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Original article

Although it is rapidly metabolized in cultured rat hepatocytes, lauric acid is used for protein acylation

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Abstract — This study was designed to examine the metabolic fate of exogenous lauric acid in cultured rat hepatocytes, in terms of both lipid metabolism and acylation of proteins. Radiolabeled [1-^{14}C]-lauric acid at 0.1 mM in the culture medium was rapidly taken up by the cells (94.8 ± 2.2% of the initial radioactivity was cleared from the medium after a 4 h incubation) but its incorporation into cellular lipids was low (24.6 ± 4.2% of initial radioactivity after 4 h), due to the high β-oxidation of lauric acid in hepatocytes (38.7 ± 4.4% after the same time). Among cellular lipids, lauric acid was preferentially incorporated into triglycerides (10.6 ± 4.6% of initial radioactivity after 4 h). Lauric acid was also rapidly converted to palmitic acid by two successive elongations. Protein acylation was detected after metabolic labeling of the cells with [11,12-^{3}H]-lauric acid. Two-dimensional electrophoresis separation of the cellular proteins and autoradiography evidenced the incorporation of radioactivity into 35 well-resolved proteins. Radiolabeling of several proteins resulted from covalent linkage to the precursor [11,12-^{3}H]-lauric acid or to its elongation product, myristic acid. The covalent linkages between these proteins and lauric acid were broken by base hydrolysis, indicating that the linkage was of the thioester or ester-type. Endogenous myristic acid produced by lauric acid elongation was used for both protein N-myristoylation and protein S-acylation. Therefore, these results show for the first time that, although it is rapidly metabolized in hepatocytes, exogenous lauric acid is a substrate for the acylation of liver proteins.

lauric acid / fatty acid metabolism / β-oxidation / fatty acid acylation of proteins / cultured rat hepatocytes

1. INTRODUCTION

Lauric acid (C12:0) usually accounts for very small amounts of total fatty acids in animal tissues but is more abundant in milk fat (3–5 wt% of total fatty acids) or in copra and palm oils (39–54 wt% and 44–51 wt%, respectively) [1].

Several aspects of dietary lauric acid metabolism in the cell have been well-documented. As a medium-chain fatty acid, it is known to be rapidly digested when provided in the diet [2], especially when it esterifies the sn-2 position in dietary triglycerides (TG) like bovine milk TG [3], and is easily β-oxidized by both mitochondrial and

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peroxisomal pathways [4]. In the liver, it can be esterified into cellular lipids, probably preferentially into the TG fraction, and converted to longer saturated fatty acids [4]. In addition, we recently demonstrated that in the absence of a competing substrate, lauric acid is desaturated at the Δ9 position, producing C12:1 n-3 in rat liver [5]. Finally, it is an excellent substrate for ω hydroxylations mediated by various cytochrome P450 enzymes [6].

Concerning its physiological effects, dietary saturated fats containing lauric acid (C12:0), but also myristic acid (C14:0) and palmitic acid (C16:0), have been known for a long time to increase plasma total- and LDL-cholesterol concentrations in animals and humans [7–10]. This increase seems to be largely explained by a decrease in hepatic LDL receptor activity [11, 12] and an increase in LDL-cholesterol production rate. A recent study analyzing the effects of increasing amounts of dietary myristic acid in hamsters showed, however, that the increase in total plasma cholesterol only reflects an increase in the level of HDL-cholesterol [13]. New mechanisms involving exogenous lauric acid have also recently been shown to induce the expression of genes through the modulation of Toll-like receptor 4 in macrophages [14].

Some other aspects of the utilization of dietary lauric acid following its intake, remain, however, largely unknown. Particularly, the cellular fate of exogenous lauric acid as a potential substrate for the acylation of proteins has not been studied, and the importance of lauric acid in the diet for this function has not been investigated, even though, fatty acid acylation of proteins is a functionally important modification [15, 16]. The functional significance of the acyl moiety may be to mediate protein subcellular localization, protein-protein interaction or protein-membrane interactions required for the expression of the biological activities of many acylated proteins [17]. Fatty acid acylation of eukaryotic proteins has been divided into two classes [18]. Myristoylation (N-acylation) refers to the co-translational covalent attachment of myristic acid, via an amide linkage, to the NH_2-terminal glycine residue of several eukaryotic cellular proteins [19]. Palmitoylation refers to the post-translational addition of long-chain fatty acids (mainly palmitic acid C16:0), via a thioester bond (S-acylation) to cysteine residues of some integral and peripheral membrane proteins [20]. Ester linkage (O-acylation) between fatty acids and serine residues has also been reported [21].

