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Review study on lactoferrin: A multifunctional protein

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Lactoferrin is a glycoprotein of 703 amino acids originally isolated from milk. Lactoferrin exists in body fluids and can be found in the form of free iron, monoferric and dimeric form. Scientist also found lactoferrin in the secretions of mammal’s pancreas, and in secondary neutrophil granules. Lactoferrin has wide range of functions which may range from binding of Lactoferrin with iron, in control of the availability of an enhanced immune system and as a strong bacteriostatic agent, but the exact mechanism of lactoferrin is still not clear.

Key words: Antimicrobial, lactoferrin, iron-binding protein, iron metabolism.

INTRODUCTION

Lactoferrin is an iron-binding protein which was first isolated in 1939 from bovine milk (Sørensen et al., 1939) and from human milk in 1960 (Johansen, 1960). Lactoferrin is found in high concentrations in breast milk and to some extend in smaller amounts in exocrine fluids such as mucosal secretions, bile, intestinal secretions, pancreatic juice (Ashida et al., 2004). Lactoferrin belongs to a family of proteins known as transferrin (Metz-Boutigue et al., 1984). Its most important characteristic is the development of an intense red colour when incubated in the presence of Fe³⁺ ions, which proves that LF is an analog of serum iron binding protein. Traditionally, it was considered as iron transporting protein of milk having bacteriostatic characters. The purpose of this paper is to review the structure and functions of Lactoferrin focusing on its antimicrobial properties.

Structure of lactoferrin

Human lactoferrin contains about 703 amino acid which can be resolved by chemical method (Metz-Boutigue et al., 1984) and cDNA cloning (Powell et al., 1990; Rey et al., 1990). HoloLactoferrin is a single polypeptide chain folded into two globular lobes, the N- and C- terminal each with one iron-binding site (Querinjean et al., 1971). The structure of hLF has been determined by X-ray diffraction at 2.2Å (Baker et al., 1998). There is internal homology between the N- and C- lobes at 1-338 and 339-703 which demonstrate 125 (or 37%) identical amino acid residues in the corresponding portions (Peter et al., 1995). This give rise to the theory of gene duplication which results in the formation of two lobes (domains) and giving rise to a family of proteins having molecular masses in the range of 80 KDa (Bullen et al., 1987). The isoelectric point of lactoferrin is 8.7A and is a glycoprotein (Furmanski et al., 1989). Lactoferrin exist in various isoforms three such isoforms, two with RNase activity (termed Lactoferrin–β and Lactoferrin –γ) and one which is without RNase activity (termed Lactoferrin-∞) have been isolated, all these forms can be isolated from human breast milk and in granulocytes. These isoforms have same chemical, physical and antigenic characteristics, But different greatly in functional properties.

Similarities between LF and other transferrin

All members of transferrin family have same polypeptide folding structure (Baker et al., 1993). All members of transferring family have iron binding and transport properties. Lactoferrin and transferrin are closely related to each other by amino acid composition with 59% and 49% homology between the domains of these molecules.

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(Bluard et al., 1974). The secondary and tertiary structures are also similar. Crystallographic studies reveal that upon iron-binding conformational changes occur in all members of transferrin family (Ward et al., 2005).

Transferrin can exist in any of four molecular forms (Makey et al., 1976; Makino et al., 1991) apotransferrin, monoferric transferring, either in the A- or B- forms, and diferric transferrin. The molecular mass of transferring decrease as the degree of iron saturation increases which proves that the binding of iron to transferring induces a conformational change which leads to closed iron-binding domain.

Some differences may exist between the lactoferrin and transferring. The inter lobe connecting peptide is helical in lactoferrin but in transferring it is irregular. Some key properties also differentiate lactoferrin from other transferring. Transferrin is primarily present in bloodstream and basically its function is to deliver iron to cells (Octave et al., 1983), While LF is found in exocrine secretions. Lactoferrin and transferring differs in surface properties.

**Surface properties and metabolism of lactoferrin**

The major functions of LF depend on the ability of LF to bind to other macromolecules such as proteins and DNA. These functions in turn depend on the surface properties of LF. LF has cationic nature with high isolectric point and this property differentiates LF from other members of transferring family. There are three notable concentrations of positive charge (a) – on N-terminus (residues 1-7), (b)-along the outside of the first helix (residues 13-30) and (c) in the inter lobe region, close to the connecting helix, this distribution of charge in LF is highly uneven. In human lactoferrin the first helix (residues 12-13) forms the basic and major part of the bactericidal domain (Bellamy et al., 1992), identified as the lactoferricin domain (Gifford et al., 2005). The Lfcin peptide, when released by proteolysis of the intact proteins, is a potent bactericidal agent (Senkovich et al., 2007), probably because it’s able to form amphipathic structures that disrupt cell membranes (Gifford et al., 2005). This region is a key factor in the antibacterial activity of LF by disruption of cell membrane. This bactericidal domain can also act as binding surface.

