



Beta-lactoglobulin–nutrition allergen and nanotransporter of different nature ligands therapy with therapeutic action

E.G. Varlamova^{a,*}, O.G. Zaripov^b

^a Federal State Institution of Science Institute of Cell Biophysics, Russian Academy of Sciences, Institutskaya st. 3, 142290, Pushchino, Moscow Region, Russia

^b Federal Science Center for Animal Husbandry named after Academy Member L.K. Ernst, Dubrovitsy village, house 60, 142132, Moscow region, Podolsky city district, Russia

ARTICLE INFO

Keywords:

Beta-lactoglobulin
Nanotransporter
Milk allergen

ABSTRACT

β-lactoglobulin is one of the nutrition allergens present in the milk of many mammals, with the exception of human. This protein belongs to the family of lipocalins, consisting of nine antiparallel β-strands (β-A to β-I) and one α-helix. This structure allows it to serve as a nanotransporter of various nature ligands in a pH dependent manner, which allows us to confidently consider it as a reliable carrier of drugs directly into the intestine, bypassing the destructive acidic environment of the stomach. Based on the latest data, this review describes the currently known methods of reducing the allergenicity of beta-lactoglobulin, as well as the mechanisms and methods of forming complexes of this protein with ligands, which emphasizes its importance and versatility and explains the growing interest in studying its properties in recent decades, and also opens up prospects for its practical application in medicine and pharmaceuticals.

1. Introduction

β-lactoglobulin (β-LG)–globular protein (18.4 kDa, 162 a.a.), belonging to the family of lipocalins, a group of transport proteins with a characteristic secondary structure, containing nine antiparallel β-strands (β-A to β-I) and one α-helix (Flower, 1996; Albani et al., 2014). Eight of the nine β-strands (β-A to β-H) form an antiparallel β-cylinder containing a conical central cavity, which acts as a ligand-binding site (Euston, 2013; Shafaei et al., 2017). Such a cylinder contains an inside ligand-binding site. The secondary structure of β-LG is represented by 15% α-helix, 50% β-sheet, 15–20% reverse turn. The cavity contains many residues of hydrophobic amino acids and functions as a carrier of hydrophobic molecules: fatty acids, retinol and other fat-soluble vitamins, isothiocyanates, various polyphenols, etc. (Halder et al., 2012; Keppler et al., 2014). The following amino acid residues form the binding site: Trp19, Tyr20, Tyr42, Gln44, Gln59, Gln68, Leu156, Glu157, Glu158, His161, Tyr102, Leu104 and Asp129. In addition, β-LG contains five cysteine residues at 66, 106, 119, 121 and 160 positions, which form two disulfide bonds (between Cys66 and Cys160, Cys106 and Cys119) and the free thiol Cys121 lying in the center of the β-LG structure (Brownlow et al., 1997; Burova et al., 1998; Qin et al., 1998; Sakurai et al., 2009), these cysteine residues are essential for the activation of glutathione synthase in the liver. At the N-term of β-LG, there is a loop that closes the cylinder on one side,

while the EF loop (helix–loop–helix structural domain) at low pH can close the other end of the cylinder (Le Maux et al., 2014).

There are several genetic variants of β-LG, the most common of which are the A and B variants, which differ from each other in two amino acids at positions 64 (the residue of aspartic acid in variant A, glycine–in variant B) and 118 (the residue of valine in variant A, alanine–in variant B) (Godovac-Zimmermann et al., 1996). Despite the structural similarity of these β-LG variants, there are differences in physico-chemical properties: different pI values (Yan et al., 2013), uneven stability of native dimers (Manderson et al., 1999; Mercadante et al., 2012), temperature and rate of denaturation, sensitivity to chemicals and affinity for fatty acids (Boye et al., 2004; Bouhallab et al., 2004; O'Kennedy et al., 2006; Loch et al., 2011).

It is known, that β-LG–main whey protein of most species, with the exception of humans, guinea pigs, rabbits, rodents. Interestingly, this protein was found in some primates (baboon, cynomolgus, Rhesus, and vervet monkey), which has homology to bovine protein. In addition, baboon protein is similar to glycodelin–glycosylated protein the homologue of β-LG found in the human uterus. Studies the baboon β-LG homology with that of other species have shown that: for sheep, this homology was 51.3%, for a reindeer–51.8%, for a pig–48.7%, for a horse–55.6%, for a cat–56.8%; for dolphin–54.9% (Uniacke and Fox, 2011; Loch et al., 2014). However, bovine β-LG is the most studied to date, while much less is known about sheep and goat lactoglobulins.

