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Proteome analysis of the bovine milk fat globule: enhancement in the membrane purification

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Introduction

The MFGM is the protective coat surrounding lipid globules in milk, which allows the dispersion of the fat in the milk plasma. This coat material is a tripartite structure that is partially derived from the apical plasma membrane, from the endoplasmic reticulum and from other intracellular compartments of the mammary epithelial cell. This membrane prevents flocculation and coalescence of lipid droplets and protects the fat against lipolysis (1). As MFGM acts as a natural emulsifying agent, it should be considered as a potential stabilizing agent in the preparation of certain foods, e.g. creams and emulsions, infant formulas, and reduced-fat products (2). Furthermore, in recent years, different factors with health beneficial properties (cholesterolemia-lowering factor, inhibitions of cancer cell growth…) have been detected in bovine MFGM components (3).

The protein composition of MFGM is completely different from the skim milk one. Milk is composed of numerous specific proteins (caseins, whey proteins). Even if bovine skim milk proteins are well identified and characterized, the total identification of the bovine MFGM proteins has not yet been concluded. Many proteins that are present in low concentration may be easily missed due to the overwhelming amounts of other contaminant proteins. Therefore, it is often necessary to reduce the complexity of the mixtures before the proteins can be identified.

The application of a method to obtain purified fat globules with a minor non globular-component is detailed. Additionally, four different detergents were tested: CHAPS, amidosulfobetaine-14 (ASB 14), Sodium Lauroyl sarcosinate (Sarkosyl) and Sodium deoxycholate, in order to maximize the membrane proteome extraction to be observed by following 2-DE.

MALDI-TOF MS and MS/MS was used to identify most proteins from the MFGM.

Methodology

Preparation of the milk fat globule membrane

The MFGM was extracted from fresh unpasteurised cream (Holstein milk). Cream was washed with phosphate buffer (0.01 M Na2HPO4/NaH2PO4; 0.9% NaCl; pH 7.2) and subjected to centrifugations between washes. Washed fat globules were resuspended in distilled water and allowed to crystallise for twenty hours at 4°C. Globules were churned at 4°C until fat and sera (MFGM suspension) were separated. The total sera was centrifuged twice (5000 x *g*, 15 min, 4°C) to remove the fat and freeze dried.

Sample preparation optimization for analysis of membrane proteins by 2-DE

In order to improve extraction and solubilization of the hydrophobic membrane proteins four detergents at different concentrations were tested in the sample preparation. Membrane proteins were precipitated by the addition of TCA. The pellet was then solubilized in rehydratation buffer before proteins separation by IEF and SDS-PAGE electrophoresis.

Protein spots of interest from bovine MFGM were excised from 2-DE, trypsin digested and identified by PMF and subsequent MS/MS analysis

Results

1. Extraction of membrane proteins by detergents

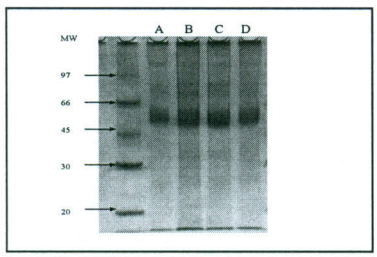

Fig1. 1D SDS-PAGE pattern of MFGM extracted with A) 0,5% Sodium Lauroyl sarcosinate, B) 4% CHAPS, C) 4% ASB 14, D) 0,5% Sodium deoxycholate

The protein bands observed in the patterns for the samples of all detergents showed a similar sequence migration of the proteins. However, zwitteronic detergents (CHAPS and ASB 14) allowed to extract the largest quantity of membrane proteins. The polypeptide pattern for MFGM proteins show three major bands that could correspond to lactadherin, butyrophilin and adipophilin. As there was no improved banding patterns over the four detergents, subsequent analysis by 2-DE were performed with the four detergents.

Samples extracted with zwitteronic detergents showed improvement over the spots patterns. No significant difference was observed between 4% CHAPS and 4% ASB 14. Membrane proteins of interest were soluble under electrophoresis conditions.

