Activation of a peroxisome-proliferating catabolite of cholic acid to its CoA ester

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We have shown that a microbial cholic acid catabolite (4*R*)-4-(2,3,4,6,6a β ,7,8,9,9a α ,9b β -decahydro-6a β -methyl-3-oxo-1*H*-cyclopenta[f]quinolin-7 β -yl)valeric acid (DCQVA), is a potent peroxisome proliferator. In this paper a possible key stage in DCQVA metabolism, the activation of DCQVA to its CoA ester, has been investigated in rat liver microsomes and particulate fractions. The microsomal reaction was dependent on CoA, ATP, DCQVA (0.2–1 mM) and protein content. The reaction was decreased by storage at 4 °C, preincubation of microsomes at 37 °C for 5 min, or inclusion of Triton X-100 in the reaction mixture. Such treatments also enhanced generation of long-chain fatty acyl-CoAs, as determined by h.p.l.c. analysis. The same effect was caused by exposing the microsomes to phospholipase A₂, suggesting that endogenous fatty acids may compete with

DCQVA for esterification with CoA. Subcellular fractionation of rat liver demonstrated that the activity of DCQVA-CoA synthesis was localized predominantly in the microsomal fraction, in contrast to long-chain fatty acyl-CoA synthetase, which was distributed among all particulate fractions. Administration of clofibrate of rats did not affect the distribution of DCQVA-CoA synthesis activity. In contrast to a 2-fold induction of long-chain fatty acyl-CoA synthetase by clofibrate treatment, the activity of DCQVA-CoA synthesis in the microsomal fraction decreased by 80%. These results suggest that DCQVA is activated by an enzyme distinct from long-chain fatty acyl-CoA synthetase. The resulting perturbation of fatty acid metabolism may be involved in the mechanism whereby DCQVA causes peroxisome proliferation.

INTRODUCTION

Induction of peroxisome proliferation is associated with induction of several fatty acid metabolizing enzymes, notably those involved in peroxisomal β -oxidation and in microsomal ω -oxidation, as well as mitochondrial carnitine acetyltransferase and 2,4-dienoyl-CoA reductase, and cytosolic long-chain acyl-CoA hydrolase [1–8]. At the same time, microsomal 1-acylglycerophosphocholine acyltransferase, which remodels the fatty acids of phospholipids, is also induced [9,10]. These changes are caused by structurally diverse chemicals [1–10] and by physiological or dietary factors, such as high-fat diets [2,11,12] or diabetes [13], that raise the level of intracellular fatty acids. Thus it has been proposed that peroxisome proliferation could be an adaptive response to a perturbation of fatty acid metabolism [1,8,12,14].

Recent studies have demonstrated that the induction of both peroxisomal β -oxidation [15] and microsomal ω -oxidation [16] is mediated by the peroxisome-proliferator-activated receptor, a member of the steroid hormone receptor superfamily [17]. However, since experiments have so far failed to demonstrate any direct binding of a xenobiotic proliferator to this receptor [17], the presence of a natural ligand has been suggested [18]. Since non-esterified fatty acids added to the culture medium can evoke the receptor-mediated expression of a receptor gene in the cells stably expressing this receptor, it has been proposed that a natural ligand may be a common metabolite of various fatty acids [19]. Since most xenobiotic proliferators are, or can metabolically be converted into amphipathic carboxylates, these compounds could be postulated to enter the metabolic pathways of naturally occurring fatty acids. Thus it has been hypothesized that the perturbation of fatty acid metabolism caused by these chemicals raises the level of endogenous ligand [19].

Activation of fatty acids to acyl-CoA esters is an essential step in the β -oxidation, elongation and esterification of fatty acids. Fatty acyl-CoAs are also used for acylation of certain cellular proteins so that they can express their functions [20]. Thus acyl-CoA synthetase appears to be a key enzyme in fatty acid metabolism and may play an important role in the proliferative response. In addition, some peroxisome proliferators, such as three 'fibrate' hypolipidaemic agents [21] and sulphur-substituted dicarboxylic fatty acid analogues [14,22], have been shown to be activated to acyl-CoA esters in rat liver. It could thus be postulated that these xenoacyl-CoAs enter and perturb the fatty acid metabolic pathway.