According to Lyer 1993 lactoferrin is produced in neutrophils and as well in iron depleted state. It proved that the steroid thyroid receptor super family works in concert to modulate lactoferrin gene expression. This led to formulation of hypothesis that levels of lactoferrin in cells are hormone dependent. After production lactoferrin transfer to its storage granules is dependent on acidification mechanism and occur through Golgi apparatus (Olsson et al., 1988). The neutrophil lactoferrin can be secreted in two way it can either be secreted into the surrounding tissues or blood (Van et al., 1974) or the granules can fuse with phagosomes (Maher et al., 1993). Degranulation factor affects the amount of LF secretion from the polymorph nuclear cells which in turn depends on the activation of guanylate cyclase, cGMP and protein kinase C (calcium dependent). It can be stimulated by interleukin – 8 and surface bound IgG and occurs in both aerobic and anaerobic conditions (Kahler et al., 1988). Usually lactoferrin plasma levels increases during infectious diseases, tumor progression, iron overload and inflammation (Kolb et al., 1989).

After the release of lactoferrin, that connects the metal ions, extracting lactoferrin occurs in one of two ways. Lactoferrin can be removed first from the areas of intraregional traffic, through what appears to be a receptor-mediated endocytosis in Phagocytic cells, with subsequent transfer of ferritin iron (Alovovson et al., 1977).

**Degradation of lactoferrin**

Lactoferrin can be removed by receptor – mediated endocytosis into phagocytic cells such as macrophages, monocytes and other cells of the reticuloendothelial system (Olofsson et al., 1977; Van et al., 1974; Van et al., 1976). Another way of lactoferrin removal is direct uptake by the liver, involving liver endothelial cells, hepatocytes and kupffer cell (Hu et al., 1993).

**Lactoferrin levels in various organs**

Lactoferrin is present in plasma in relatively low concentration with higher level being found in colostrum’s, human breast milk, and seminal plasma. Plasma lactoferrin is derived from neutrophil (Bullen et al., 1987). In granules its presence can be used to identify these granules. The number of neutrophils did not affect the plasma concentration of LF indirectly depending on how granularity and contribution of other organs such as the bone marrow and lining of the uterus and placenta, and plasma lactoferrin content (Baynes et al., 1986; Scott 1989; Mason et al., 1968).

Lactoferrin has been found in human milk and colostrum at concentration of 1 and 7 mg/ml, respectively, and in bovine milk mid lactation at levels of 0.1 mg/ml (Martur et al., 1990), 0.5 mg/ml in pooled pulmonary secretions and more than 14 mg/ml in infected parotid fluid (Brogan et al., 1975; Tabak et al., 1978). Lactoferrin plasma levels change during pregnancy as maternal plasma lactoferrin levels manifest as a progressive rise in concentration, with stabilization at week 29 of pregnancy (Sykes et al., 1982).

Pregnancy is associated with increased number of leukocytes (Andrews et al., 1951) Lactoferrin production during pregnancy may be affected by hormones (Mason and Taylor, 1979).
Lactoferrin is present in the milk of all mammalian species except dog and rat (Masson and Heremans, 1971). Human milk contains highest levels of lactoferrin as compared to bovine milk. Approximately 30% of the iron in human milk is bound to lactoferrin (Goldsmith et al., 1982). Iron body status in human milk does not depend on body iron status, but on the general state of maternal nourishment. In malnourished mothers levels of lactoferrin are lower. LF levels in breast milk are not affected by the continuous (Houghton et al., 1985). LF preterm colostrum at first and then increased concentration of colostrum production period (Hiral’s et al., 1990) decreases over.

Lactoferrin levels in amniotic fluid were found to be undetectable before the 20th week of pregnancy (Masson et al., 1968). A significant increase occur around week 30, and then it remains high until term. Lactoferrin of amniotic fluid is of decidual origin (Masson et al., 1968). Amniotic lactoferrin concentrations have highest reported levels after those of colostrums, milk, tears and seminal plasma.

Lactoferrin production in the fetus depends on gestational age and was found by immunohisto-chemical detection, from 13th weeks onwards (Reitamo et al., 1981). Some of the fetal lactoferrin comes from amniotic fluid, which has significantly higher lactoferrin levels than either fetal or maternal sera. Lactoferrin cannot cross the placenta. This is strongly demonstrated by the back of correlation between maternal and neonatal lactoferrin concentrations (Guttenberg et al., 1986).

