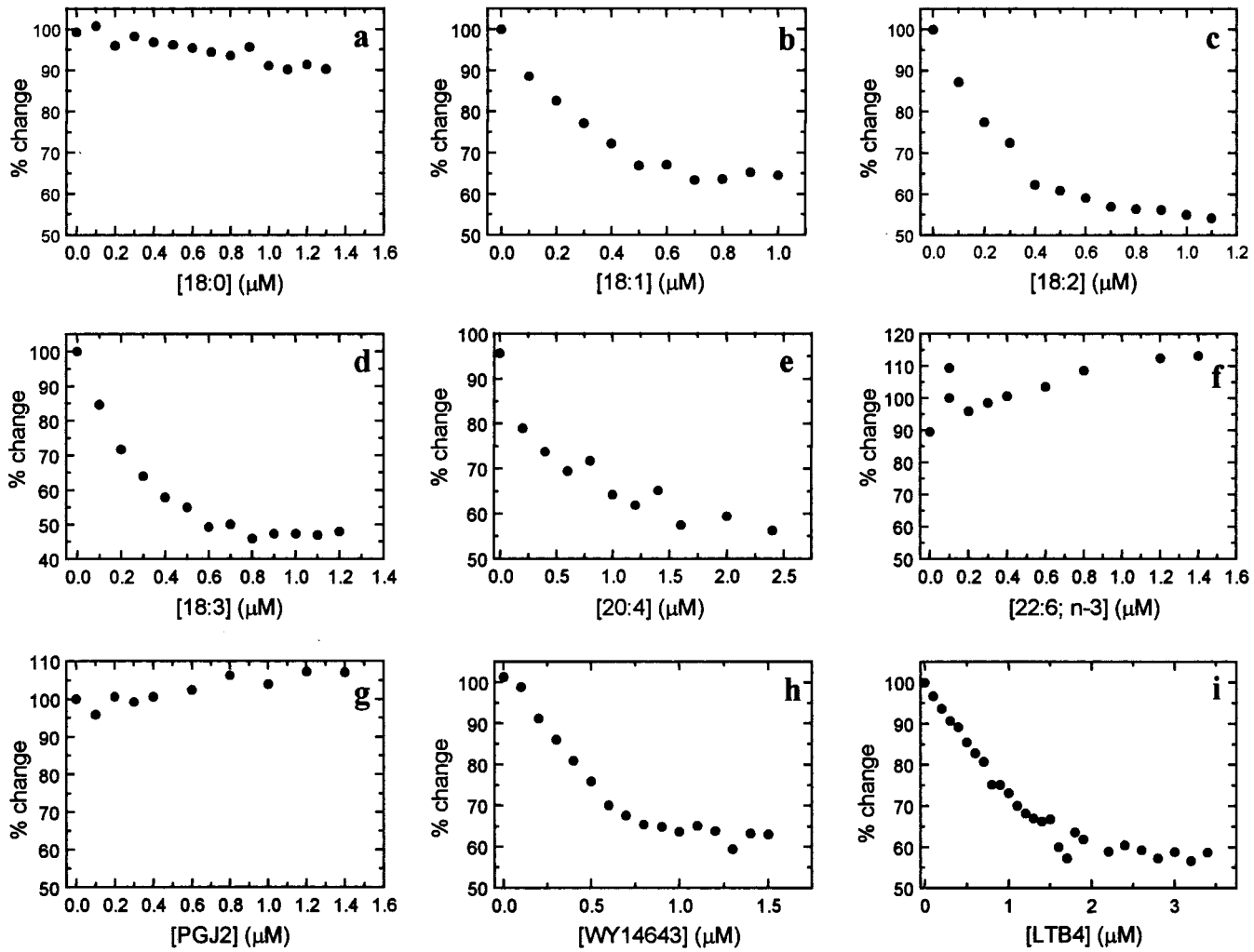

FIGURE 4: Competition titrations of the mPPARα−parinaric acid complex with various ligands. PPARα (1 μM) was complexed with parinaric acid at mole ratio corresponding to the number of binding sites measured in fluorescence titrations (see text). The complex was titrated with the appropriate ligand, and the fluorescence of the probe was measured after each addition. Ligand was added until a plateau, reflecting saturation of the protein with the competing ligand, was reached. The data are presented as percent change of initial fluorescence. Ligands tested: (a) stearic acid, (b) oleic acid, (c) linoleic acid, (d) linolenic acid, (e) arachidonic acid, (f) docosahexaenoic acid, (g) 15d-PGJ2, (h) WY14643, (i) LTB4.