We have previously reported that a microbial catabolite of cholic acid, (4*R*)-4-(2,3,4,6,6a β ,7,8,9,9a α ,9b β -decahydro-6a β -methyl-3-oxo-1*H*-cyclopenta[f]quinolin-7 β -yl)valeric acid (DCQVA), is a naturally occurring potent peroxisome proliferator [23]. In the present study we have investigated the activation of DCQVA to its CoA ester in subcellular fractions of rat liver to assess whether DCQVA-induced proliferation may involve the same mechanism as described above. Subcellular distribution, inducibility by clofibrate treatment and inhibition of DCQVA-CoA synthesis activity by fatty acids or cholic acid were studied in order to characterize the enzyme activity responsible for the formation of DCQVA-CoA and thereby assess its possible role in perturbing lipid metabolism. DCQVA-CoA hydrolysis, which could modulate DCQVA-CoA synthesis, was also studied.

EXPERIMENTAL

Materials

DCQVA was a gift from Professor Shohei Hayakawa (Okayama University, Okayama, Japan). CoA, ATP, phospho-

Abbreviations used: DCQVA, (4*R*)-4-(2,3,4,6,6a β ,7,8,9,9a α ,9b β -decahydro-6a β -methyl-3-oxo-1*H*-cyclopenta[f]quinolin-7 β -yl)valeric acid; CMC, critical micelle concentration.

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lipase A₂ (*Crotonus* venom), cholic acid, oleic acid and palmitoyl-CoA were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.); Triton X-100 was from Nakarai Chemicals Ltd. (Tokyo, Japan). Stock solutions of DCQVA (10 mM) were prepared in 15 mM NaOH, stored at 4 °C, and used within 1 week. DCQVA-CoA, cholesteryl-CoA, and oleoyl-CoA were synthesized in our laboratory by a mixed anhydride method [24], and purified by HClO₄ precipitation and subsequent washing with acetone. The CoA esters were quantified by measuring their free SH contents spectrophotometrically [25] before and after hydrolysis in 0.25 M NaOH for 30 min at room temperature. The synthesis of DCQVA-CoA was confirmed by fast-atom bombardment mass spectrometry (JEOL DX-300 mass spectrometer; Nihon Denshi Co. Ltd., Tokyo, Japan), in which a protonated ion at m/z 1055 ($[M+H]^+$) and an alkali metal addition ion at m/z 1077 ($[MNa]^+$) were observed under the following conditions: bombarding atoms, xenon (8 keV); carrier, glycerol/thioglycerol (1:1, v/v, 2 μ l). The critical micelle concentrations (CMCs) of DCQVA, oleic acid and cholic acid were determined in the assay buffer by monitoring the fluorescence enhancement of *N*-phenyl-1-naphthylamine upon being dissolved in the detergent micelles [26]. The concentration corresponding to 50% enhancement of the intensity was defined as the CMC.

Animals and preparation of subcellular fractions

Male Wistar rats weighing 120–160 g were fed on either a normal diet or a diet containing 0.3% (w/w) clofibrate for 2 weeks. Rats were anaesthetized with diethyl ether, and the livers were perfused with ice-cold 0.9% NaCl, excised and homogenized in a glass-Teflon homogenizer with cold 0.25 M sucrose. The homogenate was fractionated into nuclear, heavy mitochondrial, light mitochondrial, microsomal and cytosolic fractions by the method of de Duve et al. [27]. For the experiments in Figure 4, each fraction was redispersed and homogenized in 0.25 M sucrose containing 10 mM Tris/HCl (pH 7.4) and enzymes were assayed immediately. For the other experiments, pelleted microsomes were frozen at –70 °C, and enzymes were assayed immediately after thawing and homogenization in the same buffer.

