IN VIVO COVALENT BINDING OF $^{14}CCl_4$ METABOLITES IN LIVER MICROSOMAL LIPIDS

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Summary

Covalently bound ^{14}C from $^{14}CCl_4$ is preferentially localized in the lipids of hepatic microsomes of rats within 15 min. Label was recovered in all classes of lipids isolated from the microsomal lipid extract by diethylaminoethyl column chromatography. Among phospholipids, specific activity was the highest in the fraction containing phosphatidyl serine and lowest in phosphatidyl choline. Cholesterol esters had more than ten times the specific activity of cholesterol.

Warhol et al (1) and Villarruel and Castro (2) have reported that in rats given $^{14}CCl_4$, the majority of the label in the phospholipids of microsomal fractions was recovered in the PC fraction. In both studies phospholipids were separated and identified by TLC on silica gel. While pursuing the preliminary findings (1), we found following TLC separation of the phospholipids that more than half of the bound label in the Folch extract was lost, and that the mole ratio of phospholipid phosphorus between subgroups was inconsistent with published values (3, 4). Subsequently, we were able to substantially improve the recoveries of material by using DEAE cellulose chromatography (5) to separate lipids. PC contained a quarter of the total lipid label and almost all remaining radioactivity was recovered in the other major subgroups isolated, Ch, ChE, PE, PI, and the fraction containing PS. That fraction containing PS also contains lipid oxidation products (5) and had the highest specific activity of the phospholipid fractions. Among neutral lipids ChE had more than 10 times the specific activity of C.

Abbreviations: TLC, thin layer chromatography; DEAE, diethylaminoethyl; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PI, phosphatidyl inositol; PS, phosphatidyl serine; Ch, cholesterol; ChE, cholesterol esters.

Table 1. In vivo lipid binding pattern of ^{14}C in microsomes following $^{14}CCl_4$

Lipid fraction	Lipid content[1,2]	Per cent total lipid ^{14}C[1]	Specific activity	
			Expt 1	Expt 2
	mg/gm liver		cpm/mg lipid	
Cholesterol	0.42 ± 0.02	0.44 ± 0.27	47	140
Cholesterol esters	0.048 ± 0.009	0.91 ± 0.19	2061	1892
Phosphatidyl choline	7.6 ± 0.9	26.1 ± 1.9	324	583
Phosphatidyl ethanolamine	2.1 ± 0.8	19.3 ± 3.5	1608	1523
Phosphatidyl inositol	1.01 ± 0.28	10.0 ± 1.2	1476	1455
Phosphatidyl serine and uncharacterized material	0.38 ± 0.08	22.6 ± 3.4	10309	6392

1- Mean ± standard error of mean

2- Lipid contents derived from cholesterol and lipid phosphorus determinations

Material and Methods

Male Charles River CD rats (225 - 275 g) were given drinking water containing 0.1 per cent sodium phenobarbital for 30 days. The animals had free access to Purina Lab Chow at all times except for 16 hr prior to sacrifice. 50 uCi $^{14}CCl_4$ (5.1 mCi/mM, New England Nuclear Corp., Boston, Mass.) in 0.25 ml mineral oil was fed by stomach tube 15 min. prior to decapitation. According to methods previously described (6), the liver was homogenized in 5 volumes cold 0.25 M sucrose, the microsomal fraction obtained by differential centrifugation, lipids extracted in 2:1, $CHCl_3$:MeOH, phospholipid phosphorus content measured and radioactivity counted. Cholesterol content was measured by $FeCl_3$-sulfuric acid colorimetry following the extraction procedures described by Abell et al (7). Ch, cholesterol palmitate, PC, PE, PS, sphingomyelin and lysolecithin standards were purchased from Sigma Chemical

Co., St. Louis, Missouri. PI was obtained from Calbiochem, La Jolla, Calif.

Lipid extracts containing less than 100 mg were first purified by passage through a Sephadex G-25 column (1.5 x 7 cm.), concentrated under N_2, and separated into subgroups by column chromatography on DEAE cellulose (2.5 x 20 cm) according to Rouser et al (5). Fractions containing PI were desalted by passage through Sephadex G-25 (5). Ch and ChE in the neutral lipid fraction were further separated on silica gel H with appropriate standards by chromatography with petroleum ether:$CHCl_3$:acetone, 50:20:3. Identity and purity of the fractions containing phosphatides were determined by spotting 50 ug samples on silica gel H thin layer plates, chromatography with $CHCl_3$:MeOH:acetic acid, 25:15:4,(8) and localization with I_2 and/or ninhydrin spray. PC and PE were cleaved to fatty acid methyl esters with boron trifluoride in methanol (9).

Results and Discussion

Lipid Isolation and Recovery. Distribution of microsomal phospholipids isolated individually from 5 animals was compatible to Rouser et al (3) and Korn (4). Based on phospholipid phosphorus, the mole ratios averaged: PC 61.2 per cent; PE 16.9 per cent; PI 8.1 per cent; and PS plus uncharacterized oxidized material 3.1 per cent. Sphingomyelin and lysolecithin were not identified. In terms of the contents of the initial separate Folch extracts recoveries of ^{14}C after chromatography averaged 79 per cent, phosphorus 89 per cent, and cholesterol 107 per cent.

Label Localization. In contrast to Villarruel and Castro (2), but confirming an earlier finding of Reynolds and Yee (10) ChE contained more ^{14}C label than Ch and ChE's specific activity was more than that of Ch (Table 1). The fraction containing PS and unidentified oxidized lipids had the highest specific activity of the phospholipids based on lipid phosphorus. Localization of the ^{14}C in oxidized fractions is not illogical since CCl_4 metabolites initiate and enhance peroxidation of microsomal lipid (11, 12). Again in contrast to Villarruel and Castro's (2) reported distribution

3 hr after a tracer dose of $^{14}CCl_4$ and to our preliminary report (1) also at 15 min., PC was found to contain only a quarter of the label in microsomal lipid extracts and had the lowest specific activity of the phospholipids. The specific activities of PI and PE were equivalent and approximately three times that of PC.

All of the label in PC and PE could be further localized in the fatty acid methyl ester fractions cleaved from these phosphatides. Further characterization of the submolecular location of the binding of CCl_4 metabolites in the hydrophobic portions of the endoplasmic reticulum is proceeding in this laboratory.

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