requires the presence of unsaturations in the acyl chain (*12*, *19*). In addition to the C:18 acids, mPPARα associated with arachidonic acid (20:4), albeit with a weaker affinity. Also in agreement with previous observations, mPPARα bound the known PPARα ligand WY14643 but not PGJ2 which was reported to be a specific ligand for PPARγ (*16*). The leukotriene LTB4 bound to mPPARα with weaker affinity as compared with unsaturated C:18 fatty acids. However, it should be kept in mind that LTB4 is very labile when dissolved in water. It is thus likely that the apparent K_d obtained in this study represents an upper limit for the actual value. One discrepancy that is worth noting is that, in our hands, docosahexaenoic acid (22:6, $n-3$) was found to be a very weak ligand of mPPARα although it was reported that this fatty acid is a better transcriptional activator of xPPARα than any C:18 fatty acid (*12*), and that it binds to the *Xenopus laevis* receptor with an affinity comparable to that of LTB4 (19). This discrepancy might reflect species differences.

It was recently reported that the cis isomer of parinaric acid can bind to another isoform of PPAR, PPARγ, albeit with an apparent K_d that is 20-fold higher than that observed here for the association of *trans*-parinaric acid with PPARα (*37*). Together with the present observations, these findings suggest that parinaric acid is a useful tool for screening a large number of potential ligands for more than one isoform of PPAR. Interestingly, the magnitude of fluorescence enhancement of the probe upon binding to PPARα (Figure 3) was found to be significantly smaller than enhancements observed upon binding of this fatty acid to other proteins (e.g., *33*). As the fluorescence of lipophilic fluorophores related to the hydrophobicity of the environment in which they are dissolved, this observation suggests that the ligand-binding pocket of PPAR is more polar than binding pockets of other fatty acid binding proteins. This is well in accordance with the conclusions that the binding pocket of PPAR is able to accommodate a number of ligands with widely varying structures. It appears that the ligand-binding site of PPAR may be a larger cavity and perhaps contains more water molecules than other related receptors.

The observations reported here resolve a long-standing problem by suggesting that previously reported K_ds for formation of PPAR−ligand complexes are likely to have been erroneously high, and by demonstrating that the values of these K_ds are on the order of 5−60 nM range, well within

the physiological range. Hence, as previously noted, PPAR is a promiscuous receptor that responds to multiple signals. An important question that remains open relates to the possible physiological outcomes of the broad ligand-binding selectivity of PPAR. It is interesting to consider in regard to this question that while other nuclear hormone receptors are activated by compounds that are hormones or metabolic products of nutrients, the compounds that were found here to be the highest affinity ligands for PPAR, unsaturated C:18 fatty acids, can be obtained directly from the diet without any metabolic processing. Consequently, available information suggests that while the activities of other receptors may be regulated by modulating the activities of enzymes that catalyze the syntheses of their ligands, PPAR is unique in that it can respond directly to dietary manipulations. It has been reported in regard to this that the level of expression of genes encoding for some enzymes that are involved in lipid metabolism can be regulated by the fatty acid composition of the diet (e.g., *34*); however, the complete scope of role of PPAR in mediating these effects is not yet completely clear (*35*).

