Isolation, Purification and Characterization of Fatty-Acid-Binding Protein from Milk Fat Globule Membrane: Effect of Bovine Growth Hormone Treatment

Vitaly L. Spitsberg¹ and R. C. Gorenwit²;
¹BioVita Technologies, Inc., Ithaca, NY, ²Lactation Physiology Laboratory, Cornell University, Ithaca, NY 14853

Abstract: Fatty-acid-binding protein (FABP) was purified from bovine milk fat globule membrane (MFGM) by ion-exchange chromatography on DEAE-Sepharose and by gel-filtration on Sephadex G-50. Purified FABP was similar to bovine mammary gland heart (H)-type FABP, mammary derived growth inhibitor (MDGI). It inhibited growth of mammary epithelial cells at nanogram concentrations, had a relative molecular mass of 15 kDa, as determined by SDS-PAGE, had two isoforms with pi around 5.0 and cross-reacted with antibody to mammary gland H-FABP. The content of FABP in MFGM, obtained from growth hormone (GH)-treated cows, was not essentially different from that of MFGM obtained from untreated cows. However, the level of in vitro phosphorylated FABP of MFGM, obtained from GH-treated cows, was diminished in comparison to the sample of MFGM, obtained from untreated cows. The role of the insulin receptor in the phosphorylation of FABP in mammary gland secretory epithelial cells is suggested.

Key words: Milk fat, globule membrane, bovine growth, protein

Introduction

The role of fatty-acid-binding protein (FABP) in fatty acid transport and lipid metabolism is well known (Bass, 1988; Spener et al., 1992). However, recently accumulated experimental findings have allowed us to suggest that FABP can also play a role in regulation of cell proliferation and differentiation, at least, in the mammary gland (Grosse and Langen, 1990; Bohmer et al., 1984; Bohmer et al., 1997; Yang et al., 1994). It has been established that bovine mammary gland contains a 14.5 kDa protein that inhibits the growth of bovine mammary gland cells and some breast cancer cell lines. This protein was purified and named mammary derived growth inhibitor (MDGI) (Bohmer et al., 1985; Bohmer et al., 1987; Grosse and Langen, 1990; Grosse et al., 1991). Direct protein sequence analysis of MDGI revealed 95% homology to bovine H-FABP (Bohmer et al., 1987). However, the sequence of MDGI, deduced from the presumably cloned bovine MDGI cDNA, was identical to bovine H-FABP (Grosse et al., 1991). Despite this last finding and the fact that recombinant H-FABP had a similar cell growth inhibiting effect as MDGI (Yang et al., 1994), bovine mammary gland FABP, as isolated and purified according to the protocol of Bohmer et al. (1985) or to the improved procedure of Grosse et al. (1991), was still called MDGI, suggesting the existence of a specific message for MDGI.

Recent studies by Specht et al. (1996) Borchers et al. (1997) helped resolve the controversy of the primary structure of MDGI. These studies demonstrated that the bovine mammary gland contains at least two types of FABP, namely, H-FABP and adipocyte (A)-type FABP, Comparison of the sequences of H-FABP and A-FABP allowed researchers to conclude that the direct protein sequencing analysis of MDGI, reported previously, had led to the erroneous identification of amino acid residues in, at least, 7 positions. This was a result of the presence of contaminating amounts of the co-purified A-FABP in the analyzed MDGI preparation. However, this research group (Borchers et al., 1997) demonstrated that H-FABP, but not A-FABP, was involved in growth inhibition and differentiation in the mammary cell, and was localized in secretory epithelial cells of mammary gland tissue. A-FABP was found only in myoepithelial cells of the mammary gland. Therefore, based on the study of Grosse’s research group (Grosse et al., 1991) and Specht et al. (1996) and Borchers et al. (1997), we have designated the bovine mammary gland 15 kDa FABP with cell growth inhibitory property as bovine mammary gland H-FABP/MDGI. FABPs related to bovine MDGI were also identified in mammary gland of humans (Shi et al., 1998) and mice (Binay et al., 1992; Bansal and Medina, 1993; Treuer et al., 1994).

