

Nutritional and Physiologic Significance of α -Lactalbumin in Infants

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α -Lactalbumin is the major protein in breast milk (20–25% of total protein) and has been described to have several physiologic functions in the neonatal period. In the mammary gland, it participates in lactose synthesis, thereby creating an osmotic “drag” to facilitate milk production and secretion. α -Lactalbumin binds divalent cations (Ca, Zn) and may facilitate the absorption of essential minerals, and it provides a well-balanced supply of essential amino acids to the growing infant. During its digestion, peptides appear to be transiently formed that have antibacterial and immunostimulatory properties, thereby possibly aiding in the protection against infection. A novel folding variant (“molten globule state”) of multimeric α -lactalbumin has recently been discovered that has anti-infective activity and enhances apoptosis, thus possibly affecting mucosal cell turnover and proliferation. Cow milk also contains α -lactalbumin, albeit less than human milk (2–5% of total protein in bovine milk), and protein fractions enriched with α -lactalbumin may now be added to infant formula to provide some of the benefits of human α -lactalbumin.

Key words: α -lactalbumin, human milk, cow milk, infant formula

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Introduction

α -Lactalbumin is a protein that is present in the milk of all mammals. It is a component of the lactose synthase complex in the mammary gland and is consequently present in relatively high concentrations in milk of species with high lactose concentration, including human milk. α -Lactalbumin has an amino acid composition that

contributes substantially to meeting the essential amino acid requirements of newborn infants and may therefore be important in infant nutrition. The intact protein, as well as various forms of α -lactalbumin, such as multimers and fragments resulting from partial digestion, has been suggested to have various biologic activities (Figure 1). Because bovine α -lactalbumin preparations of various degrees of purity now are commercially available and may be added to infant formula to increase its α -lactalbumin concentration, it is important to evaluate the nutritional and physiologic significance of such fortification. In this review, we address what is known about the biochemistry and biological functions of α -lactalbumin, and discuss suggested implications of α -lactalbumin-enriched infant formula.

Chemical and Molecular Properties of α -Lactalbumin

The gene for α -lactalbumin is located on chromosome 12.¹ It is a single-chain polypeptide of 123 amino acids in both human and cow's milk,² corresponding to a molecular mass of 14,070 D in human milk and 14,178 D in cow's milk. Both proteins contain four disulfide bonds and the amino acid sequence homology between human and bovine α -lactalbumin is 74%. The primary structure of α -lactalbumin is very similar to that of c-type lysozyme;³ it has been suggested that the two proteins have evolved from a common ancestral gene. The amino acid composition of human and bovine α -lactalbumin is shown in Table 1.⁴ The protein is relatively high in tryptophan (4–5%), as well as in lysine (11%) and cysteine (6%). α -Lactalbumin contains two major domains:⁵ the α -domain, which contributes four α -helices, and the β -domain, which contains a β -sheet and loop regions.⁶ Its isoelectric point is 4.2 to 4.6 and it is highly soluble in water and salt solutions.

When exposed to low pH, such as would occur in the stomach, α -lactalbumin can be transformed into what is called a “molten globule” state. This structure is an intermediate between the native state and the fully unfolded state, and is likely to be important for the proteo-

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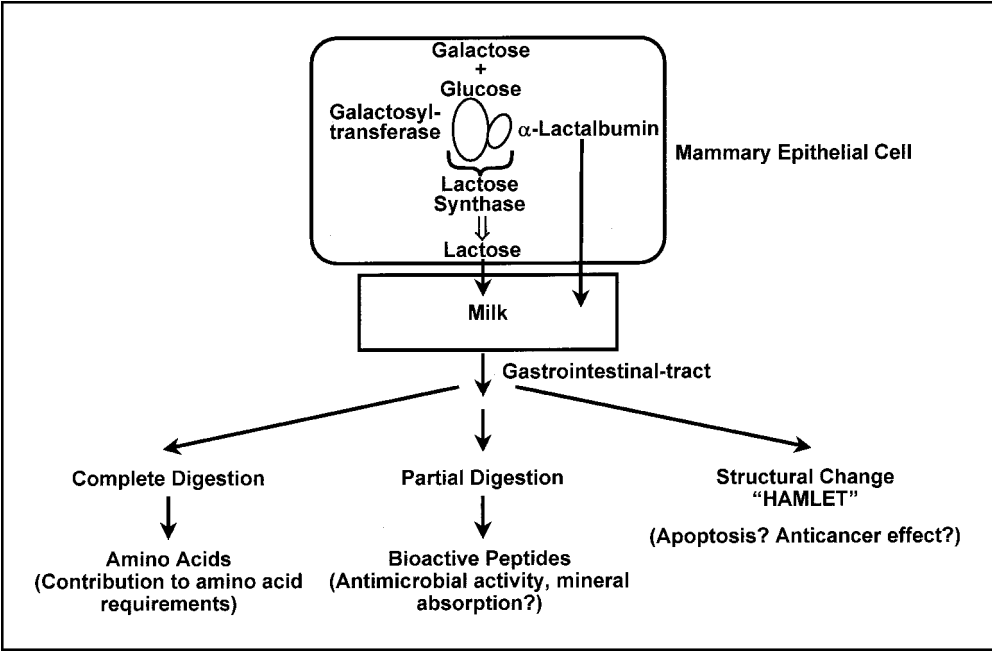