In two previous studies on the metabolism of myristic acid and palmitic acid in cultured rat hepatocytes, we emphasized on several major differences between saturated fatty acid utilization by liver cells in terms of both lipid metabolism [22] and fatty acid acylation of proteins [23]. The present paper provides evidence that although it is very rapidly metabolized in cultured rat hepatocytes, lauric acid is used for protein acylation.

2. MATERIALS AND METHODS

2.1. Chemicals

Bovine serum albumin (BSA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), Williams' medium E (W 4125), insulin (bovine), dexamethasone, collagenase and lauric acid were purchased from Sigma (St. Louis, MO). A penicillin-streptomycin antibiotic mixture was provided by Eurobio (Les Ulis, France). Fetal bovine serum (FBS) was obtained from J. Boy (Reims, France). [1-^{14}C]-lauric acid was purchased from Amersham Biosciences (Les Ulis, France) and [11,12-^{3}H]-lauric acid was from American Radiolabeled Chemicals (St. Louis, MO). Solvent and other chemicals were obtained from VWR International (Fontenay-sous-Bois, France). High purity reagents for an HPLC application came from Fisher Labosi (Elancourt, France).

Falcon Primaria Petri culture dishes were used (AES, Combourg, France). The reagents for the electrophoretical application were purchased from Amersham Biosciences and BioRad (Marnes La Coquette, France).

2.2. Cultured rat hepatocytes

The experimental protocol was in compliance with European Union guidelines for animal care and use. Sprague-Dawley male rats (250 g body weight) obtained from the breeding center R. Janvier (Le Genest-St Isle, France) were food-deprived 12 h prior to hepatocyte preparation. Hepatocytes were obtained by collagenase perfusion in situ, as previously described [22]. The culture medium (Williams'E) was supplemented with 26 mM $NaHCO_3$; 12.5 mM HEPES; 15 µM BSA; 50000 IU·L^{-1} penicillin, 50 mg·L^{-1} streptomycin; 1 µM insulin and 1 µM dexamethasone. For plating only, the culture medium was supplemented with 7% (v/v) FBS. After plating, the cells were maintained in a humidified incubator at 37 ºC under 5% CO_2 in air. After 4 h, the plating medium was changed to a serum-free culture medium.

2.3. Incubation with radiolabeled lauric acid

Hepatocytes were incubated with radiolabeled lauric acid, either [1-^{14}C]-lauric acid to study the lipid metabolism or [11,12-^{3}H]-lauric acid (labeled on the 11 and 12 carbon positions to avoid excessive ^{3}H-acetyl-CoA production by β-oxidation) to investigate protein acylation. A [1-^{14}C]-lauric acid albuminic complex was prepared as previously described [5]. The final lauric acid concentration in serum-free Williams'E medium was 0.1 mM and the final specific radioactivity was 183 MBq·$mmol^{-1}$. At 24 h of culture, incubation was initiated by the replacement of the culture medium with 2 mL of [1-^{14}C]-lauric acid containing medium per dish. Incubation with [11,12-^{3}H]-lauric acid was initiated at the same time of culture by replacing the culture medium by 1 mL of serum-free fresh medium containing the tritiated fatty acid (4 nmol, 3.7 MBq per dish). Incubations were carried out at 37 ºC in a 5% CO_2 atmosphere.

2.4. Measurement of β-oxidation products

In order to measure the β-oxidation rate, we determined the incorporation of ^{14}C from [1-^{14}C]-lauric acid into CO_2 and acid-soluble metabolites, after a 4 h incubation. The production of $^{14}CO_2$ was measured as previously described [22]. Briefly, at the end of the incubation with [1-^{14}C]-lauric acid, hepatocytes and medium were transferred into a small glass vial containing 250 µL $HClO_4$ (7 M). Each vial was sealed with a rubber cap fitted with a plastic center well. Hyamine hydroxide (Sigma, St Louis, MO) was added to the suspended plastic well by piercing the cap with a syringe. $^{14}CO_2$ trapped in hyamine hydroxide during 2 h was precipitated and counted (Packard Tri-Carb 1600 TR, Meriden, CT). The production of radiolabeled acid-soluble metabolites was also measured as previously described [22]. Hepatocytes and medium were transferred into 250 µL $HClO_4$ (7 M) containing tubes, placed at 0 ºC for 15 min and centrifuged (2500 × *g*, 10 min, 2 ºC). The supernatant was washed 3 times with hexane to remove any remaining ^{14}C-labeled fatty acids and radioactivity was counted.

