# Interaction of β-Lactoglobulin with Retinol and Fatty Acids and Its Role as a Possible Biological Function for This Protein: A Review

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## ABSTRACT

β-Lactoglobulin is the major whey protein in the milk of ruminants and some nonruminants, such as pigs and horses. Although β-lactoglobulin was first isolated 60 yr ago, no function has been definitely ascribed to β-lactoglobulin. Recent x-ray crystallographic studies have advanced knowledge of the structure of β-lactoglobulin, which is homologous with that of retinol-binding protein and lipocalycins; the function of these proteins seems to be participation in the transport of small hydrophobic substances. By analogy, this protein has been suggested as having a role as a transporter of fatty acids and retinol. This review reassesses the function of β-lactoglobulin in light of the large amount of information that has accrued in the last few years. In particular, this review concentrates upon studies of the binding of retinol and fatty acids to β-lactoglobulin, including the binding constants and number of binding sites, the location of the binding sites, and the influence of chemical modifications in the interaction of the protein with both ligands. This study also describes studies of the influence of β-lactoglobulin on several biological processes that may be relevant to the possible biological role of this protein.

(**Key words**: β-lactoglobulin, retinol, fatty acids, biological role)

**Abbreviation key**: **FABP** = fatty acid-binding protein, **RBP** = retinol-binding protein.

## INTRODUCTION

Milk whey contains a heterogeneous group of proteins that can be derived from blood or synthesized in the mammary gland. These proteins have an important nutritional function by providing amino acids required by the young animal. In addition, some proteins may have a more specific function in the mammary gland or in the newborn. Thus, α-lactalbumin is the B subunit of lactose synthetase, the enzyme that catalyzes the addition of galactose to glucose to produce lactose (8). Immunoglobulins serve to transfer passive immunity to the neonate (43). Lactoferrin may play a role in controlling iron absorption or in the selection of the intestinal flora in the newborn intestine (69). Although various roles have been proposed for β-lactoglobulin, including involvement in the phosphorus metabolism in the mammary gland (23) or in the transfer of passive immunity to the newborn (80), no function has been definitively ascribed to this protein.

β-Lactoglobulin is the major protein in the whey of ruminant species. Its concentration varies throughout lactation; it is higher in the first colostrum (between 18 to 20 mg/ml), and becomes stable during the 2nd wk postpartum (about 4 mg/ml) (57).

Although β-lactoglobulin had been considered to be exclusive to ruminant milk for many years, it is also found in the milk of nonruminants, such as horses (32), pigs (14), dogs, dolphins (61), kangaroos (33), cats (35), and whales (78). However, milks from humans (7, 49), rodents, and lagomorphs (36) appear to be devoid of β-lactoglobulin. That β-lactoglobulin is absent from the milk of some species needs to be remembered when the possible biological function of β-lactoglobulin is being considered.

At the normal pH of milk (pH 6.5), ruminant β-lactoglobulin occurs as a dimer, with a molecular weight of 18,000 per monomer, but

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β-lactoglobulin from pigs, horses, and donkeys exists as monomer. The state of association of β-lactoglobulins from other species is still uncertain. The monomer of ruminant β-lactoglobulin consists of a polypeptide chain of 162 amino acids containing five cysteine residues, four of which are involved in forming intrachain disulfide bridges (30). The homologous proteins in milk from monogastric species vary somewhat in length. Thus, variant II of horse β-lactoglobulin and cat β-lactoglobulin II has 163 amino acids (35), but the pig β-lactoglobulin variant I and kangaroo β-lactoglobulin only contain 159 and 155 amino acids, respectively (14, 33).

In the milks of ruminants, the association of β-lactoglobulin in solution changes as a function of pH. Below pH 3.5 and above 7.5, the dimers dissociate into monomers. At pH 8.6 and above, β-lactoglobulin undergoes a polymerization that progresses with time and that seems mainly to be due to the oxidation of the sulfhydryl groups (45).

