THE PRESENCE OF PLASMA MEMBRANE ENZYMES ON THE SURFACE OF BOVINE MILK FAT GLOBULES

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1. Introduction

Evidence that milk fat globules are enveloped in plasma membrane during their secretion from the lactating mammary cell has been reviewed and corroborated [1]. This evidence derives primarily from ultrastructural and compositional studies. By electron microscopy it was adduced that developing fat droplets within the cell do not have a limiting membrane. As the droplets leave the cell they engage and become surrounded by plasma membrane. Secreted droplets are invariably observed to be bound by a membrane. The nature of the lipids and proteins in this surface coating closely resemble those from plasma membrane of the lactating mammary cell [1]. The presence of acid and alkaline phosphatases [2, 3] and of Na^+,K^+-activated ATPase [3] in milk fat globule membrane (MFGM) indicate that it may contain a typical spectrum of plasma membrane-type enzymes. We report here the presence of 5′-nucleotidase, nucleotide pyrophosphatase and of Mg^{2+}-activated ATPases in MFGM. These are marker enzymes for the plasma membrane [4–7]. Milk fat globules also afford an opportunity to study 'sidedness' in the plasma membrane of the lactating cell. An initial exploration of this matter is presented.

2. Methods

The procedure of Dowben et al. [3] was used to prepare MFGM. Bovine milk (10 l) immediately upon withdrawal from the animal was separated at 38° to 40° into cream and skim milk with the aid of a laboratory scale centrifugal cream separator. The cream (50% fat) was washed three times by dispersion in 3 to 4 volumes of 0.25 M sucrose containing 0.002 M $MgCl_2$. The washed cream was cooled to 12° and churned in a small Hobart-type mixer. In this process membrane is released from the fat globules which then adhere to each other to form butter. The buttermilk containing the membrane material was centrifuged at 100,000 *g*/hr to yield a pellet of MFGM.

Milk fractions including MFGM prepared from several samples of milk by this procedure were assayed for 5′-nucleotidase [4], nucleotide pyrophosphatase [6] and ATPases [8].

3. Results and discussion

All three enzymes could be detected in raw milk (table 1). There was an enrichment in activity of the three enzymes in fractions in which MFGM was concentrated or purified; i.e., cream, washed cream, washed cream buttermilk and MFGM. We could not demonstrate in milk or fractions thereof, including MFGM, a Na^+-dependent ATPase as reported by Dowben et al. [3]. The Mg^{2+}-dependent ATPases which we did find were insensitive to ouabain and were activated by addition of dithiothreitol. The pH optimum for 5′-nucleotidase in washed cream buttermilk was 7.4 (tris buffer) with specific activity of 540.0 nmoles/min/mg of protein.

Table 1
Plasma membrane enzymes in milk fractions.

Source	Buffer (pH)	Cofactors	Activity[a]
ATPase, substrate ATP-γ-^{32}P			
Raw milk	Tris (7.4)	DTT[b], K^+, Mg^{2+}	2.1
Raw milk	Tris (7.4)	DTT, K^+, Mg^{2+}, Na^+	2.0
Washed cream	Tris (7.4)	DTT, K^+, Mg^{2+}	10.7
Washed cream-buttermilk	Tris (7.4)	DTT, K^+, Mg^{2+}	18.6
	Tris (7.4)	DTT, K^+, Mg^{2+}, Na^+	16.4
MFGM[c]	Tris (7.4)	DTT, K^+, Mg^{2+}	15.0
MFGM[c]	Tris (7.4)	DTT, K^+, Mg^{2+}, Na^+	13.6
Nucleotide pyrophosphatase, substrate FAD			
Raw milk	AMPr[d] (9.0)	Mg^{2+}	39.4
Washed cream	AMPr (9.0)	Mg^{2+}	42.0
Washed cream-buttermilk	AMPr (9.0)	Mg^{2+}	53.5
MFGM	AMPr (9.0)	Mg^{2+}	159.0
5′-Nucleotidase, substrate 5′-AMP			
Raw milk	Glycine (8.9)	Mg^{2+}	13.0
Washed cream	Glycine (8.9)	Mg^{2+}	192.0
Washed cream-buttermilk	Glycine (8.9)	Mg^{2+}	202.0
MFGM	Glycine (8.9)	Mg^{2+}	205.0

[a] nmoles/min/mg protein
[b] DTT = dithiothreitol
[c] MFGM = milk fat globule membrane
[d] AMPr = 2-amino-2-methyl-1,3-propanediol

Since the milk fat droplet (98 to 99% glyceride) emerging from the cell engages the inside of the plasma membrane and, after envelopment, exposes only the outer surface of that membrane, it is possible to use the enzymatic activity of the three fractions: washed cream (outer membrane surface), washed cream buttermilk (both inner and outer surfaces) and MFGM (both surfaces) as a guide to the location of the enzymes. From the pertinent data (table 1) we suggest that 5′-nucleotidase is located on the outer surface of the membrane since both of the samples in which inner membrane surface should have been exposed; i.e., washed cream buttermilk and MFGM, exhibited about the same level of enzyme activity as the washed cream (outer surface only). On the other hand exposing both surfaces of the membrane substantially increased the Mg^{2+}-activated ATPases over that in the washed cream suggesting distribution of this enzyme in both surfaces. Data for the nucleotide pyrophosphatase are not entirely consistent in that pelleting membrane material from washed cream buttermilk greatly enhanced the activity. We consider it possible that this enzyme is largely confined to the inner membrane surface and that inhibitory glycerides attached to this surface are removed during centrifugal preparation of the MFGM pellet.

Milk fat globules represent a physiologic source of plasma membranes from mammals. With this source difficulties and artifacts of tissue fractionation procedures can be minimized. Milk fat globules offer a useful approach by which to investigate the distribution of the various lipids, proteins and enzymatic

functions on the inner and outer surfaces of the plasma membrane. However, the possibility that membrane components may undergo rearrangement during secretion and subsequent manipulation represents a limitation of this approach.

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References

[1] T.W. Keenan, D.J. Morré, D.E. Olson, W.N. Yunghans and S. Patton, J. Cell Biol. 44 (1970) 80.
[2] K.M. Shahani, J. Dairy Sci. 49 (1966) 907.
[3] R.M. Dowben, J.R. Brunner and D.E. Philpott, Biochim. Biophys. Acta 135 (1967) 1.
[4] P. Emmelot, C.J. Bos, E.L. Benedetti and P. Rumke, Biochim. Biophys. Acta 90 (1964) 126.
[5] T.K. Ray, Biochim. Biophys. Acta 196 (1970) 1.
[6] J.R. Skidmore and E.G. Trams, Biochim. Biophys. Acta 219 (1970) 93.
[7] O. Touster, N.N. Aronson Jr., J.T. Dulaney and H. Hendrickson, J. Cell Biol. 47 (1970) 604.
[8] H. Weil-Malherbe and R.H. Green, Biochem. J. 49 (1951) 286.