Food Chemistry **34** (1989) 71–78

Molecular Species of Choline Glycerophospholipids and Ethanolamine Glycerophospholipids in Goat Liver

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(Received 18 October 1988; revised version received and accepted 7 December 1988)

ABSTRACT

Twelve and fifteen molecular species were detected in the three subclasses of choline glycerophospholipids (CGP) and ethanolamine glycerophospholipids (EGP), respectively. In the alkenylacyl glycerophosphocholine (GPC), 16:0–18:2 (17·5%) was the major species followed by 18:0–18:2 (13·7%), 18:1–18:1 (12·3%) and 16:0–20:4 (12·2%). 16:0–20:4 (18·7%) was the highest species in the alkylacyl GPC, while 18:0–18:2 (20·8%) was most abundant in the diacyl GPC. The major molecular species were 16:0–20:4, 16:0–18:2, 18:0–20:4 and 18:0–18:2 in both alkylacyl and diacyl analogues of CGP. The highest molecular species were 16:0–18:1 (20·2%) in the alkenylacyl, 16:0–18:2 (20·0%) in the alkylacyl and 18:0–18:1 (23·5%) in the diacyl subclasses of EGP. The four major molecular species in all three subclasses of EGP were 16:0–20:4, 16:0–18:2, 16:0–18:1 and 18:0–18:1. The fatty acids 16:0, 18:0 and 18:1 at the sn*–1–position combined with various fatty acid species at the* sn*–2–position depending on the subclass of CGP or EGP.*

INTRODUCTION

The goat is a very popular source of meat in oriental countries. Traditionally, goat meat is thought to have medicinal benefits as a tonic. Also the goat adapts well to harsh environmental conditions such as a lack

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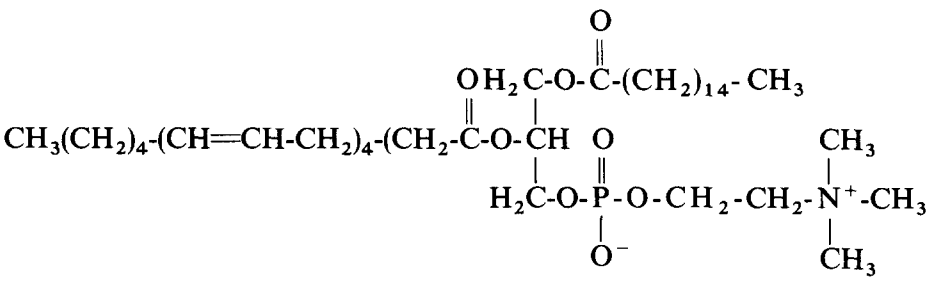
Food Chemistry 0308-8146/89/$03·50

of adequate nutrients, temperature changes and uncomfortable surroundings.

To date, very little research has been carried out on the biological characteristics of the goat. Since liver primarily regulates most metabolism in all species, it was of interest to observe the profiles of cellular membranes in the goat liver. Biological membranes are basically made up of lipid bilayers which are composed of various phospholipid classes with different molecular species. The molecular species compositions of phospholipids are altered in response to environmental changes in order to control the membrane functions. Differences in the molecular species compositions of cellular membranes exhibit striking differences in the physico-chemical properties of these membranes, and thus affect the physiological functioning of these membranes. For example, the phospholipase A_2 enzyme selectively hydrolyzes arachidonic acid (20:4*n*-6) from the *sn*-2-position of the molecular species 16:0–20:4 of 1-palmityl-2-arachidonyl-*sn*-glycero-3-phosphocholine for the synthesis of prostaglandins and other metabolites needed by the cell (Holub & Kuksis, 1978; Houslay & Stanley, 1982; Yeo *et al.*, 1989).



(1-palmityl-2-arachidonyl-*sn*-glycero-3-phosphocholine)

Phospholipid molecular species have been separated by various methods, including HPLC–GLC (Blank *et al.*, 1983, 1984; Justin *et al.*, 1985; Nakagawa & Waku, 1986; Robinson *et al.*, 1986; Purdon *et al.*, 1987), GC–GC/MS (Myer *et al.*, 1978; Myer & Kuksis, 1982), and HPLC–MS (Jungalwala *et al.*, 1984; Pind *et al.*, 1985; Bernett *et al.*, 1985; Kim & Salem, 1987). However, there are disadvantages in using each of these chromatographic methods; for instance, the resolution of the molecular species may not always be reproducible. This study employs the HPLC–GLC method of Nakagawa & Horrocks (1983), which is very reproducible and efficient in analyzing phospholipid molecular species, although that takes time.