2.5. Lipid and fatty acid analysis

At the end of the incubation with [1-^{14}C]-lauric acid, the medium was taken off and kept for lipid extraction. The cells were washed with PBS (150 mM NaCl; 5 mM Na Phosphate; pH 7.4) and harvested in PBS. After centrifugation, the cell pellet was kept for lipid extraction. Lipids from the medium and from the cells were extracted with hexane/isopropanol (3:2 v/v) as previously

described [22]. Cellular lipids were saponified for 30 min at 70 °C by 1 mL of 2 M KOH in ethanol. The fatty acids (FA) were liberated by acidification and extracted with diethylether. Total cellular FA were then subjected to liquid scintillation counting.

For lipid species analysis, lipid extracts from the cells and the medium were submitted to Thin Layer Chromatography (TLC) separation [22]. Cellular phospholipids (PL), cellular TG and secreted TG were collected and saponified as described above. Radioactivity of the FA from each lipid species was counted.

For individual FA analysis, FA from each lipid extract were converted to fatty acid naphthacyl esters and separated on HPLC (Alliance integrated system, Waters, St-Quentin-en-Yvelines, France) as previously described [24]. The elution of naphthacyl derivatives was monitored by UV absorbance at 246 nm (Tunable absorbance detector 486, Waters). Peaks corresponding to radiolabeled FA were collected (Fraction collector, Waters) and subjected to liquid scintillation counting.

2.6. Acylation of cell protein

At the end of incubation with [11,12-^{3}H]-lauric acid, the medium was removed, the cells were washed twice with PBS containing 1 mM phenylmethylsulfonylfluoride (PMSF) and 10 μg·mL^{-1} aprotinin, and harvested in the same solution. After centrifugation, cellular lipids which were non-covalently bound to proteins were extracted three times with hexane/isopropanol (3:2 v/v). After each lipid extraction, the proteins were separated from the solvent by centrifugation (4000 × *g*, 10 min, 4 °C). The final delipidated protein pellet was dried under a stream of N_2 and saved for electrophoretic analysis. Two-dimensional (2-D) electrophoresis was carried out as previously described [23]. Briefly, protein samples (300 μg) were resuspended in urea-buffer (7 M urea, 2 M thiourea, 4% (v/v) CHAPS, 0.3% (v/v) DTT, and 2% (v/v) IPG buffer 4-7). After 20 h focusing in the first dimension (48 kVh), the IEF (isoelectric focusing) gels were loaded for the second dimension (SDS-PAGE, 12% w/v acrylamide separating gel). After staining with Coomassie Blue, the gels were treated with Enhance (Perkin Elmer Life Science, France) for fluorography, dried and then exposed to Hyperfilm MP (Amersham Biosciences) at –80 °C for periods varying from several days to weeks. The radiolabeled spots corresponding to the resolved proteins were localized by superimposing the fluorographs and the original gels.

2.7. The release and identification of fatty acids covalently bound to proteins

Following 2-D electrophoresis, the proteins were electroblotted on a nitrocellulose membrane in a semidry electroblotter (Ancos, Denmark) using 39 mM glycine, 1.3 mM SDS, 20% (v/v) methanol, 48 mM Tris, pH 8.4 as the buffer. The resolved Ponceau Red-stained proteins corresponding to the previously detected radiolabeled spots were excised from the membrane and washed three times with 1 mL chloroform/ methanol (2:1 v/v) to remove any remaining non-covalently bound radiolabeled lipids. Five pieces of nitrocellulose containing the same protein were pooled for subsequent fatty acid analysis [23]. To release thioester- and ester-linked FA, the protein was first saponified in 1 mL of 2 M KOH in ethanol at 70 °C for 4 h under nitrogen. Following the addition of 1 mL 3 N HCl and 4 mL H_2O, liberated FA were extracted with diethylether. The subsequent release of the possibly remaining amide-linked FA was realized by adding 4 mL 12 N HCl to each tube followed by hydrolyzation at 100 °C for 15 h under nitrogen. Released FA were then extracted with 4 mL diethylether. FA released from each protein by these two successive treatments were converted to fatty

acid naphthacyl esters and separated on HPLC as described above [24].