Enzyme assays

Synthesis of DCQVA-CoA was assayed at 37 °C in a mixture containing 0.1 M Tris/HCl (pH 8.0), 1 mM dithiothreitol, 2 mM CoA, 0.15 M KCl, 15 mM MgCl₂, 10 mM ATP, 1 mM DCQVA and 0.25–0.35 mg of microsomal protein in a total volume of 200 μ l. After a 10 min incubation the reaction was stopped by adding, in order, 0.4 ml of acetonitrile, 0.1 ml of 1 M phosphoric acid and 0.3 ml of 25 mM potassium phosphate (pH 5.6). The mixture was centrifuged at 1200 *g* for 10 min and 100 μ l of the supernatant was injected into an h.p.l.c. apparatus (LC-6A liquid chromatograph; Shimadzu, Kyoto, Japan) to determine the amount of DCQVA-CoA synthesized. The conditions were as follows: column, α Bondapak C₁₈ (8 mm $\times$ 100 mm; Waters Associates); solvent, 25% acetonitrile in 25 mM potassium phosphate (pH 5.6); flow rate, 1.2 ml/min; detection, u.v. at 254 nm. DCQVA-CoA, CoA and DCQVA eluted at 9 min, 3.1 min and 12.2 min respectively. The peak of DCQVA-CoA was identified by alkaline hydrolysis (0.25 M NaOH for 20 min at room temperature), which resulted in the disappearance of the peak and the concomitant appearance of the CoA and DCQVA peaks. The recovery of chemically synthesized DCQVA-CoA (12 nmol) added to the complete reaction mixture which was

then processed as described above without incubation was 85–90%.

Fatty acyl-CoA synthetase was assayed by the method of Tomoda et al. [28], except that sodium oleate was used as a substrate and Triton X-100 was not included in the assay unless otherwise indicated. The reaction mixture contained 0.1 M Tris/HCl (pH 8.0), 5 mM dithiothreitol, 0.15 M KCl, 15 mM MgCl₂, 10 mM ATP, 0.25 mM sodium oleate and microsomal protein (100–150 μ g) in a total volume of 200 μ l. The incubation was carried out at 37 °C for 1 min, and terminated as described for DCQVA-CoA synthesis. The product was analysed by h.p.l.c. as described above for DCQVA-CoA, except that elution was carried out with a 10 min linear gradient from 40 to 60% acetonitrile in 25 mM potassium phosphate (pH 5.6) followed by a 10 min isocratic run with 60% acetonitrile in 25 mM potassium phosphate (pH 5.6). The flow rate was 1.0 ml/min. Oleoyl-CoA eluted at 12.8 min under these conditions.

Cholesteryl-CoA synthetase activity was assayed in a manner similar to that for DCQVA-CoA synthesis, except that 20–150 μ M sodium cholate was used as substrate. Elution of cholesteryl-CoA from h.p.l.c. was performed with a 2 min isocratic run with 25% acetonitrile in 25 mM potassium phosphate (pH 5.6), followed by a 10 min linear gradient from 25 to 50% acetonitrile in 25 mM potassium phosphate (pH 5.6) and then a 10 min isocratic run with 50% acetonitrile in 25 mM potassium phosphate (pH 5.6) at a flow rate of 1.2 ml/min. Cholesteryl-CoA eluted at 11.1 min.

DCQVA-CoA hydrolysis was measured at 37 °C in a mixture containing 0.1 M Tris/HCl (pH 8.0), 12 nmol of DCQVA-CoA and 0.05–0.2 mg of protein in a total volume of 200 μ l. After a 5–10 min incubation the reaction was stopped and analysed by h.p.l.c. in the same manner as for the DCQVA-CoA synthetase described above. The activity was measured under conditions where 20–60% of the DCQVA-CoA had disappeared and the reaction was linear relative to protein content.

