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Peroxisomal–microsomal communication in unsaturated fatty acid metabolism

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Abstract The addition of 1-acyl-*sn*-glycero-3-phosphocholine (1-acyl-GPC) to peroxisomes decreased the production of acid-soluble radioactivity formed by β-oxidation of [1-^{14}C]arachidonate due to substrate removal by esterification into the acceptor. This peroxisomal-associated acyl-CoA:1-acyl-GPC acyltransferase activity was due to microsomal contamination. The production of acid-soluble radioactivity from [1-^{14}C]7,10,13, 16-22:4, but not from [3-^{14}C]7,10,13,16- 22:4 was independent of 1-acyl-GPC, with and without microsomes. By comparing rates of peroxisomal β-oxidation with those for microsomal acylation, it was shown that the preferred metabolic fate of arachidonate, when added directly to incubations, or generated via β-oxidation, was esterification by microsomal 1-acyl-GPC acyltransferase, rather than continued peroxisomal β-oxidation.

Key words: Microsome; Peroxisome; Arachidonic acid; 7,10,13,16-Docosatetraenoic acid; β-Oxidation; Acylation

1. Introduction

It is well established that peroxisomes partially β-oxidize long chain acids and the resulting chain-shortened catabolites may then move to mitochondria where β-oxidation is completed [1]. Many long chain unsaturated fatty acids are also partially β-oxidized and the chain-shortened products are subsequently incorporated into membrane lipids [2–5].

We recently refuted the commonly accepted but untested hypothesis that 7,10,13,16,19-22:5 was metabolized to 4,7,10,13,16,19-22:6 by a microsomal acyl-CoA-dependent 4-desaturase. These studies suggested that 7,10,13,16,19-22:5 was metabolized to 6,9,12,15,18,21-24:6 in the endoplasmic reticulum. The 24-carbon acid must then move to a site of β-oxidation, where it is chain shortened to 4,7,10,13,16,19-22:6, which is then incorporated into membrane lipids [4]. Other studies have shown that 7,10,13,16-22:4 is metabolized to 4,7,10,13,16-22:5 via an analogous pathway [6].

Several types of evidence suggest that the partial β-oxidation–acylation process regulates the intracellular movement of fatty acids between the endoplasmic reticulum and peroxisomes. Martinez [7] reported that liver phospholipids of a patient with Zellweger's disease, unlike those from controls, contained low levels of esterified 4,7,10,13,16-22:5 and 4,7,10,13,16,19-22:6. This finding suggests that peroxisomes are required for the synthesis of 4,7,10,13,16-22:5 and 4,7,10,13,16,19-22:6. When 7,10,13,16-22:4 was fed to rats raised on a diet devoid of fat, only small amounts of the dietary acid were incorporated into membrane lipids, but large amounts of 5,8,11,14-20:4 were esterified [3]. When fibroblasts from control patients, as well as those with Zellweger's disease, were incubated with [3-^{14}C]7,10,13,16-22:4, both types of cells metabolized the substrate into acid soluble compounds. However, it was only possible to detect esterified 5,8,11,14-20:4 in lipids from the control cells [8]. The findings suggest that the partial β-oxidation process is confined to peroxisomes.

We recently carried out a series of mixing experiments with peroxisomes and microsomes, using [1-^{14}C]- and [3-^{14}C]-labeled 7,10,13,16-22:4 as model substrates. The conclusion of these studies was that 7,10,13,16-22:4 was rapidly β-oxidized to 5,8,11,14-20:4 in peroxisomes and that the preferred metabolic fate of 5,8,11,14-20:4 was to move out of peroxisomes to the endoplasmic reticulum where it was esterified into 1-acyl-GPC by acyl-CoA:1-acyl-GPC acyltransferase [9]. If peroxisomes also contained this enzymatic activity, it is possible that when 5,8,11,14-20:4 is produced, it is esterified in peroxisomes and the resulting diacyl-GPC might then be carried to other intracellular organelles by phospholipid carrier proteins. The studies reported here were carried out to distinguish between these two possibilities.