2.8. Protein and DNA measurements

The cellular protein content of a culture dish was determined by a modified Lowry procedure [25]. The DNA content was measured by a fluorometric method [26] using bisbenzimidazole (Hoechst H33258).

3. RESULTS

3.1. Clearance of [1-^{14}C]-lauric acid from the culture medium and ^{14}C incorporation into fatty acids of cellular and secreted lipids

Cultured rat hepatocytes (3 independent cultures) at 24 h of culture were incubated with lauric acid. Figure 1 presents the clearance of [1-^{14}C]-lauric acid from the culture medium and the incorporation of ^{14}C from lauric acid into FA of cellular lipids and secreted TG as a function of the incubation time with cultured hepatocytes. The results are expressed as the % of the initial radioactivity added to the medium and recovered in each fraction.

The disappearance of [1-^{14}C]-lauric acid in the culture medium showed that the substrate was rapidly taken up by the cells. After a 4 h incubation, only 5.0 ± 2.2% of the initial precursor remained in the medium. The incorporation of ^{14}C into total cellular FA increased during the first 2 h of incubation and then remained at a steady-state level during the remaining 10 h (about 24% of the radioactivity initially added to the medium). The distribution of ^{14}C from lauric acid between the FA of the lipid species showed a rapid incorporation into cellular TG at first (12.1 ± 2.9% after 2 h). The initial incorporation of ^{14}C into the FA of cellular PL was slower. In the 2–12 h interval, the

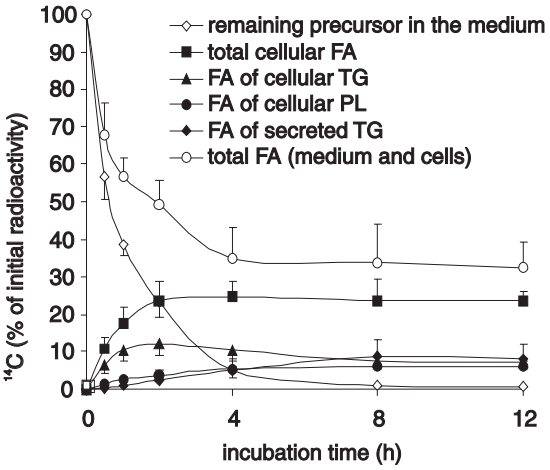

Figure 1. Clearance of [1-^{14}C] lauric acid from the culture medium and ^{14}C incorporation into fatty acids of lipids as a function of incubation time with cultured rat hepatocytes. Incubation with [1-^{14}C]-labeled lauric acid (100 μmol·L^{-1} and 183 MBq·mmol^{-1}) was started by addition of 2 mL Williams' medium E containing the radiolabeled fatty acid complexed with BSA into each cell culture dish. Lipid and fatty acid analyses are described in Materials and Methods. The results are expressed as the percent of the initial radioactivity added to the culture medium and recovered in each fraction. Each value is the mean ± SD of 3 independent cell cultures. Protein content was 1.46 ± 0.36 mg·dish^{-1} and DNA content was 25.10 ± 8.72 μg·dish^{-1}.

radioactivity of cellular TG fatty acids decreased (7.2 ± 2.0% after 12 h), while that of PL fatty acids was still increasing (6.0 ± 1.3% after 12 h). The incorporation of ^{14}C into the FA of secreted TG reached 8.2 ± 4.0% of the initially added radioactivity after 12 h of incubation.

3.2. β-Oxidation of [1-^{14}C]-lauric acid

The production of $^{14}CO_2$ and radiolabeled acid-soluble metabolites was measured after 4 h incubation using the same 3 cell cultures. Total lauric acid oxidation represented 38.7 ± 4.4% of the initially added radioactivity, including a $^{14}CO_2$ production of 0.5 ± 0.1% and a radiolabeled acid-soluble metabolite production of 38.2 ± 4.5%.