Fatty acyl-CoA hydrolase was assayed spectrophotometrically by monitoring the release of free CoA [6]. The reaction mixture contained 0.1 M Tris/HCl (pH 8.0), 20 μ M palmitoyl-CoA, 0.2 mM 5,5'-dithiobis-(2-nitrobenzoic acid) and 20–500 μ g of protein in a total volume of 1 ml. The molar absorption coefficient for the reduced form of DTNB is 13600 M⁻¹·cm⁻¹ at 412 nm.

The subcellular marker enzymes glutamate dehydrogenase, catalase and NADPH:cytochrome *c* reductase and peroxisomal β -oxidation were assayed as described previously [29]. Protein was determined by the method of Lowry et al. [30] using BSA as a standard.

RESULTS

Conversion of DCQVA into DCQVA-CoA by microsomes

The conversion of DCQVA into DCQVA-CoA by liver microsomes was investigated. H.p.l.c. analysis of the reaction products obtained after incubating DCQVA with the microsomal fraction resolved a peak which migrated with the same retention time as authentic DCQVA-CoA (Figures 1a and 1b). The occurrence of this peak was completely dependent on the addition of DCQVA (Figure 1c), microsomal protein, ATP and CoA (Figures 2a–2c), and it disappeared after alkaline hydrolysis, demonstrating that the microsomal fraction was capable of synthesizing DCQVA-CoA.

DCQVA-CoA synthesis was linear for the first 10–15 min and subsequently peaked by 30 min (Figure 2d). Upon longer incubation the h.p.l.c. peak area representing DCQVA-CoA decreased, suggesting subsequent hydrolysis of the newly synthesized DCQVA-CoA by microsomes. The rate of DCQVA-

Figure 1 H.p.l.c. of the reaction mixtures of the DCQVA-CoA synthetase assay

Microsomal DCQVA-CoA synthetase was measured at 37 °C for 10 min. The chromatograms of chemically synthesized DCQVA (1.5 nmol) (a), standard assay mixture (b), and assay mixture without DCQVA (c) are shown.

CoA synthesis under the standard assay conditions was 2.30 ± 0.40 nmol/min per mg of protein ($n = 5$ preparations), while hydrolysis of chemically synthesized DCQVA-CoA proceeded at a rate of 2.18 ± 0.45 nmol/min per mg ($n = 5$ preparations).

DCQVA-CoA synthesis was quite unstable; storage of microsomal suspensions at 4 °C resulted in a 55% and 75% loss of activity within 1 day and 3 days respectively. This was in contrast to microsomal long-chain fatty acyl-CoA synthetase (oleoyl-CoA synthetase) activity, which was unchanged for up to

Table 1 Effect of Triton X-100 and phospholipase A₂ on microsomal DCQVA-CoA synthetase activity

Assay mixtures for DCQVA-CoA synthetase and oleoyl-CoA synthetase were incubated for 10 min and 1 min respectively in the presence of various concentrations of Triton X-100. Assay mixtures for DCQVA-CoA synthetase containing phospholipase A₂ (from *Crotonus venom*) were incubated for 10 min with or without 2 mM Ca²⁺. Values are means $\pm$ range of two independent experiments. n.m., not measured.

Triton X-100 (%)	Acyl-CoA synthetase activity (nmol/min per mg)	
	DCQVA-CoA	Oleoyl-CoA
0	2.40 ± 0.21	44.3 ± 2.7
0.025	1.94 ± 0.32	n.m.
0.05	1.18 ± 0.20	n.m.
0.1	0.22 ± 0.05	47.4 ± 4.0
0.3	0.14 ± 0.04	76.5 ± 5.7
0.5	0.11 ± 0.04	81.2 ± 1.7