2. Materials and methods

2.1. Chemicals

ATP, NAD$^+$, NADP$^+$, CoASH, HEPES, *N*-tris(hydroxymethyl-2-aminosulfonic acid, 5,5′-dithiobis-(2-nitrobenzoic) acid, dithiothreitol, and essential fatty acid-free bovine serum albumin were from Sigma. Lactate dehydrogenase and Nycodenz (Accudenz) were obtained, respectively, from Boehringer Mannheim and Accurate Chemicals and Scientific Corp. 1-Palmitoyl-*sn*-glycero-3-phosphocholine (1-acyl-GPC) was from Avanti, while arachidonic acid and 7,10,13,16-22:4 were obtained from Nu-Check Prep. [1-^{14}C]5,8,11,14-20:4 as well as [1-^{14}C]- and [3-^{14}C]-labeled 7,10,13,16-22:4, were made by total synthesis [4,9].

2.2. Isolation of peroxisomes and microsomes

Sprague–Dawley rats were maintained on a chow diet containing 0.5% clofibrate (Dyets, Bethlehem, PA) for 8 days prior to being sacrificed. Liver peroxisomes were isolated by centrifuging the light mitochondrial fraction through a Nycodenz gradient [10] as previously described [9]. The peroxisomal pellet was suspended in the incubation medium which contained 130 mM KCl, 20 mM HEPES (pH 7.2) and the protein concentration was adjusted to 3 mg/ml using the Coomassie blue reagent (Pierce Chemical Co.) to assay protein. The purity of peroxisomes was determined by marker enzyme analysis as described elsewhere [9]. Peroxisomes were also analyzed for microsomal contamination by measuring NADPH-cytochrome *c*-reductase activity [11]. Microsomes were isolated by centrifuging the light mitochondrial fraction at $23,000 \times g$ for 15 min and then at $110,000 \times g$ for 1 h. The microsomes were suspended in the HEPES-KCl buffer and stored at −80°C.

2.3. Acylation of 1-acyl-GPC

The CoA derivatives of 5,8,11,14-20:4 and 7,10,13,16-22:4 were made via their mixed anhydrides [12]. Maximum rates of acylation were

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determined spectrophotometrically [13]. The incubation mixture consisted of 50 μM acyl-CoA, 300 μM 1-acyl-GPC, 2 mM 5,5′-dithiobis-(2-nitrobenzoic) acid, and 200 μg of protein in 1 ml of 0.1 M Tris-HCl (pH 7.4). Only 25 μg of protein was used to measure the acylation of arachidonoyl-CoA into 1-acyl-GPC by microsomes.

2.4. Peroxisomal β-oxidation

Peroxisomes (300 μg of protein/ml) were incubated at 37°C in a shaking water bath in a medium that contained 130 mM KCl, 20 mM HEPES, 0.1 mM EGTA, 0.5 mM NAD^+, 0.1 mM $NADP^+$, 0.2 mM CoASH, 10 mM $Mg^{+2}ATP$, 0.1 mM dithiothreitol, 20 mM pyruvate, 2 units of lactate dehydrogenase and 100 μM [1-^{14}C]- or [3-^{14}C]-labeled fatty acid that was bound to bovine serum albumin so that the fatty acid to albumin molar ratio was 2:1. Other incubations also contained 100 μM 1-acyl-GPC with or without 300 μg of microsomal protein. At various times 200 μl aliquots of the incubation mixture were removed and added to an equal volume of 5% $HClO_4$. After centrifugation, 200 μl aliquots were counted to measure acid-soluble radioactivity. Rates of β-oxidation were then calculated from the specific activity of the substrate which was always 2 Ci/mol [14]. An identical protocol was followed to determine how the addition of 1-acyl-GPC, with or without microsomes, modified the rate of β-oxidation. In order to quantify the esterification of fatty acids into 1-acyl-GPC, the incubation mixture was extracted by the method of Folch et al. [15]. The bottom organic layer was taken to dryness. The lipids were dissolved in $CHCl_3$ and applied to a Pasteur pipette packed with Unisil (Clarkson Chemical Co., Williamsport, PA). Neutral lipids and phospholipids were recovered by eluting the column with 10 ml of $CHCl_3$ and MeOH, respectively. The solvents were removed under nitrogen. Two ml of $CHCl_3$/MeOH (2:1, by vol.) were added and an aliquot was removed and counted. The remainder of the phospholipids were interesterified and the methyl esters were separated by reverse phase high performance liquid chromatography as previously described [9].