The inhibitory action of H-FABP, or MDGI, on cell proliferation can be mimicked by synthetic peptides related to the 11-amino acid C-terminus of this protein (Grosse et al., 1991; Yang et al., 1994). This C-terminus has structural homology to Type I repeat of thrombospondin (TSP) (Spitsberg et al., 1995). It is thought that the Type I repeat domain of TSP is responsible for the physiological effects of TSP through its binding to surface glycoprotein CD36 (Greenwald et al., 1992). Glycoprotein CD36 is significantly expressed in epithelial cells of the lactating mammary gland (Greenwald et al., 1992). It is suggested that the cellular action of FABP/MDGI, as a differentiation factor, can be exerted through an apocrine loop mechanism (Brandt et al., 1988). i.e. through the secretion by mammary gland cells of FABP/MDGI into extracellular spaces with subsequent binding of FABP/MDGI to CD36 (Spitsberg et al., 1995). This would then lead to the triggering of specific signal transduction pathway(s) involved in cell differentiation. Recent studies also suggested that FABP/MDGI can function as a tumor suppressor gene product (Huynh et al., 1996).

FABP, similar to mammary gland FABP/MDGI, has been found in bovine milk fat globule membrane (MFGM) (Brandt et al., 1988). However, this membrane-associated FABP was not isolated from MFGM in its intact form, and, therefore, its physicochemical nature and biological activity has not been defined.

In this work we report the purification, characterization and biological activity of FABP from bovine MFGM. Amino acid analysis, SDS-PAGE, isoelectric focusing, Western blotting, two-dimensional electrophoresis (2D E) and inhibitory cell growth assay demonstrated the similarity of FABP from MFGM to H-FABP/MDGI of bovine mammary gland tissue. In this work, we also showed that treatment of cows with bovine growth hormone (GH) appears to influence the level of in vitro FABP phosphorylation within the MFGM.

Materials and Methods

Milk from non-hormone treated and bovine recombinant growth hormone (rGH, Posilac, Monsanto, St.Louis, MO, USA) treated cows was obtained from the Cornell University Dairy Teaching and Research Farm. Milk from hormone-treated cows was collected from those cows which had received injections of rGH (500 mg of Somatropin zine) every two weeks over a six month period. MFGM was prepared according to Spitsberg et al. (1995). Rabbit polyclonal antibodies to bovine mammary gland-derived FABP/MDGI were obtained according to Spitsberg et al. (1995). DEAE-Sepharose CL-6B and Sephadex G-50 (fine) were from Sigma Chemical Co. (St.Louis, MO, USA). Precast polyacrylamide slabs for isoelectric focusing (Ampholine PAGplate, pH 4.0 - 6.5; A = 5%,
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C = 3%) were from Pharmacia (Uppsala, Sweden). Ultrafiltration membranes (pore size, 0.45 μm) were from Bio-Rad Laboratories ( Hercules, CA, USA). Gamma-32P-ATP (3000-6000 Ci/mmol) was purchased from Amer sham, Inc. All other reagents were high purity grade and were purchased from various vendors.

Isolation and purification of FABP from bovine MFGM: All purification procedures were carried out at 2-4 °C. MFGM was obtained from composite bulk tank milk according to the protocol of Spitsberg et al. (1995). The MFGM was suspended in 50 mM imidazole buffer (pH 8.0) at about 10 mg/mL. This suspension was ultrasonicated by four strikes (each of 15 sec duration) at maximum output. After that, α-mercaptoethanol was added to the suspension to a concentration of 15-20 mM. The suspension was mixed for 10-15 min and was subjected to centrifugation at 60,000 x g for 1 h. The resulting supernatant was applied to a DEAE-Sepharose column (2 x 15 cm) equilibrated with 50 mM imidazole buffer, pH 8.0 (IB). The FABP fraction was eluted from the column by IB, containing 50 mM NaCl. The FABP fraction was eluted by ultrafiltration with membrane UM1 (molecular weight cutoff < 3 kDa) to about 6 mL, and this protein solution was applied to a Sephacryl G-50 column (90 x 2.5 cm; Vo = 150 mL), equilibrated with 50 mM Tris-HCl buffer, pH 7.4, containing 140 mM NaCl (TBIS-buffer). The eluted protein fraction/peak with Kd = 1.6 (Fig. 1) was collected and concentrated by ultrafiltration, and stored at -70 °C. The isolation and purification of FABP from MFGM was repeated three times using the protocol described above. The average yield of FABP was approximately 400 μg of protein per isolation procedure.

