Constancy of Unsaturation in Molecular Species of Cardiolipin and Phosphatidyl Ethanolamine from Beef Heart Mitochondria[1]

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Abstract

The diglyceride acetate products of the acetolysis of beef heart mitochondrial cardiolipin were separated into three fractions, trienes, tetraenes and pentaenes, by argentation chromatography. Tetraenes accounted for 64 per cent of the total diglyceride acetates and linoleic acid accounted for 93 per cent of the total acyl residues of this fraction. There was an inverse linear relationship between the oleic and linoleic acid contents of cardiolipin preparations. Six diglyceride acetate fractions were obtained from beef heart mitochondrial phosphatidyl ethanolamine. The major fraction, tetraenes, accounted for 54 per cent of the total diglyceride acetates. Stearic and arachidonic acids accounted for 89 per cent of the acyl groupings in this fraction. There was a high degree of consistency in the total unsaturated fatty acid content of cardiolipin preparations and in all diglyceride acetate fractions derived from cardiolipin. This consistency was also evident in phosphatidyl ethanolamine preparations.

In a preliminary communication we reported that the fatty acids were nearly randomly distributed between the α- and β-positions of beef heart cardiolipin (8). Argentation chromatography of the diglyceride acetates prepared by acetolysis of cardiolipin revealed the presence of three molecular species. The major species, tetraenes, contained more than 90% linoleic acid (8). Wood and Harlow (19), by gas chromatographic separation, similarly found one predominant diglyceride in the Phospholipase C digestion products of rat liver cardiolipin. In continuing our earlier studies, analyses of multiple cardiolipin preparations revealed a great deal of constancy in molecular species distribution. Further, this constancy was also observed in the total unsaturated fatty acid content of both native cardiolipin and the molecular species derived therefrom. These results are reported herein along with those obtained from parallel analyses of beef heart mitochondrial phosphatidyl ethanolamine (PE). Next to cardiolipin, PE contains a higher proportion of unsaturated acyl residues than do other beef heart mitochondrial phospholipids and contains high levels of arachidonic acid (1,2,6). Analysis of PE was thus of particular interest in view of the hypothesis of De Pury and Collins (4) that arachidonyl species are more firmly bound to mitochondrial structural protein than are other species. This hypothesis is correlated in part with

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our recent observation that the highly unsaturated cardiolipin is tightly bound to cytochrome oxidase and is essential for function of the enzyme (1, 2).

Materials and Methods

Beef heart mitochondria were isolated by the method of Löw and Vallin (10). Cardiolipin and PE were isolated from washed total lipid extracts (1) by preparative thin-layer chromatography (8). Analysis by two-dimensional thin-layer chromatography (12) revealed these lipids to be free of other polar lipid contaminants. To minimize autotoxidation, samples were stored in a nitrogen atmosphere at −20° C and a small amount of butylated hydroxyanisole was added to all solvents used. Diglyceride acetates were prepared by acetolysis at 145° C for 40 hours in a nitrogen atmosphere according to Renkonen (14). In certain cases, PE was mixed with sphingomyelin (chromatographically pure, Sigma Chemical Company), digested with Phospholipase C from *Clostridium welchii*, and the diglycerides thus obtained were acetylated (14). The diglyceride acetates were first purified by thin-layer chromatography (8) and then separated by argentation chromatography on thin layers of Silica gel G containing silver nitrate (10:3 by weight). Plates were developed in chloroform containing from 0.75 to 1.25% methanol (9, 13). Lipid bands were localized, recovered (8), methylated and analyzed by gas chromatography (7). Relative concentrations of various diglyceride acetates was determined by ester analysis of the material eluted from silver nitrate plates (18).

Acetolysis was chosen for the preparation of diglyceride acetates as a matter of convenience. It is known that intramolecular migration of fatty acids occurs under these conditions (11, 15). However, intermolecular migration does not occur during this reaction (14) and, since positional distribution of fatty acids was not determined, the diglyceride acetates were suitable for analysis of molecular species distribution. As a check on the method, diglyceride acetates prepared by acetylation of the products of Phospholipase C digestion of PE (in the presence of sphingomyelin) gave results nearly identical to those obtained with the acetolysis product of the same PE sample in all analyses reported herein. Cardiolpin was not digested by the enzyme under these conditions. Although there was oxidative degradation on acetolysis, as evidenced by darkening of the reaction mixture, the colorless diglyceride acetates isolated had a fatty acid composition identical to that of the starting lipid.