β-Lactoglobulin is quite resistant to hydrolysis in vitro by proteases such as pepsin, trypsin, and chymotrypsin. However, cleavage of the disulfide bonds significantly increases the susceptibility of β-lactoglobulin to proteolysis (66). Kella and Kinsella (42) suggested that the resistance of this protein to peptic digestion could result from increased internal hydrogen bonding at acid pH (2.0), making the susceptible peptide bonds less accessible to the enzyme. In contrast, β-lactoglobulin is less resistant to tryptic and chymotryptic hydrolysis, possibly because it undergoes conformational changes above pH 7.5 that result in exposure of strategic cleavage points for these enzymes (13, 66).

Similarly, β-lactoglobulin seems to be quite resistant to gastric digestion in vivo and apparently remains mostly intact after it passes through the stomach (82). There is also evidence of absorption of some intact β-lactoglobulin in humans, as this protein can be detected in trace amounts in maternal milk from women who have consumed milk from cows (49).

The amino acid sequences have been determined for β-lactoglobulins of several species (30), and several DNA sequences (1) and gene structures (2) have been reported. Comparison of the amino acid sequences of ruminant β-lactoglobulins reveals a high degree of positional identity (91 to 99%), but the identity between pig and horse β-lactoglobulin I and II is surprisingly lower (37 and 68%, respectively). Although pairwise comparisons of the primary structures, except for kangaroo β-lactoglobulin, show at least 40% identity, the entire group of β-lactoglobulin sequences has only 13% of residues common to all species. Most of the substitutions have occurred at residues 84 to 103 and residues 148 to 163, which are located on the surface of the molecule (29, 30).

The conformational structure of four distinct crystals of β-lactoglobulin, lattices K, X, Y, and Z, have been studied by x-ray crystallography (34, 48), and the structures of lattices X, Y, and Z have been determined (48). Each of these forms has so far given rise to electron density maps at a resolution of 2.8 A or better. Interpretation of these maps has shown that the structure consists of nine strands of antiparallel β-sheet and one short α-helix (54, 71). The core of the molecule is made up of a very short α-helix segment and eight strands of anti-parallel β-sheet, which wrap around to form an antiparallel β-barrel (48).

## PROTEINS HOMOLOGOUS TO β-LACTOGLOBULIN

The three-dimensional structure of β-lactoglobulin is essentially the same as that of human retinol-binding protein (**RBP**), the main protein involved in the transport of retinol in serum (6, 16, 31). Furthermore, over the past decade it has become apparent that a series of proteins exists with tertiary structure probably close to that of β-lactoglobulin and RBP, despite the fact that these proteins show only 25 to 30% common residues (31). This family, the lipocalin or lipocalycin family, includes apolipoprotein D, a serum protein that appears to participate in the transport of cholesteryl esters; purpurin, a protein located in neural retina that is thought to be involved in binding and transporting of retinol; Bowman's glands protein from olfactory epithelium, which may bind and transport odorants; bilin-binding protein, which binds biliverdin; and others for which ligands have not yet been identified, such us pregnancy-associated endometrial α-globulin and androgen-dependent epididymal protein, among others (29, 36, 60, 70, 81).

Although the sequence homology might seem to be marginally significant, all of these proteins have two stretches of sequence that are strongly similar: residues from 26 to 29 and residues from 124 to 126. In the three-dimensional structure, these residues are located in the same region, which has been proposed to be part of the ligand-binding site (29).

Recently, the fatty acid-binding protein (**FABP**) family is another identified group of small proteins that bind hydrophobic molecules but that are, by contrast, almost exclusively intracellular. These proteins have a similar structural framework but involve a barrel with 10 strands instead of 8. This protein family includes cellular RBP, which are thought to function in the intracellular transport of retinol, cellular retinoic acid-binding proteins, $P_2$ myelin protein, and intestinal fatty acid-binding protein (24, 52, 70).

Structural analysis of both families has shown that large parts of the lipocalin and FABP structures are quantitatively equivalent, indicating a very close structural relationship and suggesting that together they form a structural "superfamily", sharing essentially the same fold. Furthermore, lipocalin and FABP families also share a common N-terminal sequence motif conserved in its conformation and location within the fold. This finding lends support to the possibility that both families also share an evolutionary relationship (24, 52). Thus, it would be logical to refer to these two classes of small proteins as octinins (8-stranded) and decinins (10-stranded) because both can reasonably be referred to as lipocalycins (4, 24, 70).