Isolation and purification of bovine mammary gland FABP/MFG: Bovine mammary gland FABP/MFG was prepared by the protocol, designated as Method B, of Grosse et al. (1991). This method is more advanced than the method previously published by Bohmer et al. (1995). In Method B, the following improvements were made: the concentration of reducing agent, β-thioglycerol, in the buffer, used for gel-filtration and ion-exchange chromatography, was increased from 10-3 M to 10-2 M. At the same time 10% glycerol was added to this buffer. The volume of the DEAE-Sepharose column was increased from 2 mL to 26 mL. The buffer, containing increased amount of β-thioglycerol and glycerol, provides conditions sufficient to prevent unnecessary aggregation of proteins during purification. The increased amount of the ion-exchanger provides a better condition for separation of the different types of FABP present in mammary gland tissue.

Amino acid analysis: Amino acid analysis of isolated and purified FABP from MFGM and bovine mammary gland tissue was done by the standard protocol at the Biotechnology Facilities of Cornell University.

Isoelectrofocusing: Iselectrofocusing was done according to the procedure recommended by the vendor (Pharmacia) with precast polyacrylamide slabs, containing ampholines to support the pH gradient from pH 4.0 to pH 6.5. Protein (2-3 μg) was applied to each lane. After isoelectrofocusing, the slabs were fixed using 40% methanol, containing 7% acetic acid, for 16 h. The slabs were then processed for silver staining according to the procedure of Gooderham (1984).

Cell growth inhibitory activity of FABP derived from MFGM: The determination of cytotoxic activity, i.e. inhibition of cell growth, of MFGM-derived FABP was done essentially as described by Spitsberg et al. (1995). MAC-T cells and primary mammary gland epithelial cells were used in these experiments. The experiments were repeated four times and the standard mean error (SME) was about ± 5%.

Protein phosphorylation: In vitro protein phosphorylation of MFGM proteins was done according to Spitsberg and Gorewit (1997). Samples of MFGM were prepared from the milk of cows treated with recombinant bovine GH and from milk of non-treated cows (controls), as indicated by Spitsberg et al. (1995). The examining of in vitro phosphorylation of 18 kDa protein within bovine MFGM was done in three different stocks of the experiments. In the first series we analyzed five samples of MFGM obtained from GH-treated cows and five samples from non-hormone treated cows. In the second series, three samples from the GH-treated cows and one sample of MFGM from non-hormone treated cows were analyzed, and we, also, analyzed two samples of MFGM prepared from the bulk milk, obtained from one of the local dairy farms, which was using injections of recombinant GH (Posilac, Monsanto) for increased milk production.

Other procedures: SDS-PAGE and Western immunoblotting were performed as described by Spitsberg et al. (1995). Non-denatured (12%) polyacrylamide gel electrophoresis was done with the buffer system used for SDS-PAGE, with no addition of SDS to the buffer gel.

Two-dimensional electrophoresis (2D-E) was done according to Anderson (1991). After 2D-E the gel slabs were subjected to Western immunoblotting (Spitsberg et al., 1995). Protein concentration was determined by Bradford's method (Bradford, 1976). Densitometric analysis of the autoradiograms was done with the Saggita Color LE scanner (Otronix) and with the Image analysis program, developed by Molecular Dynamics (Sunnyvale, CA, USA).

