The Relationship of Milk Phospholipids to Membranes of the Secretory Cell

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ABSTRACT

Forty-two per cent of the lipid phosphorus in milk was found in skim milk lipoprotein; the other 58% occurs in the milk fat globule membrane (MFGM). Investigation of these two sources of lipid phosphorus revealed that they involve the same individual phospholipids, in essentially the same proportions with similar fatty acid compositions. Both contain sphingomyelin and cerebrosides in levels characteristic of those found in plasma membranes. Other points of resemblance between MFGM and skim milk lipoprotein, have been shown previously. Infusion of (^{14}C) palmitate into the mammary gland of a lactating goat produced more extensive labeling of all the phospholipid classes in the skim milk lipoproteins than in those in the MFGM during the following 24 hr. When (^{14}C) palmitate was infused into the jugular vein of a lactating goat, a precusor-product-type relationship was observed between specific activities of the skim milk and MFGM polar lipids. These results render the MFGM an unlikely origin of the skim milk lipoprotein. Other possible sources of this latter lipoprotein are Golgi vesicle membranes or plasma membrane of the lactating cell.

INTRODUCTION

Consideration of morphological and biochemical evidence (1-4) leaves little doubt that the milk fat globule membrane (MFGM) originates directly from plasma membrane which envelopes the globule at secretion (2). A question is then raised regarding lipoprotein in the skim milk phase. This material accounts for one third to one half of the lipid phosphorus of milk (5-7) and it bears considerable likeness to MFGM. The proportions of the various phospholipids in the two and the fatty acid compositions of the phospholipids are similar (7,8). Similar enzymatic activities have also been demonstrated in the two sources (9-11). The skim milk lipoprotein may simply be material that is shed from the MFGM, but radioisotope tracer experiments indicate that this explanation is inadequate.

MATERIALS AND METHODS

In the analyses of phospholipids in fat globules and skim milk, freshly drawn milk samples were obtained from cows (Holstein) and goats (Nubian). They were divided into fat globules and skim milk by centrifugation at 30,000 to 35,000 x *g* for 1 hr at 5 C. Lipids were recovered from these samples by Roese-Gottlieb extraction (12). Solvents were removed by rotary evaporation under reduced pressure at room temperature. Polar lipids were separated from crude lipid extracts by silicic acid column chromatography (13). The polar lipids, eluted with methanol, were recovered by evaporation as above and immediately redissolved in an accurately measured volume of chloroform. These lipids were separated by two dimensional thin layer chromatography (TLC) (14). Levels of phospholipids were determined by phosphorus analysis of total lipid fractions or of areas scraped from thin layer plates (15). Identities of phospholipids separated by this procedure have been established (2,14,16). Fatty acid compositions of phospholipids separated by TLC were determined by gas chromatography of methyl esters as described previously (2,17).

To explore the relationship between the lipoproteins of skim milk and the MFGM, we conducted two tracer experiments with Na-1-(^{14}C) palmitate (Applied Science, State College, Pa.). In the first experiment, a goat in mid lactation was completely milked and the tracer (50 μC, 1 μM), in 5 ml of water, was infused into the mammary gland via a teat canal. Milk from that side of the gland was collected at hourly intervals for the first 6 hr, then at 9, 12 and 24 hr postinfusion. Fat globules were separated from skim milk in these milk samples by centrifugation at 6 200 x *g* for 6 min at -2 C. The plastic tubes (50 ml) of centrifuged milk were completely frozen at -30 C and then sawed just below the compacted layers of milk fat globules. In a second infusion experiment, 100 μC of Na-1-(^{14}C) palmitate (57 μC/μM, Amersham/Searle, Des Plaines, Ill.) was bound to 20 mg of bovine serum albumin

in 5 ml of water and injected into the jugular vein of a completely milked lactating goat. Milk samples were collected at 2 hr intervals for the first 12 hr, then at 24 hr post infusion. These milk samples were separated into MFGM and skim milk fractions by published methods (2,7). Isolation of MFGM was accomplished by freezing centrifugally compacted layers of fat globules at 4 C for 24 hr, thawing and melting the material at 40 C, diluting with several volumes of water and sedimenting the membrane material into a pellet at 36,000 x *g* for 2 hr. Extraction of the lipids, isolation and separation of phospholipids and determination of lipid phosphorus in these fractions were accomplished by the methods cited here. Specific activities of phospholipids were calculated from radioactivities and phosphorus contents of total polar lipid fractions or of the appropriate areas scraped from two dimensional thin layer chromatograms. Specific activities per microgram P were converted to a phospholipid basis by the factor, X 40.