Phospholipase A ₂ (units)	DCQVA-CoA synthetase activity (nmol/min per mg)	
	– Ca ²⁺	+ Ca ²⁺
0	2.13 ± 0.22	2.05 ± 0.16
0.2	2.06 ± 0.22	1.64 ± 0.15
0.4	2.27 ± 0.24	1.19 ± 0.17
0.8	2.11 ± 0.30	0.93 ± 0.13
1.2	2.18 ± 0.24	0.89 ± 0.13

5 days. Preincubation of microsomes in the assay mixture without substrate and cofactors at 37 °C for 5 min resulted in a 55% loss of DCQVA-CoA synthetase activity. Adding 2 mM Ca²⁺ at the preincubation step increased the loss to 70%. Triton X-100, which is usually included in the assay mixtures for fatty

Figure 2 Dependence of DCQVA-CoA synthetase activity on microsomes (a), CoA (b), ATP (c) and incubation time (d)

Assays for (a)–(c) were run for 10 min at 37 °C under the conditions described in the Experimental section.

Figure 3 Effects of oleic acid (a) and cholic acid (b) on microsomal DCQVA-CoA synthetase activity

Activity was measured at increasing substrate concentrations in the absence (○) and presence of 20 μM (●), 40 μM (□) and 60 μM (■) of oleic acid or cholic acid. $[S]/v$ versus $[S]$ plots of the curves are shown in the insets.

Figure 4 Subcellular distribution of acyl-CoA synthetases and hydrolases in the livers of normal (solid line) and 0.3% clofibrate-treated (dotted line) rats

(a) DCQVA-CoA synthetase; (b) DCQVA-CoA hydrolase; (c) oleoyl-CoA synthetase; (d) palmitoyl-CoA hydrolase. Liver homogenates from three animals were combined, and fractionated into nuclear (N), heavy mitochondrial (M), light mitochondrial (L), microsomal (P) and cytosolic (S) fractions. The experiment was repeated and essentially similar results were obtained.

acyl-CoA synthetase [28,31] and xenoacyl-CoA synthetase [21], markedly inhibited DCQVA-CoA synthesis, whereas this compound enhanced oleoyl-CoA synthesis (Table 1).

Re-analysis of assay mixtures for DCQVA-CoA synthesis under the chromatographic conditions used for resolution of oleoyl-CoA resulted in the elution of a group of peaks between 10 and 13 min. The appearance of the peaks was dependent on incubation, but not on the presence of DCQVA. Inhibition of DCQVA-CoA synthesis by storage, preincubation of microsomes

or the addition of Triton X-100 was accompanied by increases in the intensity of these peaks. Three of the peaks had the same retention times as those of linoleoyl-CoA (10.1 min), palmitoyl-CoA (11.9 min) and oleoyl-CoA (12.8 min). Thus, although further identification was not attempted, it seemed probable that these peaks were long-chain fatty acyl-CoAs synthesized from endogenous non-esterified fatty acids in the microsomes.

Exposure of the microsomes to phospholipase A_2 (from *Crotonus venom*) also caused inhibition of DCQVA-CoA

synthesis (Table 1). This inhibition was dependent on both Ca^{2+} and the concentration of phospholipase A_2 , and was accompanied by increases in the intensity of the peaks corresponding to long-chain fatty acyl-CoAs on the h.p.l.c. chromatogram.

Oleic acid added to the assay mixture also inhibited DCQVA-CoA synthesis (Figure 3a). However, the levels of oleic acid required were slightly below (20 and 40 μM) or at (60 μM) the CMC. When measuring the CMC, addition of DCQVA (0.2–1 mM) caused enhancement of the fluorescence intensity of the probe, suggesting mixed micelle formation, and this may be a cause of the complicated pattern of kinetics (Figure 3a, inset). The apparent K_m for DCQVA synthesis was 0.65 ± 0.07 mM ($n = 4$). DCQVA over the concentration range 0.5–2 mM slightly (< 22%) inhibited oleoyl-CoA synthetase when assayed with 20 μM oleic acid (results not shown).