3.3. Conversion of [1-^{14}C]-lauric acid to other fatty acids

By using the same 3 cell cultures, the labeled fatty acids incorporated into cellular lipids and secreted TG were identified and their individual radioactivity was determined as a function of incubation time. Radiolabeled saturated and monounsaturated fatty acids were detected after incubation with [1-^{14}C]-lauric acid but only the main

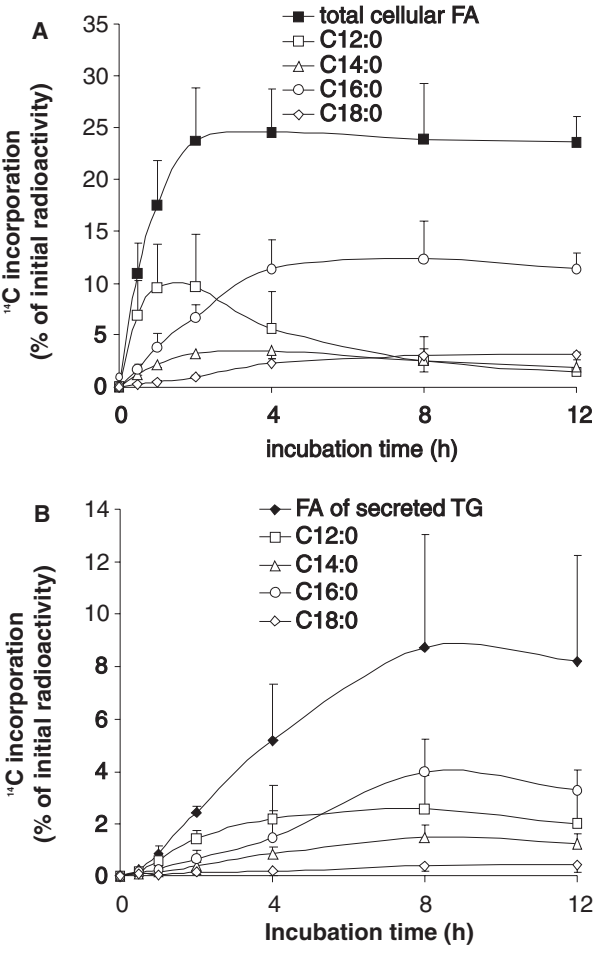

Figure 2. Conversion of [1-^{14}C] lauric acid to longer fatty acids and incorporation of the new synthesized radiolabeled fatty acids into cellular lipids (A) and secreted TG (B) as a function of incubation time with cultured rat hepatocytes. The experimental conditions were the same as in Figure 1.

radiolabeled fatty acids (C12:0, C14:0, C16:0 and C18:0) are presented in Figure 2. The incorporation of lauric acid in cellular lipids increased during the first 2 h of incubation (Fig. 2A) and during 8 h in secreted TG (Fig. 2B). Then lauric acid decreased during the remaining incubation period in total cellular fatty acids (1.5 ± 1.2% of the initially added [1-^{14}C]-lauric acid was recovered after 12 h incubation) and remained relatively constant in secreted TG (2.0 ± 1.2% of initial radioactivity after 12 h). Lauric acid was rapidly and strongly converted to longer saturated fatty acids. A single elongation of the substrate produced radiolabeled myristic acid which first increased (3.5 ± 0.8% of initial radioactivity after 4 h in cellular lipids and 1.5 ± 0.5% after 8 h in