Figure 1. Physiologic significance of α -lactalbumin.

lytic digestion of α -lactalbumin. The molten globule state contains a high degree of native-like secondary structure, whereas some of the tertiary structure of the native α -lactalbumin is missing.^{7,8} Other conditions may also transform the protein into its molten globule state, such as alkaline pH, moderate heat treatment, or addition of metal ions, such as zinc.^{9,10} The volume of the molten state α -lactalbumin is approximately 6% larger than its native form and the α -domain largely retains its structure in the molten globule state, while the β -domain is less structured. Also, the β -domain does not appear to be necessary for the formation of the molten globule state, whereas at least part of the α -domain is needed. The four disulfide bonds do not appear to have any significance for formation of the molten globule state because site-directed mutagenesis replacing the cysteine residues with alanine did not affect the ability of α -lactalbumin to form the molten globule state.¹¹

There is no post-translational modification of α -lactalbumin in most species, but in some species, glycosylation at Asn 45 has been reported.¹² α -Lactalbumin in the milk supply may be lactosylated.¹³ When care is taken to preserve the native structure of α -lactalbumin during processing, lactosylation can be minimized. MALDI-TOF (matrix-assisted laser desorption/ionization, time-of-flight) mass spectroscopy indicated that at the most one residue is added per protein molecule,¹⁴ which does not appear to affect the proteolytic digestion of α -lactalbumin. Levels of lactosylation have also been reported in commercial whey products. For example, Hau and Bovetto¹⁵ reported some but not all α -lactalbu-

Table 1. Percentages of Amino Acids in Bovine and Human α -Lactalbumin¹

Mole %	Bovine α -lactalbumin	Human α -lactalbumin
Essential		
Arginine	1.1	1.1
Cysteine	5.8	5.8
Histidine	2.9	2.0
Isoleucine	6.4	9.7
Leucine	10.4	11.3
Lysine	10.9	10.9
Methionine	0.9	1.9
Phenylalanine	4.2	4.2
Threonine	5.0	5.0
Tryptophan	5.3	4.0
Tyrosine	4.6	4.6
Valine	4.2	1.4
Non-essential		
Alanine	1.5	2.5
Aspartic acid	10.6	9.8
Glutamic acid	6.4	7.4
Glycine	2.4	2.4
Proline	1.4	1.4
Serine	4.3	5.0
Asparagine	6.4	3.2
Glutamine	5.4	6.4
Totals		
Amino Acids	100.0	100.0
Percent Coverage		

Amino acid sequence data from the ExPASy Molecular Biology Server.

min molecules have one or two lactose residues when commercial samples of whey were analyzed. At such levels, protein quality should not be adversely affected. Lactosylated α -lactalbumin has been shown to bind to *E coli* heat-labile enterotoxin,^{16,17} and this binding is eliminated by treatment with β -galactosidase, which removes terminal galactose. It can also inhibit binding of enterotoxin to GM1 ganglioside and decrease the cellular effects of this toxin,¹¹ suggesting that it may act as an analogue for intestinal enterotoxin receptors. Similarly, enteropathogenic *E coli* (EPEC) bacteria require lactosamine sequences for adherence¹⁸ and lactosylated α -lactalbumin may therefore inhibit binding of EPEC to intestinal cells¹⁹ and prevent infection (see below).

α -Lactalbumin normally binds one calcium ion ($K_d \sim 10^{-7}$ M); this binding dramatically changes the tertiary structure of the molecule from an open flexible form to a tight, compact globular structure (Figure 2), resulting in a major difference in size (Stokes' radius) from 50 Å to 35 Å.² There is also a second calcium-binding site (Figure 2A),²⁰ which may not be occupied in vivo. Binding of calcium to α -lactalbumin does not appear to be essential for binding to galactosyltransferase (the catalytic unit of lactose synthase) or for the enzymatic activity of the lactose synthase complex. Other divalent metal ions such as zinc (Figure 2B), manganese, and cobalt can also bind to α -lactalbumin,²¹ but this does not appear to occur in vivo. Although virtually all α -lactalbumin molecules in milk appear to exist in the calcium-bound form, this amount of calcium is low in proportion

to the total calcium concentration of milk (0.1–0.15% in human milk; much less in cow's milk) and it is therefore unlikely to be of significance with regard to calcium nutrition of the newborn.²²

