Sub Name: FOOD MICROWBIOLOGY
Sub Code: BVFPS203
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ANTIMICROBIAL SUBSTANCES IN MILK

INTRODUCTION

Milk is an established and healthy food source of energy, proteins, vitamins, and minerals. In addition to its value as a nutrient source, interest has arisen in the ability of milk to kill bacteria and in how this knowledge can be applied to mastitis control, human health, and functional foods for people. A number of proteins found in milk under various conditions exhibit antimicrobial activity. For example, immunoglobulins (antibodies) are protective proteins that are important in the transfer of passive immunity from the mother to the neonate. The young of many mammalian species are not born with an effective immune system. The immunoglobulins protect the neonate from infection until their own immune system is developed. Immunoglobulins are a component of the natural defense mechanism. They are synthesized in response to the presence of foreign particles, such as bacteria and viruses. The synthesis process is specific for the foreign particle that is present, resulting in an immunoglobulin structure that is capable of recoginzing the foreign particle and eliminating it from the body. Immuno- globulins are found in high concentrations in colostrum, the first milk, and in low concentrations in milk. In addition to the immunoglobulins, other proteins found in milk are thought to have antimicrobial activities. Four of these proteins will be reviewed in this article: lactoferrin, lactoperoxidase, lysozyme, and N-acetyl-ß-D-glucosaminidase (NAGase).

LACTOFERRIN

Lactoferrin, an iron-binding glycoprotein, was first isolated from cow's milk and subsequently from human milk. Lactoferrin is present in large quantities in mammalian secretions such as milk, tears, saliva, and seminal fluid, as well as in some white blood cells. Lactoferrin is one of the minor proteins naturally occurring in cow milk at an average concentration of about 0.2 grams/liter. In colostrum, the lactoferrin content can be as high as 0.5 to 1 grams/liter. During the dry period, lactoferrin concentration in mammary secretions from dry cows increases until about 30 days after drying off. The highest lactoferrin concentration found in cow mammary secretions is about 50 to 100 grams/liter. In human milk and colostrum, the reported concentrations of lactoferrin are 2 to 4 grams/liter and 6 to 8 grams/liter, respectively. In its natural state, lactoferrin is only partly saturated with iron (5 to 30 percent). Lactoferrin has many proposed biological functions, including antibacterial/ anti-inflammatory activities, defense against gastro-intestinal infections, participation in local secretory immune systems in synergism with some immunoglobulins and other protective proteins, provision of an iron-binding antioxidant protein in tissues, and possibly promotion of growth of animal cells such as lymphocytes and intestinal cells. A role for milk lactoferrin in iron absorption by the intestine has long been postulated, but remains unproven.

Most micro-organisms need iron for growth and lactoferrin has the potential to inhibit the growth of bacteria, and even kill them by depriving them of iron. The effectiveness of the antibacterial activity of lactoferrin depends on the iron requirement of the organism, the availability of exogenous iron, and the concentration and degree of iron-saturation of lactoferrin. It has been shown that 'natural' lactoferrin is bacteriostatic against a wide range of micro-organisms, including gram-negative bacteria with high iron require- ments (coliforms, which are major mastitis pathogens), and also against some gram-positive organisms such as *Staphylcoccus aureus* (also a major mastitis pathogen), bacillus species, and *Listeria monocytogenes*. Lactic acid bacteria in the stomach and intestine have low iron requirements and are generally not affected. There is also evidence that on certain streptococcal mutants and *Vibrio cholerae*, lactoferrin can exert a direct, bactericidal effect that is independent of iron-deprivation.

LACTOPEROXIDASE

Peroxidase enzymes can kill bacteria by oxidative mechanisms. Peroxidase activity occurs in various exocrine gland secretions including saliva, tears, bronchial, nasal, and intestinal secretions, as well as in milk. Milk peroxidase is known as lactoperoxidase, which is one of the non-immunoglobulin protective proteins and a prominent enzyme that plays a role in protection against microbial invasion of the mammary gland. Each lactoperoxidase molecule contains one iron atom. Bovine milk contains concentrations of about 0.03 grams/liter. In bovine colostrum, the lactoperoxidase content is very low, but increases rapidly after 4 to 5 days postpartum. The level of lactoperoxidase activity in human milk is about 20 fold lower than that in bovine milk.

Lactoperoxidase itself has no antibacterial activity. However, together with hydrogen peroxide and thiocyanate, lactoperoxidase forms a potent natural antibacterial system, the so-called lactoperoxidase system. Both hydrogen peroxide and thiocyanate are naturally distributed in animal and human tissues, although they are generally in very low concentrations. The antibacterial effect of the lactoperoxidase system is mediated by the reaction of hydrogen peroxide and thiocyanate under lactoperoxidase catalysis and the resultant generation of short-lived hypothiocyanate, which is thought to be a major antibacterial substance. The antibacterial property of the lactoperoxidase system is based upon inhibition of vital bacterial metabolic enzymes brought on by their oxidation by hypothiocyanate.

