Some Practical Implications of the Milk Mucins[1,2]

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ABSTRACT

Two mucins, large carbohydrate-rich proteins, enter milk from the lactating cell surface by way of the milk fat globule membrane. These mucins relate to a number of practical considerations including physical and flavor properties of milk, mastitis and economically important traits of cattle, and diarrheal disorders of humans and calves (scours). A greater understanding and more effective use of the milk mucins will require additional research.
(**Key words**: milk mucins, MUC1, MUC-X)

INTRODUCTION

Basic research on the epithelial mucins of milk and mammary glands over several decades has produced information, some of which is particularly meaningful in terms of the cow and her milk. In addition to providing new insights about milk, these findings have created fresh research and development possibilities. Because literature on the milk mucins has been comprehensively reviewed recently (8), this presentation will attempt an orientation on what the milk mucins are and how they relate to practical considerations about the cow and milk.

WHAT ARE THE MILK MUCINS?

Mucins are proteins to which a substantial amount of carbohydrate (usually >30%) is covalently linked. This carbohydrate serves to order water structure, among other functions, so that the aqueous phase surrounding mucins is extraordinarily viscous. Two general types of mucins exist: those that are secreted, such as from goblet cells of the intestine, and those that are integral components of membranes, such as in the plasma membrane of epithelial cells. The milk mucins are of the latter type. They exist initially on the apical (secreting) surface of the lactating epithelial cell and gain entrance to milk when the apical plasma membrane surrounds fat globules at their secretion. There are two milk mucins: MUC1, about which much is known, including the amino acid sequence from its cDNA, and MUC-X, about which limited information is available. Thus, the main focus here will be on MUC1. Quantitatively, the two mucins are minor components of milk. Proteins make up about 1% of milk fat globules, and, in fresh, uncooled milk, about 70% of the mucins are in the fat globule membrane; the remainder is associated with membrane material in the skim milk. Although the mucins as globule constituents have not been quantified precisely, they appear to make up about 5 to 10% of the globule protein.

MUCIN STRUCTURE

The MUC1 (and presumably MUC-X) of human milk exists as a relatively long filament projecting up to 1 μm from the surface of the milk fat globule [see Figure 1 in (1)]. Initial observations indicated that such filaments cannot be seen on bovine milk fat globules, but it was later deduced that MUC1 of bovine globules is unstable and is easily removed by the cooling and washing involved in preparing globules for microscopy, which is one of the unique characteristics of the bovine milk mucins with practical implications. Why MUC1 of bovine milk dissociates readily from the milk fat globule is not known. The MUC1 of human milk, which is much more stable in that regard, is known to be a heterodimer (6). In the course of intracellular synthesis, it is cleaved by a protease, but the two segments remain bound, and the integral membrane configuration is retained. With respect to its position in the apical plasma membrane, this cleavage point is extracellular, and strong detergent (SDS) is required to dissociate the dimeric structure. If bovine MUC1 proves to have such a dimeric structure, it may be much more easily dissociated than human MUC1. However, the whole bovine dimeric structure might be released from the membrane by cooling.

A further remarkable feature of the milk mucins is their peculiar type of genetic polymorphism. The peptide chain in the extracellular filament of MUC1 contains a segment of 20 amino acids that is tandemly

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repeated and is genetically variable in the number of repeats. As a consequence, the two genes (alleles), one inherited from each parent, may and usually do dictate proteins of different lengths. Thus, from the milk of a given cow, one or two bands of MUC1 may be observed by SDS gel electrophoresis, and the band or bands may vary in mobility from those of other cows.

The MUC1 of both human and bovine milk contains approximately 50% carbohydrate. The MUC-X of human milk contains about 80% carbohydrate. Similar to MUC1, MUC-X exhibits a codominant-gene-type polymorphism, but bovine milk exhibits what appears to be a nonpolymorphic MUC-X. As for human MUC-X, after SDS gel electrophoresis, bovine MUC-X is found in the stacking gel (3.5% acrylamide), but, unlike the human MUC-X, the bovine MUC-X is always a single band. Interestingly, MUC-X of equine milk is polymorphic. The most plausible candidate for identity of MUC-X is MUC4, a human mucin containing a tandem repeat of 16 amino acids that was observed to vary in number from approximately 145 to 395 in 18 unrelated individuals (7). The MUC4 shows significant homology with the mucin ASGP in murine milk (11). Similar to bovine MUC-X, ASGP shows no tandem repeat-type polymorphism. A clearer understanding of MUC-X, especially with regard to species differences, requires additional research.

FUNCTIONS OF MUCINS

Mucins are physical barrier molecules on the surface of cells. Thus, they physically protect the cells and tend to prevent invasions by microorganisms. Mucin binding to specific microorganisms can inhibit their replication and may facilitate their being phagocytosed. Epithelial cells lining the gastrointestinal tract, nasal passages, organs, and glands throughout the body present moist, exposed surfaces that can be disrupted or breached by microorganisms and chemicals including enzymes. The mucins are a first line of defense against these agents. The high sialic acid content of the mucins confers a strong negative charge to epithelial cell surfaces, which may aid in keeping ducts and alveoli open. There is almost three times as much sialic acid in MUC1 of bovine milk compared with that of human milk [Table 1 in (8)]. To the extent that a strongly negative cell surface charge can prevent cancer cell adhesion and aggregation, MUC1 may be a factor in the virtual nonexistence of mammary tumors in the cow. For further discussion of mucin functions, see Patton et al. (8).