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REFERENCES

1. Ricote, M., Li, A. C., Wilson, T. M., Kelly, C. J., and Glass, C. K. (1998) *Nature 391*, 79−82.
2. Hanley, K., Jiang, Y., He, S. S., Friedman, M., Elias, P. M., Bikle, D. D., Williams, M. L., and Feingold, K. R. (1998) *J. Invest. Dermatol. 110*, 368−375.
3. Tontonoz, P., Nagy, L., Alvarez, J. G. A., Thomazy, V. A., and Evans, R. M. (1998) *Cell 93*, 241−252.
4. Chawala, A., Schwarz, E. J., Dimaculangan, D. D., and Lazar, M. A. (1994) *Endocrinology 135*, 798−800.
5. Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994) *Cell 79*, 1147−1156.
6. Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spigelman, B. M. (1994) *Genes Dev. 8*, 1224−1234.
7. Wahli, W., Braissant, O., and Desvergne, B. (1995) *Chem. Biol. 2*, 261−266.
8. Desvergne, B., and Wahli, W. (1995) in *Inducible Gene expression* (Baeuerle, P. A., Ed.) pp 142−176, Birkhauser, Boston.
9. Reddy, J. K., Goel, S. K., Nemali, M. R., Carrino, J. J., Laffler, T. G., Reddy, M. K., Sperbeck, S. J., Osumi, T., Hashimoto, T., Lelwani, N. D., and Rao, M. S. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 1747−1751.
10. Aoyama, T., Peters, J. M., Iritani, N., Nakajima, T., Furihata, K., Hashimoto, T., and Gonzalez, F. J. (1998) *J. Biol. Chem. 273*, 5678−5684.
11. Palmer, C. N. A., Hsu, M. H., Griffin, K. J., and Johnson, E. F. (1995) *J. Biol. Chem. 270*, 16114−16121.
12. Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K., and Wahli, W. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 2160−2164.
13. Issemann, I., and Green, S. (1990) *Nature 347*, 645−650.
14. Dreyer, C., Krey, G., Keller, H., Givel, F., Helftenbein, G., and Whali, W. (1992) *Cell 68*, 879−887.
15. Bocos, C., Gottlicher, M., Gearing, K., Banner, C., Enmark, E., Teboul, M., Crickmore, A., and Gustafsson, J. A. (1995) *J. Steroid Biochem. Mol. Biol. 53*, 467−473.
16. Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995) *Cell 83*, 803−812.
17. Devchand, P. R., Keller, H., Peter, J. M., Vazquez, M., Gonzalez, F. J., and Wahli, W. (1996) *Nature 384*, 39−43.
18. Onate, S. A., Tsai, S. Y., Tsay, M. Y., and O′Malley, B. W. (1995) *Science 270*, 1354−1357.
19. Krey, G., Braissant, O., L′Horset, F., Kalkhoven, E., Perroud, M., Parker, M. G., and Wahli, W. (1997) *Mol. Endocrinol. 11*, 779−791.
20. Forman, B. M., Chen, J., and Evans, R. M. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 4312−4317.
21. Cogan, U., Kopelman, M., Mokady, S., and Shinitzky, M. (1976) *Eur. J. Biochem. 65*, 71−78.
22. Noy, N., and Xu, Z.-J. (1990) *Biochemistry 29*, 3878−3883.
23. Kersten, S., Pan, L., and Noy, N. (1995) *Biochemistry 34*, 14263−14269.
24. Chen, Z.-P., Shemshedini, L., Durand, B., Noy, N., Chambon, P., and Gronemeyer, H. (1994) *J. Biol. Chem. 269*, 25770−25776.
25. Kersten, S., Kelleher, D., Chambon, P., Gronemeyer, H., and Noy, N. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 8645−8649.
26. Kersten, S., Reczek, P. R., and Noy, N. (1997) *J. Biol. Chem. 272*, 29759−29768.
27. Lakowicz, J. A. (1983) *Principles of fluorescence spectroscopy*, Plenum Press, New York and London.
28. Spector, A. A., and Fletcher, J. E. (1978) in *Disturbances in lipid and lipoprotein metabolism* (Dietchy, J. M., Gotto, A. M., and Ontko, J. A., Eds.) pp 229−249, American Physiology Society, Bethesda, MD.
29. Ockner, R. K., Manning, J. A., Poppenhausen, R. B., and Ho, W. K. L. (1972) *Science 17*, 56−58.
30. Noy, N., Donnelly, T. M., and Zakim, D. (1986) *Biochemistry 25*, 2013−2021.
31. Kliewer, S. A., Lenhard, J. M., Wilson, T. M., Patel, I., Morris, D. C., and Lehmann, J. M. (1995) *Cell 83*, 813−819.
32. Wolf, C. R., and Palmer, C. N. A. (1998) *FEBS Lett. 431*, 476−480.
33. Sha, R. S., Kane, C. D., Xu, Z., Banaszak, L. J., and Bernlohr, D. A. (1993) *J. Biol. Chem. 268*, 7885−7892.
34. Landschulz K. T., Jump D. B., Macdougald O. A., and Lane M. D. (1994) *Biochem. Biophys. Res. Commun. 200*, 763−768.
35. Ren B., Thelen A. P., Peters J. M., Gonzalez F. J., and Jump D. B. (1997) *J. Biol. Chem. 272*, 26827−26832.
36. Norris, A. W., and Li, E (1998) in: *Methods in Molecular Biology, vol. 89*: Retinoid Protocols, (Redfern, C. P. F., Ed.) p 133, Humana Press, Totowa, NJ.
37. Palmer, C. N. A., and Wolf, C. R. (1998) *FEBS Lett. 431*, 476−480.

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