3. Results and discussion

The results in Fig. 1 compare rates of β-oxidation of [1-^{14}C]5,8,11,14-20:4 when it was incubated with peroxisomes with and without 1-acyl-GPC, as well as when 1-acyl-GPC and microsomes were incubated with peroxisomes. The addition of 1-acyl-GPC to incubations containing peroxisomes decreased the generation of acid-soluble radioactivity which was further depressed by the addition of microsomes. After 30 min the amount of radioactivity (in % of what had been added to the incubation) esterified in phospholipids of incubations containing 1-acyl-GPC with or without microsomes was 81% and 61%, respectively.

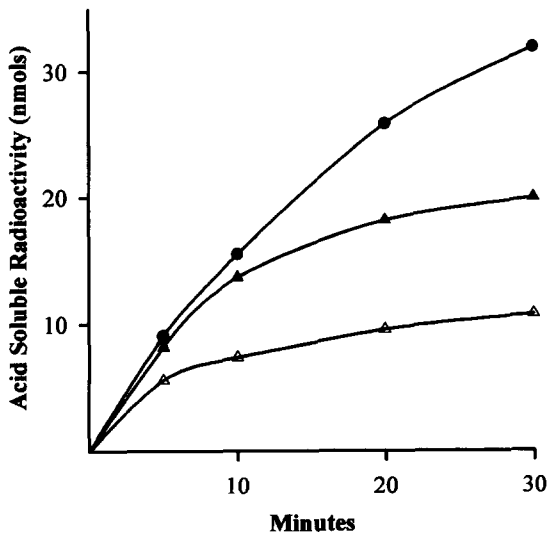

Fig. 1. The time-dependent peroxisomal β-oxidation of [1-^{14}C]5,8,11,14-20:4. Peroxisomes (300 μg of protein) were incubated with 100 μM substrate (●), as well as when incubations also contained 100 μM 1-acyl-GPC (▲), and in addition, when 100 μM 1-acyl-GPC and 300 μg of microsomal protein were included in the incubations (△).

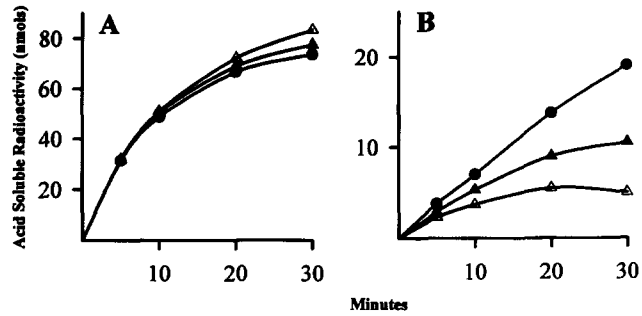

Fig. 2. The time-dependent β-oxidation of [1-^{14}C]7,10,13,16-22:4 (A) and [3-^{14}C]7,10,13,16-22:4 (B). Peroxisomes (300 μg of protein) were incubated with 100 μM substrate (●), as well as when incubations also contained 100 μM 1-acyl-GPC (▲), and, in addition, when 100 μM 1-acyl-GPC and 300 μg of microsomal protein were included in the incubations (△).