* Corresponding author.

E-mail address: 1928lv@mail.ru (E.G. Varlamova).

<https://doi.org/10.1016/j.rvsc.2020.08.014>

Received 17 June 2020; Received in revised form 8 August 2020; Accepted 28 August 2020

0034-5288/ © 2020 Elsevier Ltd. All rights reserved.

Sheep lactoglobulin is very similar to bovine, 162 a.a. are also represented, dimerizes at neutral pH values. It was shown that the EF loop in sheep's lactoglobulin can take open and closed conformations, which indicates the presence of the Tanford transition, which is characteristic of bovine β -LG (Loch et al., 2019). At low pH values, the EF loop blocks the entrance to the cylinder and access to the binding site in it; this loop position is stabilized mainly by the hydrogen bond between the carboxyl group Glu89, whose pKa is 7.3 and the carbonyl group Ser116 (Tanford et al., 1959). With an increase in pH, carboxyl deprotonation is observed, this destroys the hydrogen bond and causes conformational changes that open access to the binding site in the cylinder pocket. In addition, the presence of a more complex Tanford transition model, which also includes the GH loop and hydrogen bonds between the residues Ile84, Asn90 and Glu108 was shown by NMR (Sakurai and Goto, 2006; Sakurai et al., 2009).

2. β -lactoglobulin as a nanotransporter of various nature ligands

Recently, more and more works have been devoted to the study of β -LG as a nanotransporter of various compounds and drugs. It has been shown that β -LG can deliver directly to the intestine the widespread drug irinotecan with high antitumor activity, especially in the treatment of intestinal cancer (Potmesil, 1994; Le et al., 2012; Bijari et al., 2019). Recently, oral drug delivery systems have been most preferred over intravenous ones, however, when developing an oral drug, it is important to consider its stability and absorption in the lumen of the gastrointestinal tract. Given the physico-chemical properties of β -LG described above, it can be considered with confidence as a reliable nanotransporter of drugs directly into the intestines, bypassing the acidic environment of the stomach, which is harmful to most drugs.

Irinotecan is a camptothecin derivative with 5 aromatic rings and a lactone fragment in its structure. However, all camptothecin analogs undergo a pH-dependent rapid and reversible hydrolysis, which converts the closed-ring lactone into an inactive hydroxyl carboxylated form that does not have antitumor properties. At low pH values, the predominant structure is lactone, while at pH values close to physiological conditions, preference is given to the carboxylated form (Fassberg and Stella, 1992; Derakhshandeh et al., 2007). Intravenous injection of irinotecan has shown significant clinical activity in the treatment of colorectal cancer. In the liver this drug passes into the active form of SN-38 under the action of carboxyl esterase (Houghton et al., 1996; Drengler et al., 1999). It was shown that β -LG nanovesicles behave as a pH-dependent mechanism for the release of irinotecan, and the degree of release of this drug directly correlates with pH values. Thus, irinotecan was retained in β -LG nanovesicles at low pH values, which is typical for the acidic environment of the stomach. In the intestine, the pH of the medium is 7.4 and the maximum degree of irinotecan release was observed (Bijari et al., 2019).

Using spectroscopic analysis and molecular docking, the interaction of β -LG with 3,3'-diindolylmethane (DIM) was studied, the main driving forces of which were hydrophobic interactions and hydrogen bonds (Wang et al., 2019). DIM-bioactive substance belonging to indoles with two imido groups and two benzol rings, capable of inducing the expression of the CYP1A1 gene (Cytochrome P450, family1, subfamily A, popypeptide1), which significantly inhibits the proliferation of breast cancer cells (Reed et al., 2008). Due to its biological activity, DIM is becoming an increasingly popular agent in the pharmaceutical industry, but its low stability and poor oral bioavailability are a big problem for its widespread use (Jellinck et al., 1993). Therefore, its transfer through interaction with β -LG solves these problems.