Multimeric Forms of α -Lactalbumin

A unique form of α -lactalbumin found in human milk has been described to induce apoptosis.²³ This form was shown to contain oligomers of α -lactalbumin and to reduce leukemia cell viability by inducing apoptosis.²⁴ Structural studies indicated that the multimeric forms of α -lactalbumin were kinetically stable against dissociation into monomers and had a retained secondary structure, but also had a less well organized tertiary structure suggestive of a molten globule-like state.²⁵

Recently the same research group showed that the conversion to this special, multimeric form of α -lactalbumin, which they call HAMLET (human α -lactalbumin made lethal to tumor cells), requires partial unfolding and the fatty acid oleic acid (C18:1) as a specific cofactor.^{26,27} The authors were able to convert human milk α -lactalbumin and recombinant human α -lactalbumin (produced in *E coli*) to the HAMLET form by unfolding the molecule with EDTA (Ca^{2+} -removal) and by passing the apo-forms through a "C18:1-conditioned" column. Simply mixing C18:1 with apo- α -lactalbumin in solution did not result in the same level of activity. The HAMLET form was shown to have broad activity against cells of both human and animal origin; it induced apoptosis in

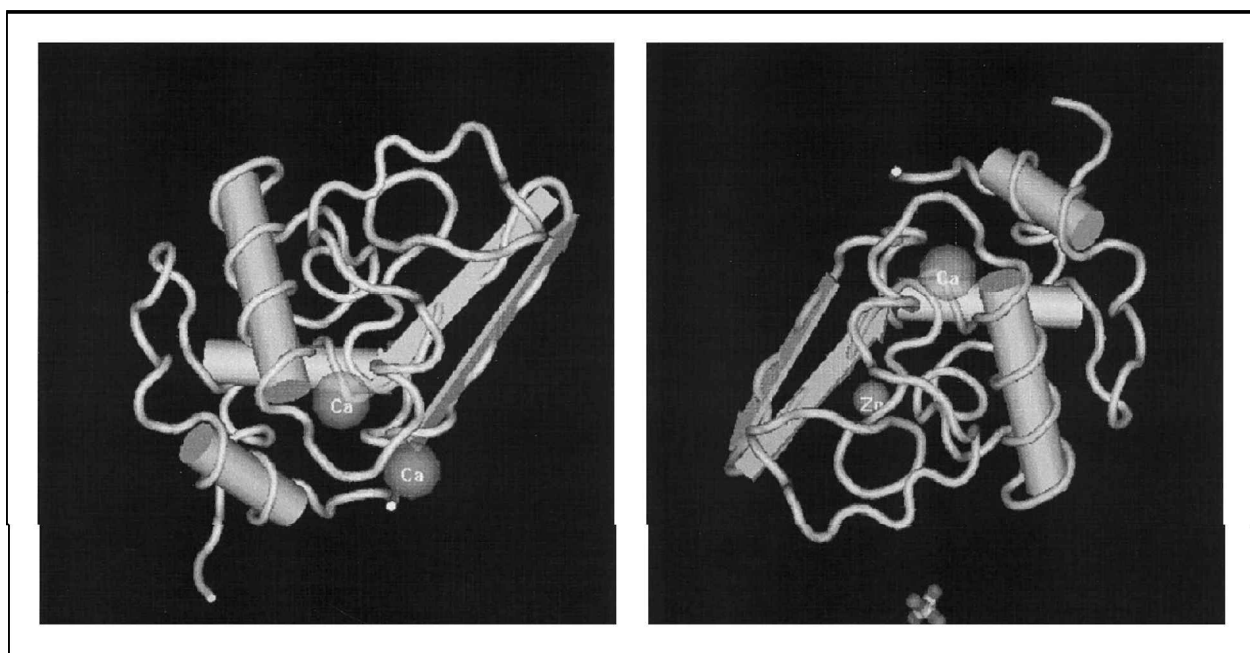


Figure 2. Tertiary structure of human α -lactalbumin. Figure 2A illustrates two calcium ions associated with α -lactalbumin; 2B illustrates the calcium/zinc-associated structure. From reference 6.

several human and murine cell lines such as the Jurkat and L1210 leukemia cell lines, the A549 lung carcinoma line, and the A-498 kidney carcinoma line. Native α -lactalbumin had no effect on these cells. The L1210 cells died rapidly when exposed to the HAMLET form (0.2 mg/mL) and DNA fragmentation was induced. Confocal microscopy showed striking differences in subcellular and nuclear uptake of HAMLET and native α -lactalbumin. Whereas HAMLET bound to the cell surface and passed through the cytoplasm to the nucleus, the native form only weakly associated with the cell surface and was not internalized. The authors speculated that HAMLET may be formed from α -lactalbumin in breast milk under conditions of the gastrointestinal tract of breastfed infants and may prevent childhood cancer in breastfed children. A recent study²⁸ demonstrated that folding variants of α -lactalbumin induce mitochondrial permeability with release of cytochrome c and lead to activation of the caspase cascade and apoptotic death, suggesting a possible mechanism for cancer prevention. HAMLET may have activities in addition to tumor suppression. Håkansson et al.²⁶ have also shown that the complex formed between the folding variant of α -lactalbumin and C18:1 has bactericidal activity against antibiotic-resistant and antibiotic-susceptible strains of *Streptococcus pneumoniae*.