The implications of these findings were that peroxisomes contained acyl-CoA:1-acyl-GPC acyltransferase activity. When microsomes were now added to the incubation, the more dramatic depression in the production of acid-soluble activity was due to enhanced substrate removal by esterification. We previously measured microsomal contamination in our peroxisomal preparation, using glucose-6-phosphatase as a marker enzyme [9]. The specific activity in peroxisomes relative to that of the total liver homogenate was 1.5. We now used a somewhat more sensitive assay for measuring microsomal contamination by determining the specific activity of NADPH-cytochrome *c*-reductase activity in both peroxisomes and microsomes [11]. We also measured acyl-CoA:1-acyl-GPC acyltransferase activity with arachidonoyl-CoA as substrate in both peroxisomes and microsomes. In two separate experiments, the microsomal contamination in peroxisomes, as determined by NADPH-cytochrome *c*-reductase activity, was 17.9% and 6.6%, respectively. When microsomal contamination in peroxisomes was assayed by measuring arachidonoyl-CoA:1-acyl-GPC acyltransferase, these values were, respectively, 16.5% and 8.7%. These results show that the esterification activity observed when peroxisomes were incubated with 1-acyl-GPC is due to microsomal contamination. In this regard it is important to note that it is difficult to prepare peroxisomes totally devoid of microsomal contamination. Das et al. [10] reported that even when liver peroxisomes, prepared from chow-fed rats, were subjected to double Nycodenz centrifugation, the peroxisomes were still contaminated with 3–6% microsomal protein.

The results in Fig. 2A show that the rate of β-oxidation of [1-^{14}C]7,10,13,16-22:4 was, in essence, the same as when incubations contained peroxisomes, with and without 1-acyl-GPC, as well as when incubations contained peroxisomes, microsomes and 1-acyl-GPC. Conversely, with [3-^{14}C]7,10,13,16-22:4 as substrate, the rate of production of acid-soluble radioactivity was lower than for the [1-^{14}C]-labeled substrate (Fig. 2B). When 1-acyl-GPC was added, the rate of production of acid-soluble radioactivity was depressed, and again, the addition of 1-acyl-GPC and microsomes further decreased the generation of acid-soluble radioactivity.

The amounts of radioactivity esterified in phospholipids from incubations containing 1-acyl-GPC with [1-^{14}C]- or [3-^{14}C]-labeled 7,10,13,16-22:4 were, respectively, 8% and 61% of what had been added to the incubations. These values were 13% and 62%, respectively, when microsomes were included in the incubations. Of the radioactivity esterified into phospholipids from incubations containing [3-^{14}C]7,10,13,16-22:4 and 1-acyl-GPC, 92% was 5,8,11,14-20:4 and only 8% was 7,10,13,16-22:4. In the presence of both 1-acyl-GPC and microsomes, these values were 81% and 19%, respectively. These data show that the second cycle of β-oxidation of [3-^{14}C]7,10,13,16-22:4 was depressed due to the esterification of 5,8,11,14-20:4 into phospholipids. Conversely, [1-^{14}C]-labeled 7,10,13,16-22:4 was not a good substrate for esterification and the presence or absence of microsomes, in addition to 1-acyl-GPC, did not decrease the production of acid- soluble radioactivity.

The rates of acylation of 7,10,13,16-22:4 and 5,8,11,14-20:4 into 1-acyl-GPC by microsomes from rats fed clofibrate were, respectively, 18 and 159 nmol/min/mg of microsomal protein. The β-oxidation studies as well as the acylation specific activities allow us to formulate a hypothesis suggesting that competition between peroxisomal β-oxidation and endoplasmic reticulum-associated acylation reactions play a major role in regulating membrane lipid fatty acid composition. The rate of β-oxidation of [1-^{14}C]7,10,13,16-22:4 at 5 min was about 21 nmol/min/mg of peroxisomal protein, while this value for [1-^{14}C]5,8,11,14-20:4 was about 5 nmol/min/mg of protein. When peroxisomes were incubated with 7,10,13,16-22:4, it was readily β-oxidized, but it was a relatively poor substrate for microsomal acylation into 1-acyl-GPC. Conversely, when [1-^{14}C]5,8,11,14-20:4 was incubated directly with peroxisomes, or generated by β-oxidation of [3-^{14}C]7,10,13,16-22:4, it was a relatively poor substrate for β-oxidation. The preferred metabolic fate of 5,8,11,14-20:4 was to move out of peroxisomes, via an as yet unknown pathway, where it was rapidly incorporated into 1-acyl-GPC by microsomal 1-acyl-GPC acyltransferase. Indeed, this type of specificity for peroxisomal β-oxidation versus microsomal acyltransferase specificity is consistent with in vivo studies. When [3-^{14}C]7,10,13,16-22:4 was injected into the tail vein of rats fed a chow diet, 96% of the esterified radioactivity was 5,8,11,14-20:4 with the remaining 4% being the substrate [16].