Thus, the overall purpose of the present study is to observe the molecular species compositions of alkenylacyl, alkylacyl and diacyl analogues of choline glycerophospholipids (CGP) and ethanolamine glycerophospholipids (EGP) in goat liver by HPLC–GLC.

MATERIALS AND METHODS

Animals. Three male goats were fed grass *ad libitum* for 3 months after lactation. They were sacrificed and their livers were excised. Each liver was homogenized by grinding in a Waring blender shortly after removal.

Lipid analyses. Total lipids were extracted from the liver by the method of Bligh & Dyer (1959). CGP and EGP fractions were separated from the total lipids by one dimensional TLC using precoated TLC plates (DC-Fertigplatten Kieselgel 60, E. Merck, Darmstadt) and a solvent system of chloroform/methanol/acetic acid/water (50:25:8:3 by vol). The separation was repeated several times to pool the CGP and EGP. The isolated CGP and EGP showed a single spot each when their purity was checked by TLC.

Molecular species separation. CGP and EGP were incubated for 3 and 6 h, respectively, at room temperature with phospholipase C (Sigma Chemical Co., St. Louis, MO) as described by Ikezawa *et al.* (1976). After incubation, the reaction was checked by TLC with petroleum ether/diethyl ether/acetic acid (60:40:1 by vol), and the diradyl glycerols from CGP and EGP were acetylated with acetic anhydride and pyridine for 3 h at 37°C. The resulting acetylated diradyl glycerols were separated into the alkenylacyl, alkylacyl and diacyl groups by HPLC with a μPorasil column (Waters Associates, Milford, MA) using cyclopentane/hexane/methyl-*t*-butyl ether/acetic acid (73:24:3:0·03 by vol) as a solvent system for isocratic elution (Nakagawa & Horrocks, 1983). The flow rate was 2 ml/min. The HPLC was equipped with two solvent pumps (Waters Associates, Milford, MA) and a variable wavelength detector (LC 75, Perkin Elmer, Norwalk, CT) set at 205 nm. The alkenylacyl, alkylacyl and diacyl glycerophosphocholine (GPC) or glycerophosphoethanolamine (GPE) subfractions were collected and their molecular species were analyzed by reverse phase HPLC on Zorbax ODS column (Dupont Co., Wilmington, MA). The molecular species of the diacyl group were eluted by the HPLC with acetonitrile/isopropanol/methyl-*t*-butyl ether/water (63:28:7:2 by vol), while the ether-linked lipids (alkenylacyl and alkylacyl groups) were separated with acetonitrile/isopropanol/methyl-*t*-butyl ether/water (72:18:8:2 by vol) at a flow rate of 0·5 ml/min. The fractions for each peak were collected several times to detect by GLC (Pye Unicam Ltd., UK). The alkenyl and alkyl chains were analyzed using a glass column packed with 10% Apiezon L on Anakrom AS (Analabs Incs., North Haven, CT) at an isothermal oven temperature of 175°C and 275°C, respectively. The alkenyl chains were detected by analysis of aldehydes which were liberated from the alkenylacyl groups by treatment with HCl fumes (Ferrell *et al.*, 1970). The alkyl groups were analyzed by making the bistrimethylchlorosilane derivatives from the alkylhydroxy glycerols which were prepared from the alkylacyl glycerols by hydrolyzing

the acyl group with 0·5N NaOH in methanol. The bistrimethylchlorosilane derivatives were produced by the reaction of the alkyl glycerols with hexamethyldisilazane and trimethylchlorosilane in pyridine for 10 min at 80°C (Nakagawa & Horrocks, 1983). All acyl chains from the alkenylacyl, alkylacyl and diacyl classes were analyzed using a glass column packed with 10% Alltech CS-10 on Chromosorb W (Alltech Associates, Deerfield, IL) at an isothermal temperature of 190°C, after methylation with 0·5N methanolic NaOH for 1 h at 65°C. The alkenyl, alkyl and acyl chains were quantified by the methylesters of C17:0 as an internal standard.

The organic solvents for the HPLC were obtained from EM Science (Gibbstown, NJ) and filtered with a 0·22 μm GS Millipore filter (Millipore Co., Bedford, MA) before use.