There is, however, no in vivo evidence that HAMLET is formed in the gastrointestinal tract of infants. Acid secretion in infants is much lower than in adults and the stomach pH is frequently approximately 4 to 5 even up to 6 months of age.²⁹ It is therefore uncertain whether unfolding of α -lactalbumin would occur. Whereas C18:1 is likely to be released from milk fat during lipid digestion, in vitro mixing of apo- α -lactalbumin and C18:1 did not result in a fully active HAMLET, although running a sample through a "C18:1-conditioned" column did.²⁷ Thus, it is uncertain whether in vivo conditions in infants actually would convert α -lactalbumin to HAMLET. Studies on gastric and duodenal aspirates from breastfed infants of different ages should resolve these questions. Such studies would also provide information on the stability of HAMLET against proteolytic digestion in vivo. Based on the similarities in structure, the possibility of bovine α -lactalbumin forming a similarly active form should also be explored.

In order to affect apoptosis, α -lactalbumin must be internalized by the cell. A study in Caco-2 cells by Caillard and Tomé³⁰ suggests that this may occur. When α -lactalbumin was introduced at the apical side of a monolayer of Caco-2 cells at concentrations similar to or lower than in human milk (0.2–3 g/L), the protein bound and was found to be internalized. Part of the internalized

protein was transported across the monolayer (5%) to the basolateral side, whereas the major part (70%) was degraded intracellularly. Intact human α -lactalbumin has been found in serum of breastfed human infants,³¹ but this only occurs at very early age; by the time the infant is weaned α -lactalbumin is no longer detectable in serum, suggesting that α -lactalbumin is efficiently degraded. By contrast, bovine β -lactoglobulin was detected even after weaning in a large proportion of infants.

The Role of α -Lactalbumin in Lactose Synthesis

Lactose, which is synthesized by the mammary gland, is the most abundant component of both human and bovine milk. Within the mammary gland, α -lactalbumin serves as a regulator of the enzyme galactosyltransferase, which is responsible for the synthesis of lactose from galactose and glucose (Figure 1).³² Galactosyltransferase is capable of synthesizing a variety of disaccharides, but under the regulatory control of α -lactalbumin, only lactose is synthesized. Lactose is essential for milk production because it is the driving osmotic force in milk volume formation. α -Lactalbumin is transported from the inner surface of the mammary Golgi apparatus (where it exerts its regulatory role) to mammary secretory vesicles and then to the alveolar lumen during milk formation. The relationship between the concentration of milk α -lactalbumin and lactose is complex, but in general there is a direct correlation between these components; for example, milk from seals and sea lions, which has very low concentrations of lactose, also has very low α -lactalbumin concentrations.³³ It appears unlikely that the concentration of α -lactalbumin alone is regulating lactose synthesis, however, as knockout and knock-in mouse genotypes³⁴ with highly varying α -lactalbumin concentration had similar lactose concentrations (see also below).

The importance of α -lactalbumin in lactose synthesis and milk production has been demonstrated by a knockout mouse model.^{34,35} Homozygous knockout mice were fertile but the dams were not able to feed their pups because the milk they produced was so viscous that it could not be removed from their mammary glands by the normal suckling stimulus. Milk physically removed from the glands was high in lipid and protein but very low in lactose, and the milk contained no detectable α -lactalbumin. Heterozygous mice had 40% less α -lactalbumin than control mice, but the lactose concentration was only 10 to 20% lower,³⁵ possibly suggesting that the mammary gland normally has more α -lactalbumin than needed for optimal lactose synthesis. When the murine α -lactalbumin gene was replaced with the human α -lactalbumin gene,³⁴ lactation and pup survival were normal,

demonstrating that human α -lactalbumin can replace mouse α -lactalbumin in the murine lactose synthase complex. The human α -lactalbumin gene expressed 15 times more mRNA and 14 times more protein than the mouse α -lactalbumin gene, showing that the concentration of α -lactalbumin is directly correlated to gene dosage, and that the major regulators of human α -lactalbumin expression are close to or within the gene.

Recombinant α -Lactalbumin

Recombinant human α -lactalbumin has been expressed in tobacco plants and the purified protein was shown to be active in lactose synthesis when combined with galactosyltransferase.³⁶ The expression level was low (5 mg/kg of leaves), which, combined with the need for extensive purification prior to use in infant nutrition, makes the use of this form of recombinant α -lactalbumin economically unrealistic. Recombinant human α -lactalbumin has also been expressed in transgenic cows,³⁷ but this has not yet resulted in any commercial applications.