Another β -LG ligand is glabridin-isoflavonoid, found in the licorice roots of *Glycyrrhiza glabra* L. (Wei et al., 2018), which exhibits a wide range of pharmacological properties: it can inhibit melanogenesis (Yokota et al., 1998; Nerya et al., 2003), has anti-inflammatory and antioxidant properties (Belinky et al., 1998; Fukai et al., 2003; Yehuda et al., 2015). However, the practical use of glabridine is limited by its

low solubility in water, low bioavailability and incomprehensible stability. It was shown that the use of β -LG as a carrier for encapsulating glabridin facilitated its transport and preservation of bioactivity, as well as the ability of this complex to absorb DPPH radicals (2,2-Diphenyl-1-picrylhydrazyl radical) (Wei et al., 2018).

In addition, it was shown that β -LG can simultaneously transport the natural antioxidant astaxanthin (ATX) and the chemotherapeutic drug 5-fluorouracil (5-FU), moreover, by interacting with independent binding sites in the structure of β -LG. It was shown that these interactions increase the stability of β -LG by reducing the structure of β -sheet and increasing α -helix (Gholami et al., 2020). Interestingly, stronger binding of ATX and 5-FU to β -LG was observed at lower concentrations of antitumor drugs. This can be very important in the development of new drugs in synergism with this protein, especially in the treatment of colorectal cancer, since it was previously shown that 5-FU in combination with other drugs is active in the treatment of this type of disease.

The study of the combination of β -LG with dendritic (i.e. dendrimer-like) mesoporous silica nanoparticles (DMSNs), as insulin host, is very promising for targeted delivery of insulin to the intestine and its full release, which allows insulin to be protected from early release/degradation in the stomach at low pH (Juère et al., 2020).

It has also been shown that β -LG can be used to deliver DAB-1-small aminobenzoic acid derivative, which has anti-inflammatory and anti-tumor/antimetastatic properties (Hamelin-Morrisette et al., 2015; Chanphai et al., 2020). Multiple spectroscopic methods and thermodynamic analysis have shown that DAB-1 binds to β -LG more efficiently than human serum albumin (HSA) and bovine serum albumin (BSA) with the order of stability β -LG > BSA > HAS (Chanphai et al., 2020).

In a number of studies, it was demonstrated that the complex of β -LG with the trace element selenium (Se) has significant antitumor activity (Ji et al., 2019; Xu et al., 2019a, 2019b; Zhao et al., 2019). It is known that Se, selenium-containing compounds of organic and inorganic nature, as well as selenoproteins, have pronounced antioxidant and antitumor activities (Ramoutar and Brumaghim, 2010; Cai et al., 2016; Lipinski, 2016; Lü et al., 2016; Zheng et al., 2016; Shigemi et al., 2017; Varlamova and Cheremushkina, 2017; Kuznetsova et al., 2018; Varlamova, 2018; Varlamova and Goltyaev, 2018; Varlamova et al., 2019). It was shown that Se- β -LG complex can induce apoptosis of HepG2 cancer cells by disrupting the cell cycle in S and G2/M phases, which leads to obvious morphological changes in cells, DNA fragmentation, loss of adhesion, etc. (Ji et al., 2019). In addition, Se- β -LG complex inhibited the growth of MCF-7 human breast adenocarcinoma cells, but did not have toxic effects on MDA-MB-231 normal human breast cells in vitro (Xu et al., 2019a, 2019b). Se- β -LG complex is synthesized by the interaction of selenic acid groups with primary amines of arginine and lysine in the structure of β -LG under vacuum and low temperatures. Two types of the Se- β -LG complex are possible: 1-Se- β -LG and 2-Se- β -LG, having a molecular weight of 20.75 kDa and 41.06 kDa, respectively, which indicates that one molecule β -LG bound to selenic acid in the structure of 1-Se- β -LG, and two of them in the 2-Se- β -LG complex. In addition, in comparison with inorganic selenium compounds, organic complexes exhibit better absorption, have higher bioavailability and less toxicity. It was shown that the Se- β -LG complex induces apoptosis of H22 mouse hepatoma cells in vitro and in vivo, causing their significant cytotoxicity in a concentration and time-dependent manner. Moreover, this complex caused slight inhibition of proliferation of normal liver cells (LO2 line) (Zhao et al., 2019). Thus, the Se- β -LG complex is a very promising antitumor object of research for pharmacology and medicine.

Metals are known to play an important role in many biological systems. Recently, palladium (II) (oxali-palladium complex) is considered as a promising chemotherapeutic agent, primarily due to the fact that palladium complexes interact with biologically important molecules (Bach et al., 2005) and have low toxicity to the kidneys,

especially in the complex with β -LG (Ghalandari et al., 2014). This complex was investigated using various spectroscopic methods. The presence of two binding sites in β -LG and oxali-palladium complex was established, one of which is located in the internal cavity of the β -barrel, the second is located in the CD loop. The formation of this complex was carried out due to hydrophobic forces, while the β -LG structure was not undergo significant changes, which is very important for the delivery of poorly soluble drugs based on metals, especially palladium.