Cholic acid added to the assay mixture inhibited DCQVA-CoA synthesis (Figure 3b). Although the levels of cholic acid and DCQVA were far below the CMCs for our assay conditions (> 2 mM and > 20 mM for cholic acid and DCQVA respectively), the results did not conform to Michaelis–Menten kinetics (Figure 3b, inset). The addition of DCQVA (1 and 2 mM) increased both the apparent K_m for cholic acid (23 μM to 120 and 150 μM respectively) and the $V_{\max}$ (1.9 nmol/min per mg to 7.1 and 8.5 nmol/min per mg respectively).

Subcellular distribution of DCQVA-CoA synthetase and hydrolase

DCQVA-CoA synthesis was found to occur mainly in the microsomal fraction (fraction P, Figure 4a), and its distribution pattern matched that of NADPH:cytochrome *c* reductase activity (results not shown). This was in contrast to fatty acyl-CoA (oleoyl-CoA) synthetase, which was distributed among all particulate fractions (Figure 4c). The activity of DCQVA-CoA hydrolase in the heavy mitochondrial (M), light mitochondrial (L), and nuclear (N) fractions was 8–20 times higher than that of DCQVA-CoA synthesis (Figure 4b). The distribution pattern of DCQVA-CoA hydrolase (Figure 4b) was different from that of fatty acyl-CoA hydrolase measured with palmitoyl-CoA as the substrate (Figure 4d).

Administration of 0.3% clofibrate to rats in their diet caused a 21-fold induction of peroxisome β -oxidation and a 2-fold increase in oleoyl-CoA synthesis (Figure 4c) in the liver, as expected on the basis of earlier reports [6,14,32,33]. In contrast, DCQVA-CoA synthesis in the microsomal fraction was reduced (0.61 and 0.54 nmol/min per mg in microsomes from treated rats in two independent experiments, compared with 2.65 and 2.39 nmol/min per mg in microsomes from control rats). DCQVA-CoA synthesis in the M, L and P fractions was also reduced, and the distribution pattern was unchanged (Figure 4a). DCQVA-CoA hydrolase was markedly induced only in the cytosolic (S) fraction (4.1-fold) and was essentially unchanged in the M, L and P fractions (Figure 4b).

DISCUSSION

In this study we have shown that liver microsomes are capable of activating DCQVA, a peroxisome proliferator [23] and cholic acid metabolite, to its CoA ester. The data presented support the conclusion that the reaction is catalysed by an enzyme distinct from long-chain fatty acyl-CoA synthetase.

Inhibition of microsomal DCQVA-CoA synthesis by endogenous fatty acids was suggested by the finding that the reduced activity caused by preincubation, storage of microsomes at 4 °C or inclusion of Triton X-100 in the reaction mixture was accompanied by enhanced generation of long-chain fatty acyl-

CoAs. It is very likely that such treatment caused activation of phospholipase A in the microsomal membrane, and that the resulting endogenous fatty acids competitively inhibited DCQVA activation. Indeed, a latent phospholipase A in rat liver Golgi which can be activated by Triton X-100 has been reported [34]. In addition, inhibition of DCQVA-CoA synthesis by exogenous phospholipase A_2 supports this hypothesis. Although such treatment also produces lysophospholipids, which would act as a detergent and could thereby cause a reduction in DCQVA-CoA synthesis, the concomitant increase in long-chain fatty acyl-CoAs suggests that the inhibition was mainly caused by fatty acids. Furthermore, microsomes contain high lysophospholipase activity, which could rapidly decrease the level of lysophospholipids [35]. Exposure of hepatocytes to phospholipase A_2 has been reported to decrease cellular phosphatidylcholine and phosphatidylethanolamine levels by 50–60%, while hepatocytes remained viable [36], suggesting that lysophospholipids generated in the membrane were either rapidly degraded or did not cause damage to the membrane. The more efficient inhibition by endogenous non-esterified fatty acids than by exogenous oleic acid can be explained by the results of a study using unilamellar vesicles of phosphatidylcholine which showed that the source of the substrate interacting with the fatty acyl-CoA synthetase is the membrane-associated pool of fatty acids and not fatty acids in the aqueous phase [37].