Results and Discussion

Only three fractions were obtained on separation of the cardiolipin diglyceride acetates. The predominant molecular species in each fraction were trienes, tetraenes, and pentaenes, respectively (Table 1). A remarkably constant amount of each species was present in the seven mitochondrial preparations analyzed. Tetraenes were the major species encountered, representing 64% of the total (range 60 to 67%). In contrast, a total of six fractions were obtained on argentation chromato-

graphy of PE (Table 1). Saturates (minor) *plus* monoenes *plus* dienes together accounted for approximately 28% of the total. As with cardiolipin, tetraenes were the major molecular species, accounting for 54% of the total. There was a reasonable consistency in distribution of molecular species among the four separate preparations analyzed.

TABLE 1. *Distribution of molecular species of diglyceride acetates derived from cardiolipin and phosphatidyl ethanolamine of beef heart mitochondria.*

Molecular species[2]	% of Total[1]	
	Cardiolipin (7)[3]	Phosphatidyl ethanolamine (4)
Saturates—dienes	—	27.6 ± 4.25
Trienes	20.5 ± 1.86	6.9 ± 1.72
Tetraenes	63.8 ± 2.66	54.4 ± 2.97
Polyenes	15.7 ± 1.71	11.1 ± 2.28

[1]Results, expressed as % of total ester groups recovered, are reported as the average ± standard deviation.

[2]Fractions are designated as the total number of double bonds.

[3]Number of separate mitochondrial preparations analyzed.

In all cardiolipin preparations, at least 91% of the total fatty acyl groupings were unsaturated (average 95%, range 91 to 99%) (Table 2). There was, however, appreciable variation in individual fatty acids comparing different samples, particularly with regard to the major fatty acid, linoleate. There was also appreciable variation among the individual fatty acids of each diglyceride acetate species from cardiolipin comparing separate preparations (Table 2). As with the parent lipid, there was a reasonable consistency in the total percentage of unsaturated fatty acids, which on the average accounted for over 90% of the total acyl residues in all molecular species. Linoleic acid was the major fatty acid in all fractions. In the tetraene fraction linoleate accounted for, on the average, 93% of the total fatty acids.

Table 2 contains data on the fatty acid composition of PE and the more highly unsaturated diglyceride acetate fractions derived therefrom. As with cardiolipin, although there was variation in individual fatty acids, the total unsaturated acid content was nearly constant comparing different preparations and averaged 64% (range 61 to 68%). The major acids were stearate and arachidonate. This constancy in unsaturated acid content was also evident in the major subfractions analyzed. In the major species, tetraenes, stearate and arachidonate together accounted for approximately 89% of the total acyl residues. We had previously demonstrated that arachidonate is contained nearly entirely in the β-position of beef heart mitochondrial PE (8). Thus it can be concluded that 1-stearoyl-2-arachidonyl phosphatidyl ethanolamine is a major species of beef heart mitochondria.

The diphosphatidyl glycerol structure of cardiolipin precludes conclusions on the molecular species distribution of the parent

phosphatide based on results obtained with diglyceride acetates. However, the results contained herein suggest that the majority of the molecular species contain three linoleate molecules and further imply the occurrence of a molecular species containing solely linoleic acid.

TABLE 2. *Fatty acid composition of cardiolipin, phosphatidyl ethanolamine, and their diglyceride acetates*[1].