**Functions of lactoferrin**

The exact role and mechanism of action of Lactoferrin has not clearly known. Lactoferrin play a role in the host defense mechanism as well as in iron metabolism. In host defense mechanism it acts as a bacteriostatic agent. LF and it also has a bactericidal effect against fungi and viruses can spread. It also improves immunity. Lactoferrin is known to have a tendency to bind to a number of other molecules or silent receptors. Other features as possible in a normal cell growth regulation function, coagulation, including modulation of cell adhesion.

**Role in iron metabolism**

As iron absorption from breast milk is high, iron status of breast fed infants is usually satisfactory up to at least 6 months and a major part of iron in human milk is bound to LF, it was earlier suggested that LF facilitates iron absorption in breast fed infants (Fairweather et al., 1987). Lactoferrin from maternal milk is known to be absorbed in the intact form from the gut of infants (Hutchens et al., 1991). A maximum concentration of lactoferrin and bovine milk in humans than in the observation of the greater availability of iron lactoferrin in breast-fed infants can promote the absorption of iron that led to the hypothesis. However, infants fed formula supplemented with bovine lactoferrin ferrous sulfate formula of several studies regarding the iron status showed no benefit. In several reports, among others, support this hypothesis.

i.) The ability of human enterocytes to extract iron from LF (Masson et al., 1971).

ii.) The high Lactoferrin uptake by enterocytes (Masson et al., 1971).

iii.) The correlation between neonatal urinary iron excretion with milk Lactoferrin content as well as with breast milk uptake (Masson and Heremans 1971).

iv.) The transport of iron across the intestinal brush border by lactoferrin.

v.) The accumulation of iron from Lactoferrin in brush border membrane vesicles (Davidson et al., 1998).

The major role of lactoferrin in iron metabolism would appear to be in the control of iron availability. LF may perhaps affect cellular mechanism through its influence on iron availability. Iron is known to affect a host of cell functions such as DNA, and to a lesser extent RNA and protein synthesis, the expression of lymphocyte surface markers, immunoglobulin secretion, interleukin-2 receptor expression and many others (Machnicki 1991).

**Antimicrobial effects**

Lactoferrin was earlier shown to have bacteriostatic activity against pathogens such as Escherichia coli (Bullen et al., 1972). The exceptionally, strong iron binding activity ($K_{a} = 10^{24}$) of LF allows it to compete with bacteria for iron, thereby causing inhibition of their growth. Recently growth of enterobacter sakazakii, a food borne pathogen which is known to cause diarrhea in infants, was shown to be inhibited by iron unsaturated lactoferrin (apolactoferrin), but not by hololactoferrin showing that the iron sequestering capacity of LF was responsible for the activity (Wakabayashi et al., 2008). It has also been reported that by direct bactericidal activity, LF can kill effectively a wide variety of pathogens such as vibrio cholera (Arnold et al., 1980). Two cationic peptides, called Lactoferrin molecule (Wakabayashi et al., 2003) and lactoferrampin (Haney et al., 2009), respectively, have been shown to have strong antimicrobial activity in cell and animal models. Lactoferrin also show strong antiviral activity against several viruses such as cytomegalovirus (CMV), Hepatitis C Virus (HCV), Herpes simplex virus (HSV), rotavirus, adenovirus and HIV (Valenti et al., 2005). The technique behind this is not yet understood but a hypothesis exist that LF binds to bacterial receptors or mammalians cells, and blocking adhesion of the pathogens to host cells. In a recent study (Wakabayashi et al., 2003) on young children
hospitalized with acute diarrhea, oral rehydration solution with recombinant human Lactoferrin and lysozyme significantly reduced diarrhea duration, diarrhea volume, and recurrence of diarrhea. Three recent clinical studies support that Lactoferrin may prevent infections in children. A study (Haney et al., 2009) on Japanese children showed that daily supplementation with 100 mg bovine Lactoferrin resulted in significantly lower frequency and duration of vomiting and diarrhea as compared with the placebo group, although no difference in rotavirus gastroenteritis was detected.

Lactoferrin has also been shown to prevent biofilm formation. This biological function relates to the ability of inhibiting microbes from adhering colonizing and forming biofilm on host cells. Which is a crucial step in the development and persistence of infection (Singh et al., 2002) showed that iron sequestration by LF – inhibited biofilm formation by pseudomonas aeruginosa in continuously cultured mammalian cells by stimulating a bacterial motion called twitching. This motion prevents bacteria from attaching to the surface of mammalian cells and ultimately forming biofilms. This activity of LF was observed even at a very low LF concentration (20 µg/ml), which is much less than the concentration required for bacteriostatic activity.