In summary, our results show that peroxisomes from clofibrate fed rats are contaminated with microsomes even when they are purified by Nycodenz-gradient centrifugation. This contamination may lead to the misinterpretation of results, particularly when the microsomal enzyme has a high specific activity, as it does for arachidonoyl-CoA:1-acyl-GPC acyltransferase. Indeed, our studies show that this activity can be used as a sensitive marker enzyme for microsomal contamination in peroxisomes. In addition, our data using 7,10,13,16-22:4 as a model substrate show that fatty acids produced in the endoplasmic reticulum may move to peroxisomes where they are chain shortened. These products – i.e. 5,8,11,14-20:4 – must then move back to the endoplasmic reticulum where they are used as substrates for membrane lipid biosynthesis. Indeed, these results form the basis of ongoing experiments to determine what regulates the biosynthesis of 4,7,10,13,16-22:5 and 4,7,10,13,16,19-22:6. The immediate precursors of these two acids are the microsomal metabolites 6,9,12,15,18-24:5 and 6,9,12,15,18,21-24:6. If these 24-carbon acids were esterified into acceptors in the endoplasmic reticulum, it would, in essence, block their movement to peroxisomes for partial β-oxidation, respectively, to 4,7,10,13,16-22:5 and 4,7,10,13,16,19-22:6.

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References

[1] Osmundsen, H., Bremer, J. and Pedersen, J.I. (1991) Biochim. Biophys. Acta 1085, 141–158.
[2] Verdino, B., Blank, M.L., Privitt, O.S. and Lundberg, W.O. (1964) J. Nutr. 83, 234–238.
[3] Sprecher, H. (1967) Biochim. Biophys. Acta 144, 293–365.
[4] Voss, A., Reinhart, M., Sankarappa, S. and Sprecher, H. (1991) J. Biol. Chem. 266, 19995–20000.
[5] Stoffel, W., Ecker, W., Assad, H. and Sprecher, H. (1970) Hoppe-Seyler's Z. Physiol. Chem. 351, 1545–1554.
[6] Mohammed, B.S., Sankarappa, S., Geiger, M. and Sprecher, H. (1995) Arch. Biochem. Biophys. 317, 179–184.
[7] Martinez, M. (1989) Lipids 24, 261–265.
[8] Christensen, E., Woldseth, B., Hagve, T.A., Poll-The, B.T., Wanders, R.J., Sprecher, H., Stokke, O. and Christophersen, B.O. (1993) Scand. J. Clin. Lab. Invest. 53 (Suppl. 215), 61–74.
[9] Baykousheva, S.P., Luthria, D.L. and Sprecher, H. (1994) J. Biol. Chem. 269, 18390–18394.
[10] Das, A.K., Horie, S. and Hajra, A.K. (1992) J. Biol. Chem. 267, 9724–9730.
[11] Sottocasa, G.L., Kuylenstierna, B., Ernster, L. and Bergstrand, A. (1967) J. Cell. Biol. 32, 415–438.
[12] Goldman, P. And Vagelos, P.R. (1961) J. Biol. Chem. 236, 2620–2624.
[13] Lands, W.E.M., Inoue, M., Sugiura, Y. and Okuyama, H. (1982) J. Biol. Chem. 257, 14968–14972.
[14] Lazarow, P.B. (1981) Methods Enzymol. 72, 315–319.
[15] Folch, J., Lees, M. and Sloane Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509.
[16] Voss, A., Reinhart, M. and Sprecher, H. (1992) Biochim. Biophys. Acta 1127, 33–40.