RESULTS AND DISCUSSION

Twelve and fifteen molecular species were detected for CGP and EGP, respectively, with variations among the analogues. Table 1 gives the molecular species compositions of the alkenylacyl, alkylacyl and diacyl

TABLE 1
Molecular Species Composition of the Alkenylacyl, Alkylacyl and Diacyl Analogues of Choline Glycerophospholipids from Goat Liver[a]

Peak number[b]	*Molecular species*[c]	*Alkenylacyl*	*Alkylacyl*	*Diacyl*
			(mol %)	
1	18:1–22:6	2·4 ± 0·4	3·2 ± 0·3	0·5 ± 0·4
2	16:0–22:6	6·1 ± 0·2	5·4 ± 0·4	3·4 ± 0·2
3	18:1–20:4	7·7 ± 0·2	6·8 ± 0·3	8·6 ± 0·1
4	16:0–20:4	12·2 ± 0·4	18·7 ± 0·2	10·2 ± 0·1
5	18:0–22:6	3·8 ± 0·2	3·1 ± 0·3	2·8 ± 0·2
6	18:1–18:2	2·1 ± 0·1	4·7 ± 0·2	5·1 ± 0·3
7	16:0–18:2	17·5 ± 0·2	16·9 ± 0·2	14·6 ± 0·3
8	18:0–20:4	10·8 ± 0·3	13·5 ± 0·1	18·3 ± 0·1
9	18:1–18:1	12·3 ± 0·1	8·1 ± 0·2	5·0 ± 0·1
10	16:0–18:1	9·5 ± 0·5	5·3 ± 0·4	5·8 ± 0·3
11	18:0–18:2	13·7 ± 0·3	10·0 ± 0·1	20·8 ± 0·1
12	18:0–18:1	0·5 ± 0·2	2·6 ± 0·2	3·7 ± 0·1

[a] Values represent means ± SE ($n = 3$) of three determinations for the alkenylacyl and diacyl analogues, and are mole % based on a single analysis for the alkylacyl analogue because of the limited sample size.

[b] Peak numbers are in the order of resolution by the HPLC column.

[c] It is assumed that the saturated acyl chains mainly exist in the *sn*-1-position of the glycerols.

analogues of CGP in goat liver by HPLC–GLC. The major molecular species were palmitic, stearic and oleic acids combined with oleic, linoleic and arachidonic acids at the *sn*-2-position in CGP. 16:0–18:2 (17·5%) species constituted the largest percentage in alkenylacyl GPC followed by 18:0–18:2 (13·7%), 18:1–18:1 (12·3%) and 16:0–20:4 (12·2%). The 16:0–20:4 species was highest in the alkylacyl GPC, while 18:0–18:2 was most abundant in the diacyl analogue. The major molecular species were 16:0–20:4 (18·7 and 10·2%), 16:0–18:2 (16·9 and 14·6%), 18:0–20:4 (13·5 and 18·3%) and 18:0–18:2 (10·0 and 20·8%) in the alkylacyl and diacyl classes of CGP. 18:0–18:1 was the most rare combination among the 12 molecular species in the alkenylacyl (0·5%) and alkylacyl (2·6%) classes, and 18:1–22:6 in the diacyl (0·5%) class of CGP (Table 1). In the alkenylacyl analogue of CGP, 16:0 and 18:0 fatty chains at the *sn*-1-position were primarily combined with 18:2 at the *sn*-2-position, whereas 18:1 primarily paired with 18:1. The fatty acid which combined with 16:0 and 18:0 alkyl groups at the *sn*-1-position was mainly 20:4 at the *sn*-2-position in the alkylacyl analogue, and 18:1 at the *sn*-1-position combined significantly with 18:1 at the *sn*-2-position in the alkylacyl group as was the case for the alkenylacyl group. In the diacyl GPC, 16:0 and 18:0 fatty acids at the *sn*-1-position were preferentially combined with 18:2, which were followed by 20:4, at the *sn*-2-position. The fatty acid 18:1 at the *sn*-1 position combined with 20:4 at the *sn*-2-position was most prevalent in the diacyl GPC. The distribution of the molecular species with 16:0 and 18:0 fatty chains at the *sn*-1-position in the alkenylacyl group was similar to that of the diacyl group, whereas the molecular species of 18:1 at the *sn*-1-position in the alkenylacyl analogue was similar to that in the alkylacyl analogue (Table 1).