Human α -lactalbumin has been expressed in transgenic rats at high levels³⁸ and bovine α -lactalbumin has been expressed at high levels in transgenic mice.³⁹ Milk from transgenic mice contained 5 to 15 times more bovine α -lactalbumin than mouse α -lactalbumin, but concentrations of lactose, fat, and total solids, as well as mammary morphology were similar to control mice. Again, this suggests that the mammary gland normally has enough α -lactalbumin for optimal lactose synthesis. In vitro lactose synthesis by mammary gland homogenates from α -lactalbumin transgenic mice was increased compared with controls, showing that bovine α -lactalbumin can interact with mouse galactosyltransferase. Pups from transgenic mice showed increased growth although there was no significant impact on milk volume, which might suggest a growth-regulating effect of α -lactalbumin.

The Role of α -Lactalbumin in Infant Nutrition

α -Lactalbumin is a major protein in human milk; it is present in concentrations of 2 to 3 g/L and comprises approximately 25 to 35% of the total protein content.^{40,41} A recent multicenter study in nine countries showed that the concentration of α -lactalbumin is relatively constant across a variety of populations, but may be higher or lower in certain locales.⁴² The reason for this variation is not known, but it is unlikely to be due to nutritional status as both severely malnourished and well-nourished Ethiopian women had concentrations of α -lactalbumin that were similar to those of well-nourished Swedish women matched for stage of lactation.⁴⁰ Its contribution to the protein content of human milk varies with the

stage of lactation; colostrum protein contains greater than 20% α -lactalbumin in mothers delivering at term⁴¹ and 14% in mothers delivering prematurely.⁴³ During the course of lactation (in women delivering term infants), α -lactalbumin concentration slowly declines and is positively correlated with total nitrogen concentration.⁴⁰ By contrast, the α -lactalbumin in bovine milk is only approximately 2 to 5% of total protein.⁴⁴ α -Lactalbumin contains an unusually high proportion of essential amino acids (Table 1)—63% of total amino acids—which can be compared to 51% in bovine casein and 52% in cow milk protein.⁴⁵ Importantly, α -lactalbumin has a high content of cysteine, lysine, and particularly tryptophan. The low levels of both cysteine and tryptophan in bovine milk represent a challenge when designing infant formulas with appropriate amino acid content. It is the high concentration of α -lactalbumin in human milk that gives it its unique amino acid composition.⁴⁶

Cow's milk has a whey protein:casein ratio of approximately 20:80; this ratio is approximately 60:40 in human milk.³⁵ Milk-based infant formulas are usually made by adding whey protein concentrate to skim milk to make the whey protein:casein ratio more similar to that of human milk. This modification in the ratio of the whey to casein proteins results in an amino acid composition that still is quite different from that of human milk protein, however, and results in a plasma amino acid profile in formula-fed infants that in many ways does not match that of breastfed infants.⁴⁷ The reason for these differences lies in the composition of whey protein from cow's milk and human milk whey proteins. β -Lactoglobulin is a major protein in bovine whey, but is absent from human milk. α -Lactalbumin is rather a major protein in human milk, but not in cow's milk, and the high levels of some essential amino acids of this protein contribute substantially to the amino acid requirement of infants.⁴⁸ Researchers have therefore suggested that development of an infant formula with increased levels of α -lactalbumin (and decreased levels of β -lactoglobulin) would result in an amino acid composition more similar to that of human milk and a plasma amino acid profile more similar to that of breastfed infants.^{46,48} However, bovine α -lactalbumin by itself is not an appropriate source of protein for infants. Although it contains high levels of such essential amino acids as tryptophan and cysteine, it contains exceptionally low levels of arginine, and therefore cannot serve as the sole whey protein source (Figure 3). Carefully balanced mixtures of bovine proteins coming from α -lactalbumin-rich whey fractions (which are now commercially available⁴⁹) and standard bovine casein fractions present the possibility of more closely meeting the amino acid requirements of infants.