Results

Isolation, purification and physicochemical properties of FABP from MFGM: The protocol described above for the isolation and purification of FABP from MFGM allowed us to obtain 300-500 μg of highly pure FABP from MFGM, obtained from 31 of milk. The high purity of FABP was confirmed by the resolution of a single band after SDS-PAGE electrophoresis and Western blotting (Fig. 2A and B). Though the purification procedure employed in this work was quite similar to that used previously to purify FABP/MFG from mammary gland tissue (Grosse et al., 1991), the ultrasonic and addition of α-mercaptoethanol (16-20 mM) to the extraction solution were necessary for the maximum solubilization of FABP from MFGM. Attempts to improve the yield of FABP from MFGM by inclusion of Triton X-100 into extraction buffer were not successful.

The SDS-PAGE analysis of purified FABP from MFGM showed the presence of only one protein with a relative molecular mass of 16 kDa, identical to that of mammary gland FABP/MFG (Fig. 2A). Western immuno-blotting analysis of FABP showed that FABP from MFGM was immunologically similar to mammary gland FABP/MFG (Fig. 2B). Like FABP/MFG from mammary gland, the purified FABP from MFGM had two components, fast and slow, when it was analyzed by PAGE at non-denatured conditions (Fig. 2C). It is likely that the appearance of these two components was a result of the existence of two isoforms of FABP (Fig. 3). It is unlikely that the slow and fast components were a result of the nonspecific self-aggregation of FABP (Fournier and Rahim, 1983), since the aggregation would lead to the appearance of numerous bands rather than only two bands, detected with different preparations of MFGM FABP and mammary gland FABP/MFG. One of the isoforms of FABP from MFGM had a pI = 5.20, and the other had a pI = 5.35. These isoforms, appeared as a result of the charge heterogeneity of the same protein, were similar to isoforms of FABP/MFG from mammary gland (Fig. 3, lanes 2 and 3 versus lane 1). In an additional experiments done by 2D-E, in which 8 M urea was used in the first dimension (isoelectrofocusing), only two isoforms of the purified FABP from MFGM were detected at pH around 5.0. Similarly, only two isoforms at pH around 5.0 were found in purified mammary gland FABP, analyzed by 2D-E. Therefore, the 2D-E analysis of MFGM FABP and mammary gland FABP, purified by the protocol as it is
samples of MFGM obtained from non-hormone-treated cows. Therefore, this ratio can serve as an indicator of the status of the phosphorylation level of 15 kDa protein. The lesser phosphorylation of 15 kDa protein in MFGM could be explained by diminished content of FABP in MFGM obtained from hormone-treated animals. However, the amount of 15 kDa protein/FABP within MFGM, obtained from control and GH-treated cows, was quite similar in both types of MFGM, as determined by SDS-PAGE analysis (Fig. 4A). The possible reduction of the in vitro phosphorylation of 15 kDa protein within MFGM, obtained from the GH-treated cows, was confirmed in the additional experiments with three individual samples of MFGM from GH-treated cows and with two samples of MFGM, prepared from the bulk milk of one of the local dairy farms, using GH for the increased production of milk.

**Discussion**

Previously it has been shown that bovine MFGM contains FABP which is related to bovine FABP/MDGI (Brandt et al., 1996). However, in this study MFGM FABP was isolated from SDS-polyacrylamide gels, i.e. in a denatured form. Therefore, FABP obtained in this way is not suitable for examining its biological activity. Moreover, the biochemical characteristics of MFGM FABP were unknown. We felt it important to determine if MFGM-associated FABP is structurally and functionally similar to FABP/MDGI. MFGM, as it is known, consists mostly of apical plasma membrane elements of secretory epithelial cells of the lactating mammary gland (Kanno, 1990). The discovery of FABP/MDGI in this membrane may support the suggestion of the involvement of FABP/MDGI in cell growth and differentiation through an apocrine loop mechanism (Brandt et al., 1998).