Similarly, the gene encoding β-lactoglobulin has a similar organization of exons and introns to RBP, $\alpha_1$-acid glycoprotein and apolipoprotein D. In particular, a comparison between β-lactoglobulin and RBP shows that the genes encode equivalent elements of the three-dimensional protein structure within analogous exons, which suggests that these proteins are members of an ancient gene family (2).

As already mentioned, the common property of all these proteins seems to be the ability to bind small hydrophobic molecules. Ligands are bound within the central cavity of the β-barrel. The changes to the amino acid residues in the central pocket could quite possibly result in different ligand specificity. In most cases, the nature of the ligands remains to be identified; one also needs to know whether these proteins are selective for specific ligands or bind a wide spectrum of small hydrophobic molecules (4, 54, 70).

## INTERACTION OF β-LACTOGLOBULIN WITH RETINOL

In 1972, Futterman and Heller (27), using fluorescence measurements, first reported that bovine β-lactoglobulin, like RBP, formed water-soluble complexes with retinol. The fluorescence yield of retinol that complexed with β-lactoglobulin was about threefold larger than retinol in petroleum ether. The degree of fluorescence enhancement of retinol bound to β-lactoglobulin was about half that of retinol bound to RBP, indicating that retinol was bound with greater affinity to RBP than to bovine β-lactoglobulin. Using fluorescence titration and circular dichroism, β-lactoglobulin was found to display two high affinity binding sites for retinol per protein dimer, each with an association constant of $2 \times 10^8\ M^{-1}$ (26). This value is in good agreement with that determined by continuous elution analytical affinity chromatography (39). However, using equilibrium dialysis and radiolabeled retinol, the number of binding sites is in accordance with that previously determined, but the association constant appears to be four orders of magnitude less (about $1.5 \times 10^4\ M^{-1}$) (62). The different value obtained by equilibrium dialysis compared with those of the other two methods might be because the constant was determined in one case by fluorescence techniques (26) and in the other by affinity chromatography in a matrix containing immobilized *trans*-retinal (39), both of which are indirect methods. Gel filtration or reversed-phase chromatography of retinol incubated with β-lactoglobulin has shown that the β-lactoglobulin peak contains only a small amount of ligand, but most of the retinol is eluted as free retinol, indicating that β-lactoglobulin binds retinol with a relatively low affinity (51, 62).

The binding of retinol to β-lactoglobulin

39). Thus, the nonpolar portion of the ligand is largely responsible for binding, and the binding site likely includes tryptophan residues, which serve to fix the β-ionone ring of retinol (26). An electrostatic contribution to the interaction has also been suggested because of the dependence of the affinity on pH and ionic strength (79).

Chemical modification of β-lactoglobulin, by methods such as methylation, ethylation (19, 20), esterification, or alkylation (21), enhances the binding affinity for retinol by opening up a second binding site. It may therefore be assumed that the partial change of β-lactoglobulin secondary structure produced by these treatments does not destroy the structure of the retinol-binding pocket. This general effect may be tentatively explained by an increase in the overall hydrophobicity of the protein (19, 21).

The existence of a specific binding site for retinol in β-lactoglobulin has been proposed, but the location and nature of this site are still unclear. In the crystal structure of RBP, retinol was bound in a deep pocket formed by the β-barrel of the protein polypeptide chain with the β-ionone ring deep inside the protein; the alcohol group pointed toward the solvent (6, 16).

However, two independently performed crystallographic analyses of β-lactoglobulin have led to different hypotheses about the location of the putative retinol-binding pocket. Modeling studies have proposed that retinol is bound in a deep pocket within the central calyx in which the tryptophan residue at position 19 is located close to the β-ionone ring and the retinol hydroxyl group can then interact with the ε-amine of lysine at position 70 (54). An alternative proposition, based upon fluorescence spectroscopy and electron densities assigned to retinol, supports the idea that binding of retinol is quite different in β-lactoglobulin and in RBP. The retinol would be bound in a hydrophobic pocket located on the surface of β-lactoglobulin at the interface between the β-barrel and the α-helix that packs onto the barrel, and limited by phenylalanine and lysine residues at positions 136 and 141, respectively (47, 48).