Modification of the amino acid composition of in-

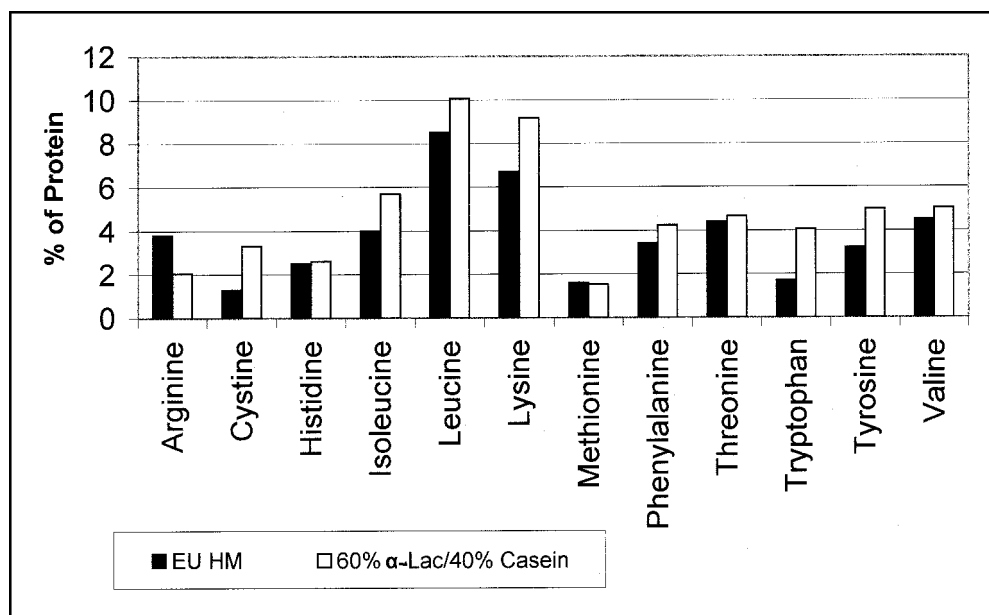


Figure 3. Amino acid composition of 60/40 whey-predominant formula with bovine α -lactalbumin as the sole source of whey protein compared with that of mature human milk as described by the European Union (EU HM). Data from reference 46.

infant formula has recently become more important; scientists have noted that infant formula may contain a higher concentration of protein than needed and that a reduction of the protein level of formula may be desirable.⁵⁰ Infant formula has for some time generally contained approximately 15 g of protein/L; this results in satisfactory growth as well as in high levels of some plasma amino acids (e.g., threonine) and blood urea nitrogen (BUN). Such high levels of BUN indicate that levels of some dietary amino acids are excessive and are therefore being catabolized. A reduction in the protein level of infant formula would thus “normalize” concentrations of the elevated amino acids and of BUN, possibly reducing metabolic stress on immature organs in the newborn, such as liver and kidneys. Although no long-term effects of this “metabolic stress” have been documented, a lower level of protein in formula has been deemed prudent. When reducing the protein content of infant formula, however, concentrations of some essential amino acids may become lower than those in breast milk; this is of concern especially if they are rate-limiting amino acids for protein anabolism or brain transmitter synthesis. In a study on infant formulas,⁵¹ concentrations of tryptophan were between 72 and 108% and concentrations of cysteine + methionine were 57 to 91% of the concentrations in human milk, respectively. Fazzolari-Nesci et al.⁵² and Hanning et al.⁵³ showed that feeding infants with liquid formula containing 15 g of protein/L resulted in plasma tryptophan concentrations lower than those of breastfed infants. By contrast, Lönnerdal and Chen⁴⁷ showed that infants fed powdered formula with

13 g of protein/L had plasma tryptophan concentrations similar to those of breastfed infants. The type of formula (i.e., casein-predominant vs. whey-predominant, liquid or powdered form, source of whey used, etc.) may determine whether feeding specific formulas will result in plasma amino acid concentrations significantly lower than those of breastfed infants.

Increasing the concentration of tryptophan in formula by adding free tryptophan has been evaluated by some investigators.^{52–54} Whereas such additions did increase plasma tryptophan concentrations of the formula-fed infants, it is likely that the absorption kinetics for the free amino acid are quite different from those of protein-bound tryptophan, which may affect its use in protein synthesis and ultimately its catabolism. Another possibility is to add a protein that is rich in tryptophan and cysteine compared with infant formula. Heine et al.⁵⁵ added bovine α -lactalbumin (80% purity) to infant formula with reduced protein content (13 g/L) at two different levels, thereby increasing the tryptophan content to 1.88 and 2.10%, respectively. In a crossover design, infants were fed these formulas or formula with “regular” protein content (18 g/L; 1.66% tryptophan). They were then compared with a control group of breastfed infants. Infants fed the low-protein formula with the higher tryptophan content (2.10%) had plasma tryptophan concentrations similar to those of breastfed infants, whereas those fed the formula with lower tryptophan content did not; infants fed formula with the “regular” level of protein did not have plasma tryptophan concentrations similar to those of breastfed infants either. The

true tryptophan concentration in human milk protein is difficult to determine, but is likely to be above 2%. If the protein content of infant formula is lowered, therefore, fortification with α -lactalbumin may be of nutritional significance in that it may increase both formula and plasma tryptophan concentrations. Heine et al.⁴⁵ previously stated that tryptophan and cysteine are low in formula and that increasing the α -lactalbumin content would result in increased plasma levels of not only tryptophan but also cysteine. Unfortunately, plasma cysteine was not measured in their formula-feeding study; formula concentrations of cysteine were increased, however, possibly leading to increased plasma levels.