In our work, we presented evidence that FABP associated with the bovine MFGM, is identical to the soluble, cytosolic form of H-FABP or MDGI of bovine mammary gland tissue (Grose et al., 1991). Firstly, we demonstrated that purified 15 kDa protein from bovine MFGM and FABP/MDGI from mammary gland tissue (Grose et al., 1991; Spitsberg and Gorevit, 1996) had similar biological activities. Both FABPs were able to suppress growth of mammary-derived cells at nanomolar concentrations. Secondly, the identity of the isolated 15 kDa protein from MFGM and FABP/MDGI was confirmed by analysis of the macromolecular species of these proteins by SDS-PAGE, non-denaturing PAGE, isoelectric focusing, Western immunoblotting and 2D-E. Since, in bovine mammary gland, only two types of FABP, namely, H-FABP, localized in secretory epithelial cells, and A-FABP, localized only in myoepithelial cells, were clearly identified, we feel strongly that the isolated and purified 15 kDa protein from MFGM is FABP of heart-type, and can be referred to MFGM-associated H-FABP/MDGI.

The identification of H-FABP/MDGI associated with MFGM, representing the apical plasma membrane of secretory epithelial cells of mammary gland, opens a new avenue for studying the expression and biochemical modification of MFGM associated H-FABP/MDGI in the mammary gland under various physiological conditions.

We analyzed whether GH-treatment of cows can affect the content of H-FABP/MDGI within the MFGM and/or the level of in vitro phosphorylation of MFGM-associated H-FABP/MDGI (Spitsberg and Gorevit, 1997). This work showed that the level of phosphorylation of 15 kDa FABP, within the MFGM obtained from GH-treated animals was affected by GH-treatment. The level of phosphorylation was less than that for MFGM obtained from non-treated animals. The content of H-FABP/MDGI of MFGM was not affected by prolonged GH-treatment of cows, although the effect of GH-treatment on total expression of H-FABP/MDGI in mammary gland tissue cannot be ruled out. Here, it is worthy to mention the recent in vitro finding of Huynh and Beamer (1998) that GH and insulin-like growth factor 1 (IGF-1) can upregulate MDGI gene expression.

The observed reduced level of in vitro phosphorylation of FABP within the MFGM, obtained from GH-treated cows, can be

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<th>Concentration (ng/ml)</th>
<th>MAC-T Cells (%)</th>
<th>Primary epithelial cells (%)</th>
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<tr>
<td>0.1</td>
<td>28.0</td>
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<tr>
<td>1.0</td>
<td>67.0</td>
<td>60.0</td>
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<td>10</td>
<td>71.0</td>
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<tr>
<td>100</td>
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indicated in the Methods, did not reveal any noticeable amount of 15 kDa band related to the A-FABP (Specht et al., 1996). The different numbers in pl for two isoforms of FABP, reported in this study, and for bovine mammary gland H-FABP, reported by Specht et al. (1996), can be explained by an inaccuracies in determination of pH gradient in isoelectrofocusing experiments rather than by differences in the nature of the investigated FABP's. What is essential in both cases, is the two isoforms of FABP in this study and Specht's study had quite similar differences in their pl's, namely, 0.15 units in our study, and 0.2 units in study of Specht et al. (1996), and these two isoforms were positioned at pH around 5.0. The possibility that MFGM FABP or FABP/MDGI, purified in our study, could represent bovine brain (B)-type of FABP, which has a high homology (90-95%) with bovine H-FABP (Schoentgen et al., 1989), can be excluded, since the B-FABP would have a pl around 6.0. Because the 11-amino acid C-terminus of B-FABP is quite different from that of H-FABP, it is unlikely that B-FABP would have cell growth inhibitory properties. On similar grounds, keratinocyte-epidermal lipid-binding protein with 48% homology to H-FABP and bovine MDGI (Kieg et al., 1993) cannot represent the MFGM FABP studied in this work.

In our work it was also found that the amino acid composition of purified MFGM FABP was quite similar to that of purified FABP/MDGI and was not significantly differed from the amino acid composition derived from the published bovine mammary gland H-FABP amino acid sequence (Borchers et al., 1997).

The analytical parameters of MFGM FABP and bovine mammary gland FABP/MDGI, purified by the Method B (Grosse et al., 1991), provided evidence that both preparations of FABP analyzed in our work belong to the H-type of FABP, i.e. they are identical to H-FABP, described by Borchers et al. (1997).