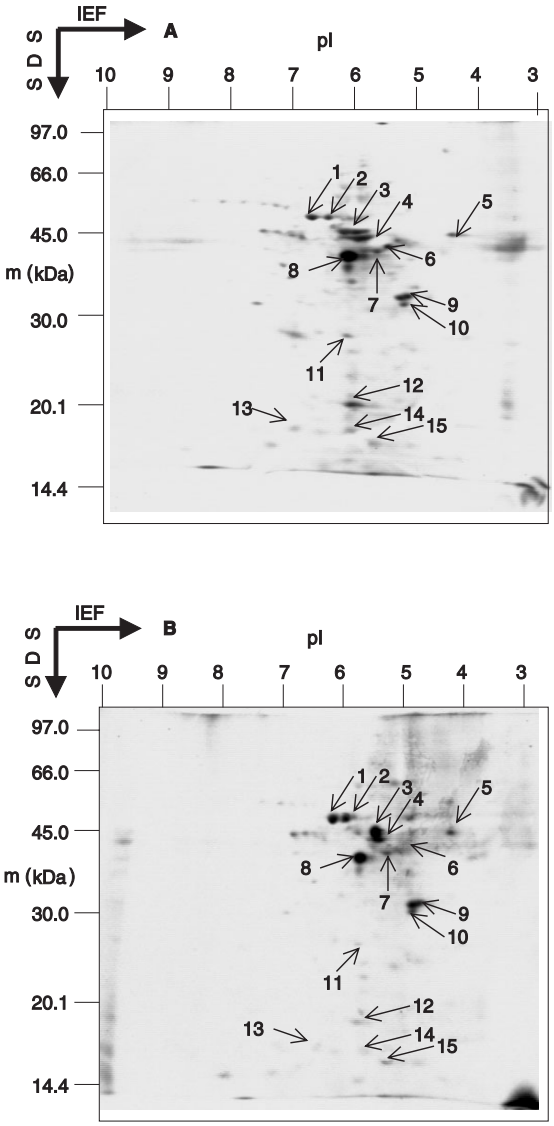

Figure 3. ^{3}H incorporation from [11,12-^{3}H] lauric acid into proteins of cultured rat hepatocytes. The cells were incubated for 3 h (A) and 6 h (B) with [11,12-^{3}H] lauric acid (4 nmol and 3.7 MBq per dish). After each of these incubation times, the cellular delipidated proteins were subjected to two-dimensional electrophoresis (IEF 3-10 followed by 12% w/v acrylamide separating gel) followed by fluorography. Each gel was loaded with 300 µg of protein. The gels were exposed to Hyperfilm MP film at –80 °C for 20 days.

secreted TG) before decreasing (respectively 2.0 ± 0.8% and 1.2 ± 0.3% after 12 h). Newly synthesized radiolabeled palmitic acid was the major radiolabeled fatty acid in cellular lipids after 3 h incubation, and in secreted TG after 5 h incubation, reaching respectively 11.4 ± 1.5% and 4.0 ± 1.3% after 12 h. Radiolabeled stearic acid was also identified, but in smaller amounts (respectively 3.1 and 3.0% in total cellular fatty acids and secreted TG after 12 h of incubation). Radiolabeled cellular monounsaturated fatty acids (C12:1, C14:1, C16:1 and C18:1) did not account for more than 2% of cellular fatty acids whatever the incubation time (data not shown).

3.4. ^{3}H incorporation from [11,12-^{3}H]-lauric acid into cellular proteins

In order to detect the acylated proteins, rat hepatocytes at 24 h of culture were incubated with [11,12-^{3}H]-lauric acid for 3 h and 6 h. The delipidated cellular proteins were subjected to 2-D electrophoresis followed by fluorography (Fig. 3). The radioactivity appeared to be selectively and reproducibly incorporated into 15 major radiolabeled proteins. More than 20 other proteins were also weakly radiolabeled.

3.5. Identification of ^{3}H-fatty acids released from radiolabeled proteins

In order to determine the kind of linkage between the radiolabel and the proteins, some of the previously well-resolved detected proteins (after 2-D electrophoresis and western blotting followed by spot excision) were submitted to alkaline hydrolysis followed by acid hydrolysis to release the possibly S- (or O-) and N-linked fatty acids, respectively. These proteins were chosen based on previous results obtained with myristic acid [23]. The identification of the released radiolabeled fatty acids was achieved by HPLC analysis (Fig. 4).