As shown in Table 2, fifteen molecular species were analyzed in the alkenylacyl, alkylacyl and diacyl classes of goat liver EGP. The major molecular species were 16:0–20:4, 16:0–18:2, 16:0–18:1 and 18:0–18:1 in the alkenylacyl and alkylacyl analogues of EGP, while 18:0–X was not detected in the analogues. 16:0–18:1 (20·2%) was the major species in the alkenylacyl analogue and 16:0–18:2 (20·0%) was the major species in the alkylacyl GPE. Distinct differences were found in the percentages of 18:0–22:6, 18:1–18:2 and 16:0–20:1 species between the alkenylacyl and alkylacyl GPE. The major molecular species was 18:0–18:1 (23·5%) in the diacyl analogue, followed by 18:0–20:4 (14·1%), 16:0–18:2 (12·5%), 18:1–18:1 (10·2%) and 16:0–18:1 (10·0%). The diacyl GPE contained very small amounts of the 18:1–18:2 (0·8%) and 18:0–X (0·7%) species. The fatty acids 16:0 and 18:0 at the *sn*-1-position primarily paired with 18:1 at the *sn*-2-position in the alkenylacyl class. 18:2 was the major fatty acid at the *sn*-2-position to pair with 18:1 at the *sn*-1-position in the alkenylacyl analogue of EGP. In the alkylacyl and diacyl analogues, 18:0 and 18:1 at the

TABLE 2
Molecular Species Composition of the Alkenylacyl, Alkylacyl and Diacyl Analogues of Ethanolamine Glycerophospholipids from Goat Liver[a]

Peak number[b]	*Molecular species*[c]	*Alkenylacyl*	*Alkylacyl*	*Diacyl*
			(*mol %*)	
1	18:1–22:6	0·6 ± 0·4	4·3 ± 0·3	2·0 ± 0·5
2	16:0–22:6	1·5 ± 0·3	4·5 ± 0·4	1·7 ± 0·2
3	18:1–20:4	4·6 ± 0·2	7·1 ± 0·3	3·1 ± 0·3
4	16:0–20:4	15·1 ± 0·3	12·9 ± 0·4	7·0 ± 0·1
5	18:0–22:6	1·8 ± 0·2	7·5 ± 0·3	3·2 ± 0·2
6	18:1–18:2	7·7 ± 0·1	0·5 ± 0·1	0·8 ± 0·1
7	16:0–18:2	16·5 ± 0·2	20·0 ± 0·1	12·5 ± 0·3
8	18:0–20:4	4·7 ± 0·1	6·3 ± 0·2	14·1 ± 0·5
9	18:1–18:1	5·3 ± 0·1	7·2 ± 0·1	10·2 ± 0·2
10	16:0–18:1	20·2 ± 0·3	12·4 ± 0·3	10·0 ± 0·8
11	18:0–18:2	2·6 ± 0·1	1·0 ± 0·2	6·2 ± 0·5
12	18:0–22:4	2·5 ± 0·2	0·6 ± 0·1	1·5 ± 0·2
13	16:0–20:1	6·5 ± 0·1	0·5 ± 0·2	2·7 ± 0·2
14	18:0–18:1	9·0 ± 0·2	11·0 ± 0·1	23·5 ± 0·3
15	18:0–X	—	—	0·7 ± 0·4

[a,b,c] See Table 1 for description.

sn-1-position coupled most frequently with 18:1 at the *sn*-2-position, whereas 16:0 at the *sn*-1-position mainly combined with 18:2 at the *sn*-2-position (Table 2).

Generally, the molecular species clearly varied between subclasses of the same phospholipid and between phospholipid classes, as indicated in the Tables. EGP had three more molecular species than did CGP. Particularly, the quantities of the 18:0–18:2 and 18:0–18:1 species were considerably different between CGP and EGP. The molecular species, 18:0–18:2, accounted for 13·7, 10·0 and 20·8% in the alkenylacyl, alkylacyl and diacyl analogues of CGP, respectively, while only accounting for 2·6, 1·0 and 6·2% in these three analogues of EGP. On the other hand, the 18:0–18:1 species was 0·5, 2·6 and 3·7% in these analogues of CGP, while giving 9·0, 11·0 and 23·5%, respectively in these three analogues of EGP.

Many research groups have analyzed the molecular species compositions of livers from various animals. However, very little information is available on the molecular species compositions of goat liver. In general, the most abundant molecular species are 16:0–18:1, 16:0–18:2, 16:0–20:4, 18:0–18:1, 18:0–18:2 and 18:0–20:4 in liver of the animal studied, although these compositions varied with animal species and phospholipid classes.

Disa & Mira (1981) studied the molecular species of CGP and EGP in goat liver. They found 6 and 8 molecular species in CGP and EGP, by using TLC which has poor sensitivity to separate lipids compared to HPLC. That study reported that the distribution of mono, di, tri, tetra and hexaenoic molecular species was 12, 21, 14, 33 and 7% in CGP and 6, 22, 5, 29 and 22% respectively, in EGP. However, twice the number of molecular species (12 and 15 for CGP and EGP) were detected in this study which employed an HPLC–GLC system to separate the diradyl groups and their molecular species of CGP and EGP. The results of this study may contribute to investigations on the physico-chemical properties of cellular membranes in goat liver.

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