RESULTS

Analysis of milks from four individual cows revealed an average of 42.4% of the total lipid phosphorus in the skim milk (range of 39.9% to 43.5%). The milk of an individual goat yielded 42.0% of the lipid phosphorus in the skim milk. The remainder of the lipid phosphorus is contained in the MFGM; none was dissolved in the fat (5,7). The same phospholipid classes were associated with fat globules and in skim milk. Only small variations were observed in the distribution of the individual phospholipids between skim milk and globules of a given milk and the average values for the four samples show close agreement for levels of the individual components in the comparison between skim milk and globules (Fig. 1). Some minor variation in phospholipids at the two sites would be expected on the basis that secretion involves the addition of plasma membrane phospholipids to those that may preexist on the fat droplet within the cell (2,3). Mono- and dihexose cerebrosides, constituents characteristic of plasma membranes and MFGM (2,17), were present in both globules and skim milk from all samples. Comparative fatty acid analyses (not shown) for phosphatidyl inositol, phosphatidyl ethanolamine and phosphatidyl choline from globules and skim milk confirmed earlier findings regarding the similarities in compositions for the two sites (7,8).

Data from the experiments comparing specific activities of phospholipids in skim milk with those of fat globules after infusion of Na-

TABLE I

Radioactivity Incorporation Into the Phospholipids of Skim Milk and of Milk Fat Globule Membrane at Various Intervals of Milking After Infusion of 50 μC of Na-1-(^{14}C) Palmitate Into the Udder of a Lactating Goat

Milking Interval, hr	Specific activity, cpm/mg									
	Phosphatidyl ethanolamine		Phosphatidyl serine		Phosphatidyl choline		Phosphatidyl inositol		Sphingomyelin	
	G[a]	S[a]	G	S	G	S	G	S	G	S
1	428	1796	0	428	1752	16096	640	6964	196	692
2	76	448	0	100	160	2964	80	720	172	800
3	36	248	40	269	172	1768	0	648	60	900
4	32	128	24	32	80	792	0	612	60	448
5	10	244	0	256	92	1054	4	476	84	564
6	12	212	0	152	116	1084	0	120	80	584
9	20	b	16	b	84	b	20	b	72	b
12	16	56	0	280	68	268	0	240	60	352
24	12	28	0	80	40	116	0	60	68	368

[a]Abbreviations: G, globules; S, skim milk.
[b]Samples accidently lost.

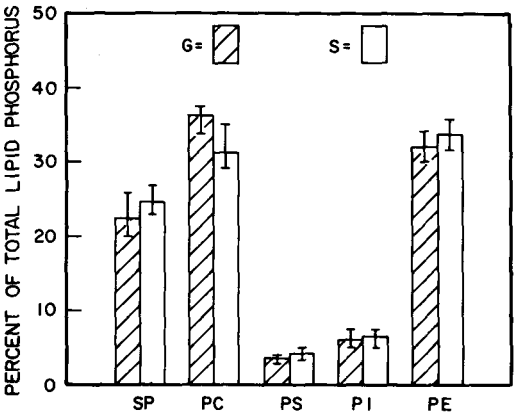

FIG. 1. Distribution of phospholipids as per cent of total lipid phosphorus in fat globules (G) and skim milk (S). The averages and ranges encountered in four individual milk samples are presented. Abbrevitions: PE, phosphatidyl ethanolamine; PI, phosphatidyl inositol; PS, phosphatidyl serine; PC, phosphatidyl choline; Sp, sphingomyelin.

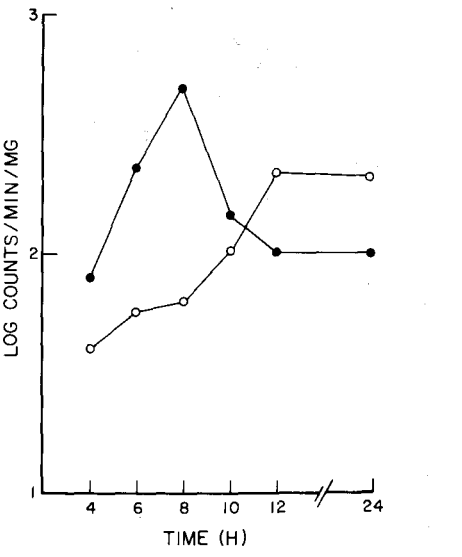

FIG. 2. Changes in specific activity of goat fat globule membrane and skim milk polar lipid fractions after intravenous infusion of 100 μC of sodium-1-(^{14}C) palmitate. ●——●, skim milk; ○——○, fat globule membrane.