Inhibition of DCQVA-CoA synthesis by endogenous fatty acids raises the possibility that DCQVA may be activated by long-chain fatty acyl-CoA synthetase. However, long-chain fatty acyl-CoA synthetases with identical molecular, catalytic and immunological properties are present in microsomes, peroxisomes and mitochondria [32,38], whereas DCQVA-CoA synthetase is localized predominantly in microsomes, suggesting that an enzyme distinct from long-chain fatty acyl-CoA synthetase is responsible for this reaction. The decrease in DCQVA-CoA synthesis caused by clofibrate, in contrast to the induction of oleoyl-CoA synthesis, also supports this hypothesis. Since cholesteryl-CoA synthetase activity is localized predominantly in microsomes [39], it is possible that DCQVA-CoA synthesis is catalysed by this enzyme. However, cholic acid did not act as a competitive inhibitor of DCQVA-CoA synthesis. Very-long-chain fatty acyl-CoA synthetase has been reported in microsomes and peroxisomes [40]. Trihydroxycoprostanic acid [41], dicarboxylic acid [42] and prostaglandins [43] are activated solely in microsomes. Although a recent report showed that freezing and thawing of the mitochondrial fraction decreased the activity of ciprofibril-CoA synthesis [44], the subcellular localization of DCQVA-CoA in the present study was unaffected by such treatment.

The capability of liver microsomes to synthesize DCQVA-CoA suggests that DCQVA could enter and perturb the fatty acid metabolic pathway. Although DCQVA-CoA synthesis activity was localized predominantly in the microsomal fraction, a recent report that palmitoyl-CoA penetrates through the peroxisomal membrane [45] raises the possibility that DCQVA-CoA could also affect peroxisomal fatty acid metabolism. Perturbation of fatty acid metabolism has been suggested to be a cause of peroxisome proliferation [1,8,12,14,18,19].

Alternatively, CoA esters of peroxisome proliferators could be a common mediator accounting for the uniform inductive response caused by structurally diverse chemicals [8,14,21,22]. Increased levels of cellular acyl-CoA esters, including xenoacyl-CoA esters, correlate with the induction of peroxisomal β -oxidation in the case of several proliferators [8]. Although dehydroepiandrosterone, a peroxisome proliferator *in vivo* [46], does not have a carboxylic acid residue and therefore cannot be

converted to its CoA ester, this compound has been shown not to activate the peroxisome proliferator-activated receptor [17,18]. Thus a different mechanism could be operating in this case. Another exception is perfluoro-octanoic acid, which reportedly is not converted to its CoA ester [47]. However, this conclusion was reached based on the absence of a second peak as h.p.l.c. but the retention time for perfluorooctanoyl-CoA was not determined, as an authentic standard was not available [47].

The small size of the cellular acyl-CoA pool [48] also suggests that acyl-CoA may be a physiologically active species, such as for example diacylglycerol, a physiological activator of protein kinase C which in turn regulates many cellular functions [49]. Indeed, CoA esters of peroxisome-proliferating hypolipidaemic drugs are potentiators of protein kinase C [50]. The cellular pool of acyl-CoA esters can also be regulated by acyl-CoA hydrolase. DCQVA-CoA hydrolase, which was found in all subcellular fractions, may have the function of preventing intracellular accumulation of DCQVA-CoA, which would be difficult to metabolize. The activity of DCQVA-CoA hydrolase in the cytosolic fraction was induced by clofibrate. Inductions of cytosolic long-chain fatty acyl-CoA hydrolase [6–8] and cytosolic clofibril-CoA hydrolase [33] by peroxisome proliferators have been previously reported, and were postulated to be an adaptive response to the enhanced level of acyl-CoA esters.

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