Recently, Chen et al. (11), using proteolysis techniques, found that the central core of the β-barrel, with α-helix removed, still bound to immobilized retinal in a manner similar to the intact protein. Furthermore, Cho et al. (12), using site-directed mutagenesis in the putative interior and surface pockets, proposed that retinol binds in the conserved interior cavity rather than the surface pocket.

However, although the overall architecture of retinol-binding sites in β-lactoglobulin and RBP may be similar, notable differences exist. The binding of retinol by β-lactoglobulin appears to be different from that by RBP on the basis of three types of experimental results. First, retinol bound to β-lactoglobulin is displaced from the complex by another retinoid, a degradation product that forms the retro-complex. This process occurs despite the protection of retinol by the protein molecule (38). Nothing analogous to this has been described for RBP. Second, oxidation rate of retinol by liver alcohol dehydrogenase was higher when retinol was presented bound to β-lactoglobulin compared to RBP (27). Third, rotational relaxation measurements suggested that retinol binds in a region of the β-lactoglobulin molecule that is flexible with respect to the rest of the protein (48).

These facts suggest that retinol in β-lactoglobulin is more exposed to the environment, despite the observation that the calyx in β-lactoglobulin appears to be deeper than in RBP (52). Thus, the retinol-binding site modeled in the central cavity of β-lactoglobulin is mostly protected from the solvent as in the RBP. However, the tip of the isoprene tail remains exposed.

Monaco et al. (48) interpreted these data to rationalize their observed surface-binding pocket. This model may represent an artefactual model of binding because the complex of retinol and β-lactoglobulin shows a rather weak electron density assigned to the retinol molecules, possibly as a result of low occupancy. Furthermore, the retinol position observed by Monaco et al. (48) may reflect the prior occupancy of the preferred binding site within the central calyx by another molecule that could displace retinol, e.g., palmitate (12, 22).

The binding of retinol to β-lactoglobulin in vitro, as well as the existence of some homology of the primary and the three-dimensional structures between this protein and RBP, has led to speculation that β-lactoglobulin may participate in the transport of retinol in the

intestine of the newborn (31, 54, 60). However, despite the indirect experimental support for in vitro interaction, the presence of retinol bound to β-lactoglobulin in milk has not so far been detected (51, 55). However, Said et al. (68) have shown that β-lactoglobulin may enhance intestinal uptake of retinol in rats. This property is shared by RBP, but other proteins, such as albumin or lactoferrin, failed to affect retinol uptake, indicating a certain degree of specificity in the enhancing effect. Furthermore, the presence of a receptor for β-lactoglobulin–like proteins at the brush border membrane of the enterocyte has been suggested (54, 68). However, the presence of a receptor for this protein would be unusual because rat milk does not contain β-lactoglobulin (36).

## INTERACTION OF β-LACTOGLOBULIN WITH FATTY ACIDS

In 1970, Spector and Fletcher (76), using the partition equilibrium of radiolabeled fatty acids, reported that β-lactoglobulin interacts in vitro with fatty acids. Furthermore, competition experiments showed that free fatty acids compete with retinol for binding to β-lactoglobulin (62). The strength of fatty acid binding to β-lactoglobulin decreases in the order palmitic, stearic, oleic, and lauric acids (76). Caprylic and capric acids are not bound (25). A model has been described that contains one primary site with an association constant of the order of $10^5$ to $10^6$ $M^{-1}$ and a large number of weak secondary binding sites with an affinity constant of $10^3$ $M^{-1}$ (25, 51, 76). The association constant is higher for fatty acids with long hydrocarbon chains and is reduced when the structure of β-lactoglobulin is altered by alkylation or esterification (25) or by exposure to urea or heat (76). Similarly, by using frontal analysis chromatography in a matrix with a structure similar to a fatty acid, the apparent association constant for β-lactoglobulin is markedly higher when the hydrocarbon chain is longer (about 20 times higher for a 16-carbon chain than for a 12-carbon chain). These findings indicate that nonpolar interactions play an important role in the binding process (25, 55, 76).