LYSOZYME

Lysozyme is an enzyme present in the milk of some species, especially human milk. There are two types of lysozyme. One type is found in the hen egg-white and is known as chicken-type or c-lysozyme. The other type is found in the goose egg-white and is known as goose type or g-lysozyme. Human and equine lysozymes are considered to be the c-lysozyme type. However, cow milk may contain both c- and g-lysozymes because both types are found in various other body fluids and in the stomach tissue of the cow. Lysozyme kills bacteria by disrupting the glycosidic bond between the two components of peptidoglycan, a constituent of the bacterial cell wall.

Lysozyme activity is nearly undetectable in cow milk, but very high in human milk (0.12 grams/liter). The concentration of lysozyme is highest in human colostrum and pre-colostral milk. The limited lysozyme activity in cow milk increases due to mastitis and high somatic cell counts. Heating cow milk at 75°C for 15 minutes destroys 25 percent of the activity of this enzyme. However, human milk lysozyme is more heat stable than cow milk lysozyme.

Lysozyme possesses antibacterial activity against a number of bacteria. This enzyme usually functions in association with lactoferrin or immunoglobulin A. Lysozyme is effective against *Escherichia coli* in concert with immunoglobulin A. It causes lysis of some species of salmonellae in association with ascorbate and peroxide, both of which are present in low concentrations in milk. Microwave irradiation can decrease the activity of lysozyme against *Escherichia coli*. In addition, lysozyme can limit the migration of neutrophils into damaged tissue and might function as an anti-inflammatory agent.

N-ACETYL-ß-D-GLUCOSAMINIDASE

N-Acetyl-ß-D-glucosamindase (NAGase) is an enzyme whose activity has been implicated as an indicator of tissue damage during mastitis. It is a lysosomal enzyme that is secreted in large quantities in the mammary gland during involution and inflammation. The NAGase enzyme has also been found in other bovine secretions, such as uterine fluids. The specific function of NAGase in the mammary gland is not known, however, recent research has suggested that NAGase may exhibit some antimicrobial activity.

During lactation, cow milk normally has low NAGase activity. Similarly, NAGase is low in mammary secretions in the early dry period, coinciding with the period of highest incidence of new intramammary infection. By the mid-dry period, however, NAGase activity is at its highest in mammary secretions, concurrent with the lowest incidence of new intramammary infection. Therefore, the high levels of NAGase activity, along with elevated lactoferrin concentrations, in the mammary gland during the mid-dry period may contribute to increased antibacterial activity found in mammary secetions at that time.

There is a relationship between the presence of pathogens in the udder and NAGase levels in milk. Marked increases in NAGase activity resulting from the presence of major mastitis pathogens have been observed. Since NAGase has been found in uterine fluids, it has been suggested that NAGase may have a role in the bactericidal function of the uterus as well. Researchers have studied the bactericidal effect of NAGase on several bacterial pathogens commonly found to infect the cow uterus. Of these pathogens, *Actionmyces pyogenes*, *Staphylococcus aureus*, *Strepto-coccus agalactiae*, and *Pseudomonas aeroginosa* were inhibited by NAGase, while the *Escherichia coli* and *Enterobacter aerogenes* were not inhibited. Although these results can not be directly extrapolated to bacterial strains that cause mastitis, they do lend support for such an antimicrobial function of NAGase in the mammary gland.

APPLICATIONS

Antimicrobial substances have important applications in the dairy, animal production, and human health industries. Based on the physiological and functional properties attributed to lactoferrin, a number of interesting and innovative possibilities for the application of this natural protective protein can be considered. Health and functional foods, sports nutrition capsules, and drinks containing lactoferrin are being developed. Lactoferrin is already being used as an ingredient in infant formulas.

The antibacterial properties of the lactoperoxidase system also have been applied to animal production and clinical medicine. For example, due to the lack of cooling equipment, many farmers in China are faced with the problem of milk spoilage during storage and transport. In order to preserve the quality of milk, Chinese scientists are teaching farmers how to activate the lactoperoxi-dase system in raw milk. Addition of a small quantity of sodium thiocyanate and sodium percarbonate added to fresh raw milk is effective at reducing milk spoilage. For a small cost (about ten cents per 100 pounds of milk) milk spoilage can be delayed without harmful effects to the milk or alteration of taste. Another application could be addition of lactoperoxidase to calf milk replacers as a substitute for anti- biotics. Improved performance of calves by the activated lacto-peroxidase system has been demonstrated.

Lysozyme's ability to limit the migration of neutrophils to damaged tissue means that it might be used as an antiinflammatory agent. Lysozyme could be used as an additive in hospital formulas, infant formulas, and feed products as a means to reduce swelling resulting from surgery or illness. Research has shown that feeding human and cow colostrum containing lysozyme to infants and calves can reduce the incidence of gastro-intestinal infections.

N-acetyl-ß-D-glucosaminadase activity assays are already being used in commercial mastitis tests as an indicator for tissue damage. If further studies reveal that it does indeed have antibacterial activity, then it might be used as a natural form of mastitis control.

CONCLUSIONS

Antimicrobial proteins naturally present in milk have the ability to kill and inhibit a broad spectrum of bacteria. The antibacterial properties of these proteins make them suitable for use in a variety of applications, including the prevention of mastitis in cattle and for improving the health of man.