2. 2-DE analysis of milk fat globule membrane

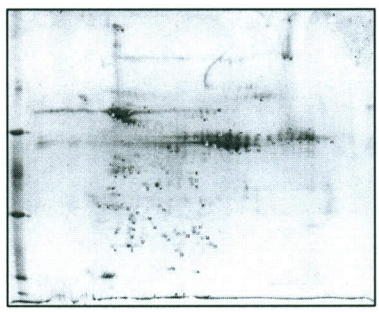

Fig 2. 2-DE of MFGM extracted with 4% CHAPS. Preparative gel was stained with Coomassie blue.

Milk fat globule proteins counts for 2-4% of the total milk protein content. Abundant proteins may mask identification of less abundant proteins of interest. In the 2-DE no spots were detected in the typical area of non-MFGM proteins. Our preparation of the MFGM allowed optimal fractionation as most of the non-MFGM proteins were eliminated.

The large majority of the MFGM proteins appeared at a p*I* between 4 and 8. Data files from Maldi analysis were searched against Mammalian protein database.

For several proteins, two or more spots differing in their p*I* were found, suggesting post-translational proteins.

A total of 79 spots matched bovine proteins. The other 3 spots were matched to homologous proteins in human (spot 5 and 6) and rabbit (spot 18).

3. Identifications of proteins by PMF and subsequent MS/MS

Spot Number	Protein name	Accession number	Function
1,7,8	Fatty acid-binding protein, heart	P10790	Intracellular transport
3	β-lactoglobulin (precursor)	P02754	Primary component of whey
5(1) (human)	Ras-related protein Rab-18	Q9NP72	Endocytosis/ recycling
6(1) (human)	Calcium binding protein p-22	Q99653	Required for constitutive membrane traffic
9	ADP-ribosylation factor 1	P84080	Protein trafficking
10	ADP-ribosylation factor 4	Q3SZF2	Protein trafficking
12	GTP-binding protein SAR 1a	Q3T0D7	Transport from the endoplasmic reticulum to the Golgi apparatus
13,14	Transforming protein RhoA precursor	P61585	Regulates a signal transduction pathway
15	Annexin III	Q3SWX7	Inhibitor of phospholipase A2, also possesses anti-coagulant properties
16	Ras-related protein Rab-11B	Q3MHP2	Protein trafficking
17	GTP-binding protein SAR 1b	Q3T0T7	Involved in transport from the endoplasmic reticulum to the Golgi apparatus
18(1) (rabbit)	Carbonic anhydrase 2 (EC 4.2.1.1)	P00919	Reversible hydration of carbon dioxide
19	Guanine nucleotide binding protein G(I)/G(S)/G(T) subunit beta 2	P11017	Involved as a modulator or Transducer in various transmembrane signaling systems
20, 61-74, 93, 94	Lactadherin precursor (Milk fat globule	Q95114	Phospholipid binding
22	Ras-related protein Rab-1B	Q2HJH2	Protein transport
24-27, 37-50, 53, 54, 76-84, 86, 87	Butyrophilin subfamily 1 member A1 precursor (BT)	P18892	Fat globule secretion
28, 29	Annexin A5 (Annexin V)	P81287	Anticoagulant protein
51	Actin cytoplasmic 1 (Beta-actin)	P60712	Cell motility
55	Guanine nucleotide binding protein G(o) subunit alpha	P08239	Modulator or transducer
56-60, 90-92	Adipophilin (Adipose differentiation-related protein)	Q9TUM6	May be involved in development and maintenance of adipose tissue
85	Heat shock cognate 71 kDa protein	P19120	Chaperone
88, 89	Xanthine dehydrogenase/oxidase	P80457	Redox reaction
95	Polymeric-immunoglobulin receptor precursor	P81265	This receptor binds polymeric IgA and IgM

(1) Protein matched to homologous

Conclusions

- Our method for the MFGM preparation allowed maximal elimination of non-MFGM proteins
- Extraction of membrane proteins by 4% CHAPS or 4% ASB 14 gave good results on a 2-DE map
- Presence of the most abundant proteins in the MFGM and further identification of MFGM minor proteins for additional completion of the proteome were confirmed.

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