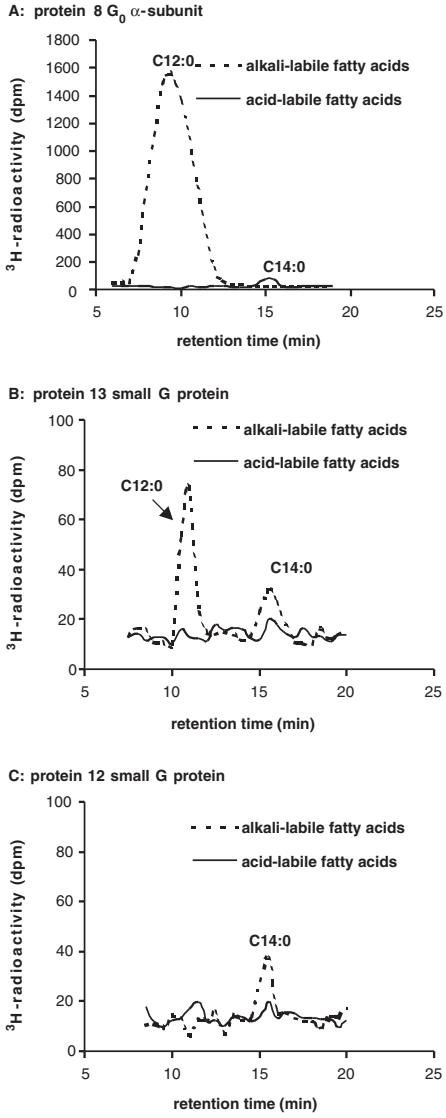

Figure 4. HPLC elution profiles of radiolabeled fatty acids released by successive alkaline and acid treatment of several radiolabeled proteins (A: protein 8; B: protein 13; C: protein 12). After incubation for 3 h with [11,12-^{3}H] lauric acid (4 nmol and 3.7 MBq per dish), 2-D electrophoresis and western blotting followed by spot excision, each protein was submitted to alkaline hydrolysis followed by acid hydrolysis to release the S- (or O-) and N-linked fatty acids, respectively. The identification of the fatty acids was achieved by HPLC analysis of each extract.

In a previous work [23], protein 8 (Fig. 3) was identified as the α-subunit of a heterotrimeric G_0 protein. After incubation with [11,12-^{3}H]-lauric acid, this protein (Fig. 4A) exhibits an alkali-labile thioester or ester linkage with lauric acid, and an amide linkage with myristic acid which is released by acid hydrolysis but not by the initial alkaline treatment. Labeled myristic acid is presumably formed endogenously through the elongation of the precursor. This result showed that this protein was both N-acylated with myristic acid and acylated with lauric acid.

In the same previous work [23], proteins 12 and 13 (Fig. 3) were identified as small G proteins. After incubation with [11,12-^{3}H]-lauric acid, protein 13 (Fig. 4B) exhibits a thioester or ester linkage with the precursor lauric acid and with its elongation product (myristic acid), lauric acid being the most abundant. Protein 12 (Fig. 4C) was acylated with the single endogenous alkali-labile myristic acid.

Several other radiolabeled proteins were analyzed (proteins 1, 3 and 5). These analyses did not provide evidence for the presence of ^{3}H-fatty acids covalently attached to the proteins (data not shown). This result suggests that radiolabeling of these proteins could be ascribed to the incorporation of ^{3}H-amino acid residues derived from the strong β-oxidation of lauric acid shown above.

4. DISCUSSION

The purpose of this work was to investigate lauric acid metabolism in cultured rat hepatocytes, in terms of both lipid metabolism and potential acylation of proteins. The cells were incubated with radiolabeled lauric acid, either [1-^{14}C]-lauric acid to study the lipid metabolism or [11,12-^{3}H]-lauric acid to investigate protein acylation.

We first investigated the incorporation into lipids and the β-oxidation rate of [1-^{14}C]-lauric acid. Although lauric acid was rapidly taken up by the cells (95% of the precursor was cleared from the medium after 4 h), the ^{14}C incorporation into total cellular fatty acids was low after 4 h incubation, representing only 24% of the initial radioactivity (Fig. 1). In order to explain this low incorporation into lipids, we measured the β-oxidation rate of lauric acid and found that about 39% of the initial precursor was oxidized after a 4 h incubation. This high rate of oxidation, consistent with a study carried out on isolated hepatocytes [4], occurred in our culture system where Williams' E medium contains 11 mM glucose and 1 μM insulin, all conditions that presumably favor lipid esterification of fatty acids rather than oxidation [22]. When similar experiments were carried out with myristic acid and palmitic acid in the same cellular model [22], we found that [1-^{14}C]-myristic acid oxidation represented 15% and [1-^{14}C]-palmitic acid oxidation represented 2.5% of the initial radioactivity after a 4 h incubation.

Then, we characterized the radiolabeled fatty acids derived from lauric acid conversion and their relative incorporation into cellular lipids and secreted TG (Fig. 2). As already shown in isolated hepatocytes [4], lauric acid was rapidly and strongly converted to longer saturated fatty acids. At the end of the incubation, radiolabeled palmitic acid (Fig. 2) was the major radiolabeled fatty acid in cellular lipids and in secreted TG. Altogether, these results show that lauric acid was rapidly metabolized especially through the influence of oxidation and elongation, and therefore rapidly disappeared in hepatocytes. After 12 h of incubation, cellular lauric acid represented only 1.5% of the initially added radioactivity (Fig. 2).