In addition, the binding of palmitate analogues, in which the carboxyl group has been modified or removed, is weaker than that of the native acidic form (76). Furthermore, with a hydrocarbon gel matrix, the affinity of β-lactoglobulin is lower than that with fatty acid-like gels containing a free carboxyl group (55). An increase in ionic strength decreases the amount of protein adsorbed, indicating that polar interactions also are important in the binding process (55). These observations are consistent with the presence of an electrostatic component in the binding involving the ionized carboxyl group of the fatty acid and a cationic protein site. Thus, Pérez et al. (55) and Spector and Fletcher (76) postulated that the binding site for fatty acids in β-lactoglobulin consists of a hydrophobic pocket with a positively charged amino acid near the entrance.

The binding of fatty acids to β-lactoglobulin causes a small change in the ultraviolet fluorescence intensity of β-lactoglobulin. This change could occur because free fatty acids interact directly with a segment of the molecule that contains tryptophan or as a result of a small conformational change in the region of tryptophan residues that are secondary to the binding of fatty acids (76).

The binding of fatty acids to β-lactoglobulin increases the resistance of the protein to proteolytic degradation (64) and to thermal denaturation (65), indicating that ligand binding may be an important factor in the stabilization of the structure of β-lactoglobulin. However, this increased stability is not observed when retinol is bound to β-lactoglobulin (64, 65).

β-Lactoglobulin, when isolated from milk of ruminants (cows, sheep, and goats) using nondenaturing techniques, also has several bound lipids, mainly triglycerides and fatty acids. The amount of total fatty acids bound is about 1.0 to 1.4 mol/mol of protein dimer. The predominant fatty acids are palmitic and oleic acids, which together account for approximately 75% of the fatty acids bound. The pattern of fatty acids bound to ruminant β-lactoglobulin resembles that of the fatty acids present in whey and milk, indicating no important differences in selectivity for individual fatty acids (18, 55).

For ruminants, the only whey proteins with the ability to bind fatty acids are β-lactoglobulin and albumin. Other major whey proteins, such as α-lactalbumin, do not have

this property (55). Although the number of high affinity binding sites for fatty acids and the values of association constants are lower for β-lactoglobulin than for albumin (75), the molar concentration of β-lactoglobulin is about 30 times higher than that of albumin; β-lactoglobulin is, therefore, the main FABP in ruminant whey (55, 57, 59).

However, β-lactoglobulins from nonruminant species, such as horses and pigs, do not possess physiologically bound fatty acids or the ability to bind them in vitro. This different property indicates that the ability of ruminant β-lactoglobulin to bind fatty acids is not shared by the homologous protein from nonruminant milk (25, 56).

The ability of β-lactoglobulin to bind fatty acids has been exploited by its use as an emulsifying agent in food technology (72) or as a fatty acid carrier in cell culture. Thus, β-lactoglobulin supports growth of lymphocytes (77) and Ehrlich ascites tumor cells (76) and enhances the uptake of free palmitate by rat hepatocyte monolayers (10). The results obtained with β-lactoglobulin are strikingly similar to those previously reported with albumin, indicating that this phenomenon is nonspecific for the protein carrier (10, 76). Although β-lactoglobulin binds less fatty acids per mole of protein than does bovine serum albumin, fatty acids are bound with lower affinity and may therefore be transferred more efficiently to cells (76).

Furthermore, β-lactoglobulin has a stimulatory effect on several physiological processes. β-lactoglobulin increases sodium-dependent amino acid uptake in synaptosomes (67) and also effectively stimulates the isomerization reaction from *all-trans*- to 11-*cis*-retinol in nuclear membranes from bovine retinal pigment epithelium (44). The *all-trans* to 11-*cis* isomerization of retinol appears to require the intermediate formation of *all-trans*-retinyl esters, which are then directly isomerized, with hydrolysis, to form 11-*cis*-retinol. Such stimulatory effects also occur from other proteins that bind fatty acids, such as bovine serum albumin and hepatic FABP (10, 44, 76, 77). These proteins have rather different types of binding sites, so it is probably the removal of fatty acids that is important. The hypothesis has therefore been proposed that β-lactoglobulin traps released fatty acids, thus protecting the membrane or the isomerase itself from the lytic effects of these substances (44, 67).