1-(^{14}C) palmitate into one side of a goat's udder are given in Table I. These data clearly establish the incorporation of the tracer fatty acid into all of the phospholipids of the skim milk and at substantially higher levels than in those of the globules. Since some slight contamination of the globule layers with skim milk was unavoidable in separating the phases, the levels of radioactivity in the globule phospholipids appears to be low indeed. The total incorporation of radioactivity into ester lipids (mainly triglycerides) of the milk during 24 hr was 8.3% of the total infused activity with 0.25% of this dose being recovered in the phospholipids. While phospholipids of the MFGM were relatively devoid of radioactivity throughout the experiment, the neutral glycerides within the globules contained greater than 90% of the incorporated activity. This is good evidence that the tracer was metabolized by the lactating cell. With the exception of the 1 hr datum for phosphatidyl choline in skim milk (Table I), none of the individual phospholipid specific activities at a given time approached those of the neutral lipids in the fat globules.

The conversion of palmitate to other labeled metabolites was not evaluated in this experiment; however, we have previously observed, under these same conditions, that between 90% and 100% of the activity incorporated into lipids remains as palmitate. Our analyses indicate that the palmitate concentrations in total and individual phospholipids from milk fat globules and skim milk are quite comparable. As shown in Figure 1 there is slightly more phosphatidyl choline in milk fat globules. For the purpose of evaluating activity data in Table I the following concentration of palmitate in the individual phospholipids can be used; phosphatidyl choline 33%, phosphatidyl ethanolamine 14%, sphingomyelin 15%, phosphatidyl inositol 13% and phosphatidyl serine 8%. It can be seen that the labeling pattern (Table I) does not strictly follow the amount of palmitate in the particular phospholipids although phosphatidyl choline has the highest levels of activity, and of palmitate.

Results from the experiment comparing the specific activities of total polar lipid fractions of skim milk and MFGM after injection of Na-1-(^{14}C) palmitate into the circulation are depicted in Figure 2. In contrast to the previous experiment where the isotope was introduced directly into the mammary gland, in this case, labeling of phospholipids was much lower due to slow entry of the administered acid into the mammary gland over a prolonged period. While the skim milk polar lipids were maximally labeled 8 hr postinfusion, the specific activity of MFGM polar lipids did not reach a maximum value until 12 hr postinfusion. In this experiment approximately 3.4% of the total dose administered was recovered in the milk lipids during 24 hr.

DISCUSSION

The findings of this study are in harmony with other findings (2,7-11) showing sub-

stantial resemblance between the lipoproteins of skim milk, MFGM and plasma membrane of the lactating cell. Our data do not support the concept that the skim milk lipoprotein arises by disintegration of MFGM. Although the results of the ^{14}C-tracer experiments pose some difficulties of interpretation, they show clearly that the skim milk phospholipids become labeled more promptly and intensively than do those of the MFGM. While a common origin of the skim milk and fat globule phospholipids may exist, the differences in the palmitate labeling patterns (Table I, Fig. 2) indicate that phospholipids from the two sites equilibrate quite differently with the tracer, and that at some point they have diverged biosynthetically.

Membranes of Golgi vesicles, which are vehicles in the secretion of the milk proteins and which interact with the plasma membrane at secretion are a logical source of the skim milk lipoprotein. The occurrence of Golgi membranes in skim milk is also suggested by the presence of UDP galactosyl transferase (18,19). This enzyme, the A protein of lactose synthetase (19), is localized in the Golgi apparatus of lactating mammary tissue (20) and represents a marker for the Golgi apparatus in hepatocytes (21,22). The shedding of plasma membrane or of some MFGM components into the skim milk are not precluded by the present findings.

The probability that the nonfat phase of milk contains small amounts of cell membrane in a physiologically dispersed state offers a unique research opportunity. Preliminary experiments (D.L. Puppione and S. Patton, unpublished) reveal the presence of skim milk lipoprotein in the ultracentrifugal density class 1.063-1.21 g/ml.

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