**Cell growth inhibitory activity of FABP derived from MFGM**

The examination of growth inhibitory activity of FABP from MFGM showed that this protein had biological activity quite similar to FABP prepared from mammary gland (Grosse et al., 1991; Spitsberg et al., 1996). A activity was exerted in the concentration range 1-10 ng/ml (Table 1).

**In vitro phosphorylated FABP**

The level of in vitro phosphorylation of FABP or 15 kDa-protein (Spitsberg and Gorevit, 1997) within MFGM obtained from cows, treated with recombinant bovine growth hormone, were examined in this work. Fig. 4. Panel B, represents the autoradiogram of the in vitro phosphorylation of MFGM proteins obtained from the raw milk of GH-treated cows and from untreated cows. The level of phosphorylation of FABP, i.e. the densitometric density (DD) of the 15 kDa bands of the autoradiogram (Fig.4, Panel B) is presented in Fig 5. The fig. 4 and 5 clearly demonstrated that the in vitro labeling of 15 kDa protein of all five samples of MFGM, obtained from the GH-treated cows, was less than in the samples of MFGM obtained from non-treated cows. The labeling of 15 kDa protein was especially reduced in the samples of MFGM analyzed in lanes 2 and 6 in Fig.4B. The phosphorylation of other major bands of MFGM, such as the 66 kDa and 61-62 kDa bands, was not markedly affected by GH treatment. We found that the ratio of the DD of the 66 kDa band to the DD of the 15 kDa band was practically constant when it was measured in five different
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Fig. 1: Elution profile of the MFOM-FABP containing fraction, obtained from DEAE-Sepharose, on Sephadex G-50 (30 x 2.5 cm). Equilibration buffer: 50 mM Tris-HCl, pH 7.4, containing 140 mM NaCl. The protein peak with $V_r/V_{0} = 1.5 - 1.6$ ($V_r = 150$ ml; $V_0 = 240$ ml) was collected and analyzed by SDS-PAGE, Western immunoblotting and cell growth inhibitory assay (see Methods). The first 10 fractions were collected at 14.5 ml/hour and subsequent fractions were collected at 5.3 ml/hour. The triangle points show the $V_r$ (left) and $V_0$ (right).

Fig. 2: Analysis of purified FABP from MFOM by PAGE and Western immunoblotting. Panel A: (12%) SDS-PAGE; lane 1: 100 ng of bovine mammary gland FABP; lane 2: 125 ng of bovine MFOM-FABP; lane 3: 100 ng â-lactalbumin (14.2 kDa); lane 4: protein molecular weight standards. The gel was stained with silver (Gooderham, 1994). Panel B: Western immunoblotting analysis of purified bovine MFOM-FABP. The proteins were separated by 12% SDS-PAGE and transferred to nitrocellulose membrane. Lane 1: about 100 ng of bovine mammary gland FABP; lane 2: about 100 ng of MFOM-FABP; Lane 3: 100 ng â-lactalbumin (14.2 kDa). Secondary antibody was anti-bovine mammary gland FABP (Spitsberg et al., 1994). Secondary antibody was conjugated goat anti-rabbit IgG-HRP. The blot was developed with 4-chloro-1-naphthol and H$_2$O$_2$. Panel C: Western immunoblotting analysis after the separation of the proteins in non-denaturing (12%) PAGE. Lane 1: 100 ng of mammary gland FABP; 2: 100 ng of MFOM-FABP. Antibodies and detection were as it is described in panel B.

Fig. 3: Analysis of FABP by isoelectrofocusing. Lane 1: 3 Î¼g of bovine mammary gland FABP; lane 2: 1.8 Î¼g of MFOM-FABP; lane 3: 3.0 Î¼g of MFOM-FABP. The gel was stained by silver.