Recently, Pérez et al. (58) reported that bovine β-lactoglobulin increases the activity of ruminant pharyngeal lipase. This lipase, also called pregastric lipase (EC 3.1.1.3), is secreted from glands in the proximal-dorsal side of the tongue, the soft palate, and the anterior portion of the esophagus (50). Its activity is higher in newborn animals and is followed by a marked decrease in activity as the animal becomes older. This lipase participates in lipid digestion in the stomach, and the optimal pH ranges from 4.0 to 6.0. Activity in the calf is stimulated by suckling, a characteristic not yet investigated in humans or rats (37). Pregastric lipase is very stable to low pH and pepsin degradation and is very important in newborn and young animals because their concentrations of pancreatic lipase and bile salts are low (46). Furthermore, although milk fat globules are resistant to the action of pancreatic lipase, they are readily hydrolyzed by lingual lipases and, thus, facilitate subsequent hydrolysis by pancreatic lipase (5). However, pregastric and pancreatic lipases are both subjected to strong inhibition by free fatty acids, which can be prevented when they are removed (5). Extensive in vitro studies using bovine serum albumin as a fatty acid acceptor have shown that bovine serum albumin can increase the activity of pregastric and pancreatic lipases (5, 41).

Remarkably, the activity of ruminant pregastric lipase increases to more than twice its level in the presence of β-lactoglobulin concentrations ranging from 10 to 20 mg/ml, which correspond to protein levels found during the colostral period (58, 60). However, the concentration of albumin required for a stimulatory effect on lipase activity (between 5 to 10 mg/ml) is much greater than that found either in colostrum or in the milk of ruminants (59). Thus, the biological role of β-lactoglobulin in ruminants could be to aid milk fat digestion in the newborn animal by promoting pregastric lipase activity (58). However, this function would not occur in other species, such as horses and pigs, in which β-lactoglobulin does not bind fatty acids (56).

In milk from other species, the existence of proteins, apart from β-lactoglobulin and albumin, that can bind fatty acids has not been

described. However, high concentrations of albumin in milk from rodents have been reported to reach 5 mg/ml for rats and 10 mg/ml for mice (28), suggesting that albumin could participate in promoting lingual lipase activity in these species, the milk of which is devoid of β-lactoglobulin. Triglycerides of lagomorph milk have a very high content of medium-chain fatty acids, particularly octanoic and decanoic acids (74); intragastric hydrolysis of milk fat is followed by selective absorption of medium-chain fatty acids directly from the gastric mucosa (3). Consequently, intragastric lipolysis in these species would not require the presence of FABP. In milk from humans and other primates, a potent lipase stimulated by bile salts has been isolated that is stable at pH 3.5 and resists degradation in the stomach. This lipase is an important compensatory factor in the intestinal digestion of milk fat in the newborn human (37); because this lipase is active in the presence of bile salts, it would not require the presence of FABP.

## INTERACTION OF β-LACTOGLOBULIN WITH OTHER SUBSTANCES

In addition to retinol and fatty acids, a wide variety of hydrophobic compounds interact with β-lactoglobulin, including triglycerides (73), alkanes (9), aliphatic ketones (53), and aromatic compounds (*p*-nitrophenyl phosphate, toluene) (23). The association constants of these substances with β-lactoglobulin have been calculated, but, in most cases, which region of the protein is involved in the binding is unclear. β-lactoglobulin possesses one primary binding site per monomer for alkanes and ketones with an association constant of about $10^3$ $M^{-1}$. The binding site is thought to be located inside a hydrophobic cavity and is unaffected by the state of association of the protein (36). Modification of the structure of β-lactoglobulin with urea, reduction of disulfide bonds or ethylation reduces binding of these compounds, reflecting the importance of the native structure in determining binding affinities (53).

Recently, Dai-Dong et al. (17) have verified that bovine β-lactoglobulin has a protecting effect on the thermal destruction of ascorbic acid in aqueous solution. This observation led to the hypothesis of the existence of a specific interaction between vitamin C and β-lactoglobulin. However, by use of equilibrium dialysis and gel filtration chromatography with radiolabeled ascorbic acid, β-lactoglobulin is not able to bind vitamin C (63). Therefore, the protective effect of β-lactoglobulin on ascorbic acid thermal destruction could be due to an antioxidant effect because of the presence of reductive thiol groups in the protein. This effect does not necessarily require direct interaction between the protein and vitamin (63).