Then, we investigated if lauric acid, even though rapidly metabolized in this cellular model, could be a substrate for the acylation of proteins in cultured rat hepatocytes. As previously described with myristic acid [23], we studied the incorporation of radioactivity from this fatty acid into cellular

proteins using 2-D electrophoresis separation followed by fluorography. In order to detect hepatic proteins acylated with lauric acid, the nature of the covalent modification (N- and/or S-linkage) by fatty acids and the identity of these fatty acids were further investigated regarding some of the well-resolved radiolabeled proteins.

The results first showed a reproducible and selective incorporation of radioactivity from lauric acid into several proteins (Fig. 3). Since the presence of radioactivity resisted the successive extensive delipidation, urea-denaturation, 2-D electrophoresis and staining procedure, it was likely that the radioactivity was attached to some proteins via covalent linkages. The kind of linkage between the radiolabel and the proteins was therefore investigated.

Only some of the proteins already identified in a previous work [23] and shown to be acylated after incubation with [9,10-^{3}H]-myristic acid were analyzed. Protein 8 was previously identified as the α-subunit of a heterotrimeric G_0 protein [23]. This protein has been shown to be dually acylated (N-terminally myristoylated with myristic acid and S-acylated with palmitic acid) in several cellular models [27]. In cultured rat hepatocytes [23], after incubation with [9,10-^{3}H]-myristic acid, this heterotrimeric G protein α-subunit was also N-myristoylated with myristic acid and acylated with palmitic acid. In the present work, after incubation with [11,12-^{3}H]-lauric acid, this protein was attached to radiolabeled lauric acid *via* a thioester or ester linkage and to radiolabeled myristic acid via a linkage which resisted the basic treatment but was broken by acid hydrolysis (Fig. 4A), which indicates that the linkage was an amide-type linkage.

Proteins 12 and 13 were previously identified as small G proteins [23], known to be firstly prenylated and then S-acylated [28] in a number of cellular models. In cultured rat hepatocytes [23], after incubation with [9,10-^{3}H]-myristic acid, these proteins exhibit an alkali-labile thioester or ester linkage with the radiolabeled precursor. In the present work, after incubation with [11,12-^{3}H]-lauric acid, these proteins were S- or O-acylated with lauric acid and/or myristic acid (Figs. 4B and 4C).

These results show that exogenous lauric acid is used for protein acylation in cultured rat hepatocytes. In this model, lauric acid can therefore substitute for palmitic acid in S- and/or O-acylation, as already shown for myristic acid [23]. S-acylated proteins have been described to be primarily attached to palmitic acid [29]. However it is still not clear whether palmitoylation is specific or even selective for palmitate. For instance, polyunsaturated fatty acids, such as arachidonic acid or eicosapentaenoic acid, have been shown to be involved in post-translational modifications of proteins in platelets [30]. It has been shown, in isolated mitochondria from rat liver, that some proteins are acylated by myristic acid [31]. This acylation is rapidly reversible and inhibited by lauric acid, suggesting that lauric acid, like myristic acid, is a potential substrate for protein acylation. In our present work, exogenous lauric acid was elongated to endogenous myristic acid that was also used for protein acylation (Fig. 4).

In our results, we did not find any C12:0 attached to the N-terminal glycine of protein 8 (Fig. 4). The possibility that lauric acid could be used for the N-acylation of proteins not analyzed in this study, cannot, however, be ruled out. N-myristoylation has generally been considered to be highly specific for myristoyl-CoA. However, in the retina, the α-subunit of the heterotrimeric G protein transducin and other retinal proteins can be heterogeneously N-acylated with lauric acid, two unsaturated fatty acids with 14 carbons (C14:1 n-9 and C14:2 n-6) and myristic acid [32]. The reason for this tissue-specific heterogeneous N-acylation is still not clear [33]. Our results suggest that rat liver myristoyl-CoA: protein N-myristoyl-transferase is highly specific for myristic acid since endogenous myristic acid coming from the elongation of lauric acid

was used to myristoylate the α-subunit of heterotrimeric G_0 protein (Fig. 4A).

In conclusion, this work provides evidence that, even if exogenous lauric acid is rapidly metabolized in rat liver cells, it is used for protein acylation. The functional significance of covalent linkage formation with different saturated or unsaturated fatty acids on the biological activities of the acylated proteins is not known. From a nutritional point of view, it is important to notice that dietary saturated fatty acids may be used for protein acylation.

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