POLYMORPHIC FORMS OF BOVINE MUC1

In an analysis of milk samples from 119 Holstein cows, the MUC1 bands on SDS gels fell into five groups according to molecular mass (5). These were (masses in kilodaltons from animals) 193 (22), 189 (83), 180 (16), 177 (73), and 156 (43). In an analysis of such bands in the five major Western dairy breeds, patterns of the Holsteins and Guernseys resembled each other, and those of the Ayrshires, Brown Swiss, and Jerseys composed another group (9). Among the Ayrshires, 17 out of 21 exhibited a single, slow-moving band of MUC1, which suggests that individuals of that breed are more closely related to each other than are those within the other breeds and that the Ayrshire breed may have emerged more recently than did the others.

One useful aspect of MUC1 polymorphism is that the alleles, as revealed by the molecular masses of the proteins, may be analyzed for their association with economically important traits of the cows, such as milk yield, fat content, and susceptibility to mastitis. A study (4) examining such parameters in Holstein cows found some association between the MUC1 phenotype and milk fat yield, milk fat percentage, and milk protein yield. Cows with the larger forms of MUC1 tended to have higher yields and higher percentages of fat and protein. With the assumption that the size of MUC1 might be related to effectiveness as a barrier against mastitis, this study (4) looked at SCC in the milk as a relevant criterion. No relationship was found. However, a case control study involving actual mastitis might provide a better approach to the problem. An interesting example of how size of MUC1 may be related to incidence of disease is evident from a recent human epidemiological study (3), which showed that stomach cancer patients tended to have smaller alleles (fewer tandem repeats) for MUC1 than did controls.

That bovine MUC1 is readily released from the fat globule membrane into the skim milk phase on cooling suggests varying degrees of loss of the mucin over time may even occur from the surface of epithelial cells in the gland. Cows that tend to shed the mucins from their alveolar cells, or that simply express less mucin in the apical membrane of these cells, may present a less effective barrier against mastitis.

MUC1 AND PROPERTIES OF MILK

As mentioned previously, MUC1 is readily dislodged from bovine milk fat globules by cooling and agitation, which is practiced when milkings are pooled from a herd. The normal handling of milk tends to transfer MUC1 into the skim phase. This change can be expected to affect the clumping of fat globules in milk and their rising to form a cream layer. In years past, a tremendous research effort was made to understand these phenomena. The milk mucins now may provide a more specific answer. The MUC1 of bovine milk has a high content (30.5%) of sialic acid in its carbohydrate, which should impart a strong, negative charge to the fat globules and, thus, make them self-repelling, whereas the loss of MUC1 containing these sialic acid residues from the globules would make the fat globules not only more neutral but might also expose hydrophobic or positively charged lipids, all of which conditions would favor clumping and rising of the globules. In this connection, caprine milk manifests a natural homogenization or resistance to creaming. In our experience, MUC1 is more firmly embedded in caprine milk globules. As for bovine milk, the MUC-1 of caprine milk is polymorphic, but its MUC-X is not (2).

With respect to exposure of lipids as a result of mucin removal from globules of bovine milk, cooling, warming, and cooling of raw milk incites lipolysis with resulting off-flavor. The first cooling should remove mucin covering the surface of the globules, and this mucin may well be a barrier to the milk lipase. The second cooling may accomplish adsorption of the lipase onto newly exposed lipid surfaces, thus enabling the enzyme to bring about lipolysis. The term spontaneous rancidity is used to describe milk that has already undergone some lipolysis as it comes from the gland. One possible explanation of the phenomenon is that the mucins on fat globules as secreted from the lactating cell are either quantitatively insufficient or easily shed, leaving lipids of the globule exposed to milk lipase.

By separation of warm milk as freshly drawn from the udder, it should be possible to obtain a cream that is rich in milk mucin and a skim that is poor in milk mucin, which is the reverse of normal processing by which cooled milk is separated, and mucin-rich skim is produced. It may be of interest to compare physical properties such as viscosity, foaming, whipping, mouthfeel, and flavor stability of cream and skim made by these two methods.

ISOLATION AND USE OF MILK MUCINS

When milk is processed into cheese, the MUC1 is disposed more or less quantitatively into the whey. Because the milk mucins are of relatively high molecular mass and are high in carbohydrate, it should be possible to isolate them from whey by filtration or by specific adsorption-elution techniques. However, no commercial methodology for doing this appears to have been published.

From a biotechnical standpoint, precise glycosylation of a protein is not easily achieved, yet there do not appear to be many well-developed uses for mucins, which are very complex structurally. A likely application for the bovine milk mucins concerns binding (and inactivation) of pathogenic microorganisms. Human milk mucins and complex carbohydrates of human milk have this capability (10), but it is probable that a different spectrum or organisms may be involved for mucins of the two species. The carbohydrate compositions of MUC1 from human and bovine milks are quite different. There is much more sialic acid and some mannose in the latter as compared with the former. On the basis of such differences, bovine milk mucins may have special benefits as a prevention for scours in calves, a costly disorder and chronic problem, which would seem to be a worthy area of research. If only a few of the many diarrheal disorders could be suppressed by fortifying infant formulas, calf feeds, and processed human foods with mucins, it would represent a large market and constructive use for the bovine milk mucins.

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