Formulas with increased levels of α -lactalbumin may have specific physiologic benefits for the infant. Tryptophan is the precursor to the neurotransmitter serotonin; consuming this amino acid at a concentration similar to that of human milk, balanced with appropriate levels of other amino acids, may be beneficial to the infant. When preparations rich in α -lactalbumin were provided to adults vulnerable to stress, depression scores were lower and cognitive performance was improved.^{56,57} Such activities could be evaluated in infants receiving formulas with α -lactalbumin levels similar to human milk.

Digestibility of α -Lactalbumin

α -Lactalbumin, commercially once known as “lactalbumin” or whey protein, was traditionally considered a highly digestible protein and, owing to its balanced amino acid composition, of high biologic value.⁴⁸ Evaluations in some experimental animals proved this theory.^{58,59} Recent studies in vitro and in infant rhesus monkeys demonstrated the need to further investigate the digestibility of α -lactalbumin. Jakobsson et al.⁶⁰ examined the in vitro digestion of bovine casein, α -lactalbumin, and β -lactoglobulin in duodenal juice obtained from 3- to 19-month-old infants. In pure form, 30 mg of casein was hydrolyzed/mL of duodenal juice/minute, whereas only 1 mg of α -lactalbumin and β -lactoglobulin was hydrolyzed. When the proteins were present in cow's milk or infant formula, hydrolysis was slower, the corresponding numbers being 16, 0.03, and 0.12 mg \cdot mL⁻¹ \cdot minute⁻¹, respectively. Pre-incubation of the samples with gastric aspirates at a pH of 4 to 5 (normal gastric pH in infants) did not affect the results probably because there was minimal or no pepsin activity at this pH. Thus, casein appeared to be digested rapidly and effectively, whereas this was not the case for α -lactalbumin and β -lactoglobulin. Results in adults, however, were different suggesting that age-related differences in gastrointestinal conditions strongly affect the digestive fate of milk proteins. Mahe et al.⁶¹ studied the digestion

time course of milk proteins in adult short-bowel subjects. They found that α -lactalbumin and β -lactoglobulin were present in jejunal effluents, but only for the first 30 minutes, whereas casein was detected for 1 to 2 hours. It is therefore possible that active gastric digestion, which occurs in adults, may increase the digestibility of α -lactalbumin and β -lactoglobulin. This is supported by observations by Sakai et al.,⁶² who incubated infant formulas with pepsin at various pHs between 1.5 and 4.0. Both α -lactalbumin and β -lactoglobulin were hydrolyzed at a pH of 1.5 to 2.5, whereas they were resistant to proteolysis above pH 3.0. The gastric pH of infants is approximately 4 to 5 up to at least 6 months of age,²⁹ which might explain the lack of effect of pepsin on α -lactalbumin. The reason for the persistence of casein in adults is not known; however, it is possible that digestion occurs, but smaller fragments with intact epitopes remain in the upper gut. It is also possible that heat treatment has a negative effect on the digestibility of casein (see below).

Studies in preterm and 6-week-old infant rhesus monkeys also indicate that digestion of α -lactalbumin and β -lactoglobulin is slow.⁶³ As much as 30 to 50% of these proteins were detected in duodenal aspirates 60 minutes after ingestion and intact α -lactalbumin was found in serum, suggesting absorption of the protein in the small intestine. α -Lactalbumin in breast milk, however, appears to be easily digested. When extracting proteins from fecal samples obtained from term infants, no intact α -lactalbumin was detected by crossed immunoelectrophoresis using antibodies against human whey proteins; lactoferrin and secretory IgA, on the other hand, were present.⁶⁴ Similarly no α -lactalbumin was detected in the stool of preterm infants fed breast milk, whereas several other breast milk proteins were found in intact form.⁶⁵

Global endpoints of protein digestibility and quality indicate that term infants can digest formulas rich in α -lactalbumin. Heine et al.⁵⁵ observed elevated plasma tryptophan levels when feeding formulas enriched with α -lactalbumin, which demonstrated excellent tryptophan bioavailability. In addition, Lien et al.⁶⁶ recently reported the results of a multicenter clinical study comparing a formula with elevated α -lactalbumin levels with a standard formula. Growth rates and protein status (serum albumin) were similar between the two groups. These results demonstrate that formulas with elevated levels of α -lactalbumin have high protein quality and are well utilized.

When feeding preterm infants human milk, so-called “human milk fortifiers” are often added to increase the protein content of the breast milk. This practice may affect the digestion of α -lactalbumin in breast milk because the fortifiers are made from cow's milk protein.