	Cardiolipin (7)[2]			
Acid	Total	Trienes	Tetraenes	Polyenes
14:0	0.3 ± 0.2	2.1 ± 2.23	0.4 ± 0.6	0.8 ± 0.66
16:0	2.3 ± 1.40	5.4 ± 1.68	1.8 ± 0.95	5.1 ± 1.86
16:1	2.5 ± 0.17	11.4 ± 1.15	1.0 ± 0.68	4.0 ± 1.16
18:0	2.1 ± 1.47	2.2 ± 1.23	0.9 ± 0.51	3.3 ± 1.37
18:1	9.9 ± 3.14	28.5 ± 1.77	1.5 ± 0.94	22.0 ± 7.05
18:2	73.9 ± 5.72	50.0 ± 6.58	93.2 ± 4.32	37.2 ± 3.86
18:3	5.8 ± 1.42	0.4 ± 0.63	1.1 ± 1.85	20.4 ± 6.61
20:3	2.0 ± 1.41			3.8 ± 1.39
20:4	1.5 ± 0.97			3.3 ± 1.38
20:5	Trace			0.2 ± 0.46
22:5				
22:6				
Total % unsaturated	95.3 ± 2.93	90.4 ± 6.78	96.7 ± 1.72	90.9 ± 3.56
	Phosphatidyl Ethanolamine (5)			
Acid	Total	Trienes	Tetraenes	Polyenes
14:0	0.9 ± 1.34	1.3 ± 1.48	0.2 ± 0.24	0.2 ± 0.87
16:0	3.5 ± 2.03	16.4 ± 9.56	1.9 ± 0.65	5.9 ± 2.48
16:1	1.0 ± 0.86	7.0 ± 2.40	0.6 ± 0.47	1.8 ± 1.41
18:0	32.5± 5.81	15.1 ± 6.36	40.3 ± 2.48	33.5 ± 6.67
18:1	4.1 ± 0.89	24.6 ± 4.18	1.8 ± 2.14	10.6 ± 2.58
18:2	11.5 ± 4.32	31.9 ± 9.45	4.7 ± 4.38	4.3 ± 1.11
18:3	2.5 ± 3.29	0.6 ± 1.25	0.5 ± 0.58	0.8 ± 1.07
20:3	2.5 ± 1.55	3.1 ± 3.78	1.7 ± 0.40	0.1 ± 0.31
20:4	35.6 ± 2.31		48.5 ± 5.60	16.1 ± 7.14
20:5	4.8 ± 0.42			18.8 ± 8.04
22:5	0.9 ± 0.80			4.2 ± 5.70
22:6	0.5 ± 1.07			3.8 ± 3.71
Total % unsaturated	63.8 ± 3.40	67.2 ± 5.06	57.8 ± 2.48	60.5 ± 8.63

[1]Weight % of fatty acids reported as the avearge ± standard deviation.

[2]Number of separate mitochondrial preparations analyzed.

The observations on the constancy in total percentage of unsaturated acyl residues in both PE and cardiolipin and the molecular species derived from them render feasible the conclusion that the physiological function of these lipids requires a closely controlled content of unsaturated fatty acids. This conclusion is substantiated by the observation that alterations in fatty acid composition can influence the properties of membranes by affecting both the attractive forces between lipids and proteins and the molecular packing of membrane lipids (4, 16).

Eichberg (5) has recently reported that nearly 50% of the cardiolipin of beef heart can be isolated as a protein complex. The high degree of unsaturation in all diglyceride acetate species from cardiolipin may explain the tenaceous binding of cardiolipin to protein as being due to extensive interaction between the double bonds of the fatty acids and hydrophobic groups within the proteins. (3, 4, 17).

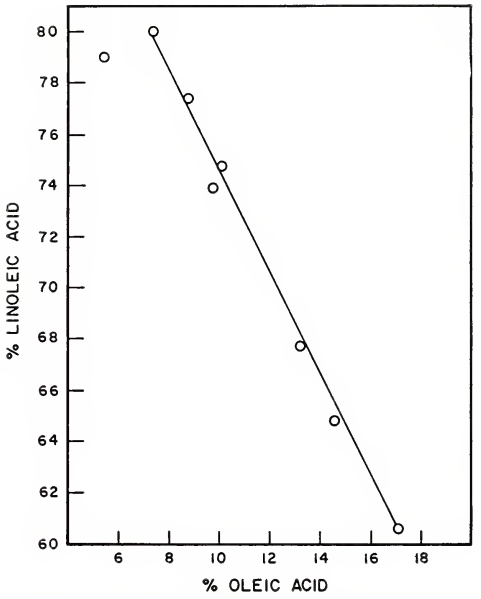

FIGURE 1. *Relationship between the contents of linoleic acid and oleic acid in beef heart mitochondrial cordiolipin. Data are weight percentages calculated by peak area analysis of gas chromatograms.*

We wish to call attention to one further observation made in the course of these studies. Although there was appreciable variation in the contents of oleic and linoleic acids comparing different preparations of cardiolipin, there was a nearly linear inverse relationship between the contents of these two acids (Fig. 1). This appears to indicate that at least a portion of one acid may be replaced by the other in cardiolipin without impairment of the psychochemical properties of the mitochondrial membrane.

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