Lactoferrin exerts its antibacterial effects by means of different mechanisms which are as:

i.) It is an iron-binding protein from iron available which limits the amount of free iron available. Iron is an essential growth factor for microorganisms (Otto et al., 1992).

ii.) It is capable of destabilizing the other membrane of gram negative bacteria (Ellison et al., 1988).

iii.) Liberation of bactericidal peptides.

iv.) Glycans of bovine LF inhibits the binding of gram-negative bacteria to cells (Teraguchi et al., 1996).

It is known that Lactoferrin and as well the peptide which is derived from bovine and lactoferrin B inhibit the growth of fungi (Bellamy et al., 1993; Vorland et al., 1998; Wakabayashi et al., 1996). The active components of Lactoferrin are assumed to be Lactoferricin (Wakabayashi et al., 1996). The mechanism of action of the lactoferrin related substances has not been fully elucidated. It has been shown that Lactoferricin B directly binds to Candida cells (Bellamy et al., 1993), and is highly effective in disrupting the cell membrane of Candida (Wakabayashi et al., 1996) showed that the anti-Candida activity of Lactoferrin or lactoferricin B in combination with clotrimazole had a synergistic effect.

Lactoferrin and its peptides have an effect against protozoa (urchany et al., 1995; Isamida et al., 1998), though the mode of action is unclear. The effect against toxoplasma gondii may be the same as for bacteria. The cell surface of T. gondii tachyzoites is known to have a strong negative charge and binds cationic substances (Cintra and de Souza, 1985). It is hypothesized that lactoferricin has the capacity to bind to the surface of the parasite in the intestinal tract, and this interaction results in loss of infectivity, resulting in disruption of the biological function of the parasite membrane (Heird et al., 1984).

**Lactoferrin and cellular proliferation**

Lactoferrin played a significant role in cellular proliferation. This suggested better gastrointestinal development in new born animals fed maternal milk as compared to newborn animals fed commercial formulas (Heird et al., 1984; Berseth et al., 1983), increased thymidine incorporation with lactoferrin supplementation of milk formulas (Berseth et al., 1983) and in vitro augmentation of thymidine incorporation into rat crypt cell DNA by Lactoferrin (Nichols et al., 1987). It was proved by the fourfold higher DNA synthesis in a mouse embryo cell line under the influence of hololactoferrin than in the same line under the influence of apolactoferrin (Zuma et al., 1989). The effect of LF on cancerous cells would appear to be inhibitory rather than stimulatory (Amouric et al., 1984). Most researchers suggest that lactoferrin can acts as a negative feedback regulatory of myelopoiesis (Garre et al., 1992). The mechanism involves the suppression of the release of cytokines such as interleukin-1, tumor necrosis factor and interleukin-2, Interleukin 6 and TNF in response to LPS monocytes (Crouch's et al., 1992; Mattsby-Baltzer et al., 1996).

Shinoda et al. (1996) described that lactoferrin has the ability to stimulate the release of neutrophil activating polypeptide interleukin-8 from human polymorph nuclear leukocytes (Shinoda et al., 1996).

**Influence of lactoferrin on immune cells and autoimmune diseases**

Lactoferrin is likely to favour the rapid recruitment of polymophonuclear monocytes from blood to the inflammatory sites (Boxer et al., 1982; Kurose et al., 1994). Iron-saturated lactoferrin inhibits myelopoiesis. Perhaps the dynamic impact factor granulocyte monocyte (GM-CSF) production (Zucali et al., 1989), which reduces the production of interleukin-1, is by suppression. It has been reported that LF can increase the cytotoxicity of natural killer cells in vitro (Damiens et al., 1998). Antibodies to lactoferrin have been found in patients with autoimmune diseases such as systemic lupus erythematosus (Sinico et al., 1993), rheumatoid arthritis with vasculitis (Coremans et al., 1993), primary sclerosing cholangitis, and many other inflammatory diseases.

**Other clinical applications**

Reported a number of clinical applications and (leukemia,
myeloid chronic98, granulocytic leukemia as a tool in the diagnosis of blood neutrophils or neutrophil lactoferrin plasma dynamics as an index of the total pool is included in determining Olofsson et al., 1977), chronic pancreatitis and calcifying (Figarella and Srls, 1975; Multigner et al., 1980), cystic fibrosis (Rayner et al., 1991), schizophrenia (Hallgren et al., 1982), and rheumatoid arthritis (Bennet et al., 1977). Lactoferrin antibodies in patients with Felt's syndrome have been reported, and the detection of antibodies may be useful in diagnosis (Coremas et al., 1993).

Conclusion

Several recent studies have supported that lactoferrin have a wide spectrum of functions. Although lactoferrin may exert most of its functions through its effect on iron availability, but the exact mechanism of its action is not yet clear. The inhibitory effect of Lactoferrin on carcinogenesis also holds promise, but further trials are needed in all these areas before the therapeutic potential can be clearly established.

REFERENCES