Fig. 4: Analysis of in vitro $^{35}$S-labeled MFOM proteins. Panel A: (12%) SDS-PAGE of bovine MFOM, obtained from GH-treated and untreated cows. Lane 1: MW (kDa) standards; Lane 2: 150 Î¼g of MFOM obtained from milk of non-hormone-treated cow (CTR control), lanes 3-6: 50 Î¼g of MFOM obtained from three individual samples of milk of GH-treated cows (BST1, BST2, BST3). The gel was stained by Coomassie blue R-250. The 15 kDa protein, previously identified only as FABP (Brandt et al., 1988; Spitsberg et al., 1995; Spitsberg and Gorewit, 1997) is present in these samples of MFOM in equal amount. Panel B: Autoradiogram of (12%) SDS-PAGE of the in vitro $^{35}$S-labeled MFOM proteins, obtained from GH-treated and non-hormone-treated cows. 50 Î¼g of proteins of MFOM were in vitro phosphorylated and subjected to SDS-PAGE (see Methods). Lane 1: MFOM from non-treated cow (control); lanes 2-6: individual MFOM from five GH-treated cows. The labelling pattern of other samples of MFOM from four untreated cows were similar to that shown in lane 1. Note that the in vitro labeling of 65 kDa protein (butyrophilin) (Spitsberg and Gorewit, 1997) was quite similar in all analyzed samples of MFOM.
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Fig. 5: Graph of the densitometric analysis of the autoradiogram in Fig. 4. Panel B. The graph demonstrates the decreased labeling of 15 kDa protein/FABP in five individual samples of MFGM obtained from GH-treated cows. The level of labeling of 15 kDa protein/FABP within control MFGM was taken as 100%.

interpreted as a result of diminished content or diminished activity of the phosphorylating FABP protein kinase in this membrane. One of the candidates for this kinase is the beta-subunit of insulin receptor since this polypeptide was detected in MFGM by Western immunoblotting (Spitsberg and Gorewit, 1997). In addition, the specific insulin-binding sites in bovine MFGM have been also reported (Smith et al., 1987). In our previous work, we demonstrated that a tyrosine residue of the 15 kDa protein of bovine primary epithelial cells is phosphorylated and it reacted positively with FABP-antibody (Spitsberg et al., 1984). There is evidence that FABP in mouse mammary gland epithelial cells is phosphorylated by the insulin receptor (Nielsen and Spener, 1993; Nielsen et al., 1994). There are a few recent publications indicating the relationships between GH and insulin receptor (Balbis et al., 1996, 1992; Leenanuruksa and McDowell, 1989). Balbis et al. (1996, 1992) demonstrated that overexpression of bovine GH in transgenic mice is associated with down regulation of hepatic insulin receptor. In other work (Leenanuruksa and McDowell, 1988), injection of recombinant bovine GH into alloxan-diabetic insulin-stabilized ewes led to increased plasma glucose, which was decreased by a double-dosed insulin infusion. If the phosphorylation of FABP takes place through the involvement of the beta-subunit of the insulin receptor, the association of FABP with membrane should occur on the cytoplasmic side of the plasma membrane and consequently on the cytoplasmic side of MFGM since MFGM represents the portion of plasma membrane encircling the secreted lipid droplets. The analysis of immunoprecipitates formed after the addition of anti-FABP or anti-CD36 to the solubilized proteins of MFGM showed that one of the candidates for the binding of FABP to the plasma membrane is the cytoplasmic domain of CD36 (Spitsberg et al., 1995). Further study will be needed to show if the complex of FABP with CD36 is also associated with the beta-subunit of insulin receptor.

The significance of the finding that the phosphorylation of FABP within the MFGM is under hormonal regulation may have a practical application. Our preliminary work (Spitsberg and Gorewit, unpublished data) indicates that there is a correlation between the level of phosphorylation of FABP within the MFGM and the level of milk production by individual animals. We suggest the degree of in vitro phosphorylation of FABP within the MFGM can serve as an indirect sign as to whether or not the animals were given biologically efficacases doses by GH. Therefore, the analysis of the in vitro phosphorylation of individual samples of MFGM could provide an understanding as to why some cows do not respond to the injection of GH with significantly elevated milk production.

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Abbreviations: MFGM - milk fat globule membrane; H-FABP - heart-type fatty acid binding protein; A-FABP - adipocyte-type fatty acid binding protein; MDGI - mammary derived growth inhibitor; GH - growth hormone; BST - bovine somatotropin; SDS - sodium dodecysulfate; PAGE - polyacrylamide gel electrophoresis.