The binding of *p*-nitrophenyl phosphate and other low molecular weight phosphates to β-lactoglobulin inhibits phosphate hydrolysis induced by milk alkaline phosphatase (40). This observation led to the speculation that β-lactoglobulin may play a regulatory role in mammary gland phosphorus metabolism (23). However, this inhibition seems to be unimportant because, at the normal pH of milk, activity of alkaline phosphatase on casein micelles is low.

Sonicated vesicles containing a complex of β-lactoglobulin and dipalmitoyl phosphatylcholine can be formed, but treatment of the protein with a helix-forming solvent is necessary because the native protein does not complex with the lipid. Circular dichroism and fluorescence spectra have revealed that β-lactoglobulin in this form contains 30% α-helix and exhibits a 10% increase in tryptophan fluorescence, but the complex apparently does not increase the hydrophobicity of these residues. An initial ionic attraction to position the lipid and protein molecules, followed by hydrophobic interactions to stabilize a complex, has been suggested as an explanation of this interaction (9).

β-Lactoglobulin also exhibits considerable adsorption to an artificial phospholipid membrane between pH 1.3 and 4.0, but none occurs at pH between 6 and 7. Circular dichroism spectroscopy showed no detectable change in conformation of β-lactoglobulin in the films, so the existence of an electrostatic mechanism at acid pH has been proposed in which positively charged β-lactoglobulin interacts with negatively charged lipids in the monolayer to form a coating about one molecule thick (15).

The ability of partially unfolded β-lactoglobulin to interact with lipids is thought to be important in food technology. A better understanding of these interactions could lead

to enhanced utilization of this protein in food systems (9, 15).

## CONCLUSIONS

Much is known about the physicochemical properties of β-lactoglobulin. However, the biological function of this protein has not yet been satisfactorily resolved despite intensive research. The stability of β-lactoglobulin to low pH (42), its resistance to proteolysis (66), and its high concentration in milk, particularly during the colostral period (57), nevertheless suggests that this protein has some specific function in the newborn. The similarity of the three-dimensional structure of β-lactoglobulin to the lipocalycin family, which is composed of proteins with the ability to bind small hydrophobic ligands, points to a transport function for β-lactoglobulin in newborn animals (16, 29).

The ability of β-lactoglobulin to bind retinol in vitro (27, 62), coupled with experimental evidence that β-lactoglobulin enhances uptake of retinol in the intestine (68), suggests that this protein could be involved in the transport of retinol to the newborn.

Conversely, the ability of ruminant β-lactoglobulin to bind fatty acids in vitro (58, 76), the presence of fatty acids that are physiologically bound to the protein in milk (18, 55), the ability of β-lactoglobulin to increase the activity of pregastric lipase (58), and the capacity of β-lactoglobulin to enhance the uptake of fatty acids (10) suggest that ruminant β-lactoglobulin could participate in milk fatty acid metabolism in the newborn.

Most fatty acids that are present in milk are found as triglycerides, which form the fat globule. Most of the retinol in milk is found esterified with fatty acids (74). However, during gastric digestion of milk lipids, the triglycerides and retinol esters are hydrolyzed by preduodenal lipases, greatly increasing the amount of free fatty acids (3, 37). Under these conditions, β-lactoglobulin would probably bind a large amount of fatty acids that would therefore displace the retinol eventually bound to β-lactoglobulin (58, 62). These observations suggest that the biological role of ruminant β-lactoglobulin is probably more closely related to milk fatty acid metabolism than to retinol transport (58).

However, the lack of interaction between horse or pig β-lactoglobulin with fatty acids indicates that the biological function of β-lactoglobulin in these species is not related to fatty acid metabolism (56). Comparison of the amino acid sequences of horse or pig β-lactoglobulin with β-lactoglobulin of cows reveals a degree of homology about 55 and 38%, respectively. In comparison, α-lactalbumin, which plays a role in the biosynthesis of lactose, shows approximately 40% homology with lysozyme, which catalyzes the hydrolysis of bacterial cell-wall polysaccharides (80). These two proteins represent an example of extreme functional divergence among proteins with a degree of homology similar to that reported for β-lactoglobulin from ruminant and nonruminant species (31). Thus, the existence of a low homology between ruminant with horse and pig β-lactoglobulins could lead to the existence of functional differences among species.

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