Lindberg et al.⁶⁷ found that both bovine and human milk α -lactalbumin were relatively resistant to digestion when human milk with fortifiers and preterm formulas were exposed to proteolysis by duodenal juice from preterm infants, a 3-year-old child, or adults; approximately 20 to 50% of the α -lactalbumin was immunologically intact after 40 minutes of digestion. Casein, however, was more rapidly digested. These observations may have implications for the design of improved fortifiers and formulas for preterm infants.

Bioactive Peptides Derived from α -Lactalbumin

During the digestion of α -lactalbumin, smaller peptides are formed that may exert biologic activities in the intestine, or elsewhere in the body, if absorbed intact. Several of these (amino acids 50–52, 99–103, and 104–108) have been shown to inhibit angiotensin-I-converting enzyme (ACE) in vitro.⁶⁸ A number of these peptides have been synthesized,⁶⁹ but their biologic effect appears to occur only at concentrations that are much higher (μ M) than those likely to be formed in the gut. An opioid peptide (amino acids 50–53) called α -lactorphin, which is structurally similar to human leu-enkephalin, has been shown to lower blood pressure in spontaneously hypertensive rats when administered subcutaneously,⁷⁰ but it is uncertain whether these effects would be observed when it is formed during digestion.

Three bactericidal domains of bovine α -lactalbumin are formed during digestion with trypsin and chymotrypsin in vitro.⁷¹ No bactericidal peptides were formed during pepsin incubation. Of those formed during trypsin digestion, one was small (residues 1–5), while the other was more complex (residues [17–31]S-S[109–114]) and included a disulfide bridge. The peptide formed by chymotrypsin digestion was also complex (residues [61–68]S-S[75–80]). The peptides were mostly active against Gram-positive bacteria (e.g., *B subtilis*), while Gram-negative bacteria were only marginally affected. Concentrations of 55 nmoles/sample were used, but it is not yet known whether these peptides are formed in vivo, and if so, at what concentrations. Pihlanto-Leppala et al.⁷² also produced enzymatic hydrolysates of α -lactalbumin and found a peptide that inhibited the growth of *E coli*; however, the concentration needed for growth inhibition (25 g/L) is much higher than the concentration usually found for antibacterial peptides (mg/L). Brück et al.⁷³ used batch culture and a two-stage continuous culture system to study the effect of α -lactalbumin-supplemented formula on mixed populations of human gut bacteria. They found that α -lactalbumin supplementation caused a significant reduction of potentially pathogenic microflora (*Bacteroides*, *Clostridia*, *E coli*), similar to

that observed in breastfed infants. However, whether this effect was due to peptides formed from α -lactalbumin was not investigated.

It is possible that peptides formed during digestion of α -lactalbumin not only have an inhibitory effect on pathogens, but also stimulate host-friendly gut microflora. Kee et al.⁷⁴ found peptide fractions from pepsin-hydrolyzed α -lactalbumin that stimulated the growth of *Bifidobacteria* in vitro. This finding needs to be followed up in vivo.

The immune-stimulating peptide, GLF, which consists of Gly-Leu-Phe (amino acids 51–53, symbols GLF), is formed at concentrations (nM) that are likely to be of biologic significance.¹⁹ This tripeptide has been shown to enhance phagocytosis by human macrophages and to stimulate polymorphonuclear neutrophil oxidative metabolism as well as phosphoinositide metabolism, all of which are important in bacterial killing.⁷⁵ Specific binding sites for the GLF peptide have been found on human phagocytic cells, such as polymorphonuclear leukocytes and monocytes.⁷⁶ When purified bovine α -lactalbumin was digested by human neonatal gastric juice, GLF, as well as several small peptides having a GLF N-terminal sequence, were identified. The amount formed was similar when pH 2 and pH 4 were used. The GLF peptide was also found in gastric aspirates from young infants fed human milk, showing that it is also formed from human α -lactalbumin in vivo. The concentration of GLF required for stimulation of immune cell activity was approximately 30 μ M, which is considerably higher than the physiologically active concentration of 1 nM.⁷⁵ The released GLF peptide may stimulate phagocytosis by two different mechanisms.¹⁹ One possibility is that it stimulates macrophages at the lamina propria of the villi, possibly aided by tripeptide transporters known to be present in the intestinal epithelium.⁷⁷ Another possibility is that GLF acts on macrophages in breast milk, which are believed to aid in the defense against infections.⁷⁸ Phagocytes in colostrum, of which macrophages constitute 80%, have been shown to kill EPEC, possibly explaining the observation that infant formula supplemented with bovine α -lactalbumin had a protective effect against induced EPEC infection in infant rhesus monkeys.⁷⁹

Conclusion

α -Lactalbumin is the predominant protein in human milk and it appears that during evolution several roles have developed for this protein. Besides its better known roles of being part of the lactose synthase complex and a source of amino acids, recent research suggests that α -lactalbumin can be physiologically active by affecting gut microflora, by enhancing mineral absorption, by

stimulating immune function, and possibly by having a role in apoptosis.

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