An inclusion complex of naringin and ternary nanoparticles was successfully prepared with excellent encapsulation efficiency and loading content (Feng et al., 2017). Naringin (4',5,7-trihydroxyflavanone-7- β -L-rhamnoglucoside-(1,2)- α -D-glucopyranoside) is a plant citrus flavonoid, especially in grapefruit. Sugar groups of naringin prevent its passive diffusion through biological membranes; therefore, it is not digested by the digestive system until it is deglycosylized (Scalbert and Williamson, 2000). This flavonoid helps strengthen the immune system by increasing the activity of catalase, superoxide dismutase, paraoxonase and other antioxidants (Mamdouh and Monira, 2004). In addition, naringin has anti-atherogenic ability, is involved in the metabolism of fatty acids, and has anticancer and antimicrobial activity. Water-soluble ternary nanoparticle material consisting of amylose, α -linoleic acid and β -LG was developed to encapsulate naringin to improve its bioavailability. In vitro studies have shown that complexation between naringin and ternary nanoparticles contributes to the retention of this flavonoid in the gastrointestinal tract, effectively encapsulating and delivering it (Kanakis et al., 2011).

The β -LG also forms complexes with catecholamines, in particular, with arachidonoyl adrenalin (AA-AD) and arachidonoyl noradrenalin (AA-NOR) (Gholami et al., 2015). These interactions were investigated using chemometric analysis, molecular docking and molecular dynamics simulation. Methods. It has been established that hydrogen bonds, hydrophobic bonds, and Van der Waals interactions play an important role in the formation of these complexes. AA-AD and AA-NOR bind to the β -LG in the calyx binding site with the participation of polar residues (Asn, Ser, Met) and hydrophobic residues (Leu, Val, Ile, Ala). Due to the high structural flexibility of catecholamines, the number of bonds with β -LG is also large. The formation of these complexes is important, because it is known that AA-AD and AA-NOR perform a number of important physiological functions: control of the excitability of the heart, control of blood pressure, vasodilation, respiration, etc.

In addition, numerous studies have demonstrated the interaction of β -LG with plant polyphenols, which represent a class of secondary metabolites with antioxidant, anti-inflammatory and antitumor activities and used for the prevention of cardiovascular, cerebrovascular, neurodegenerative diseases, etc. (Xie and Zhou, 2017; Yeh et al., 2017). In particular, it was shown that β -LG interacts with four polyphenols (resveratrol, theaflavin, chlorogenic acid and delphinidin-3-O-glucoside) through hydrophobic and hydrophilic forces (Xu et al., 2019a, 2019b). Moreover, the conformation of β -LG had changed and both β -sheet and α -helix had increased (Kanakis et al., 2011). In addition, β -LG can be used to transfer the main component of the catechin-Epigallocatechin gallate (EGCG) into the intestine, which has antioxidant and anticancer properties (Bing et al., 2013; Shin et al., 2016; Liang et al., 2017; Rady et al., 2018). However, due to instability and biodegradation in the gastrointestinal environment, as well as low bioavailability, the development of functional food products and treatment based on EGCG is a difficult task (Yang et al., 2016). In these studies, β -LG nanoparticles were used in combination with chitosan-aminosugar, a linear polysaccharide derived from chitin. Chitosan nanoparticles (CS NPs) have good absorption by the intestinal mucosa and permeability, which increases the absorption of drugs carried by CS NPs in the intestine. However, the molecular structure of chitosan is sensitive to acidic pH values, which reduces the bioavailability of drugs when taken orally (Schütz et al., 2011). EGCG loaded CS NPs coated

with or without β -LG were synthesized by ionic cross-linking method. The size of the nanoparticles of the EGCG-CS/ β -LG design turned out to be smaller in size and had better encapsulation efficiency compared to nanoparticles without β -LG. The presence of β -LG contributed to a decrease in the level of EGCG release in the stomach by approximately 60–75% and an increase in the stability of the antioxidant activity of EGCG (Dai et al., 2020).

Recently, the dynamic mechanism of the interaction of tea polyphenol “catechin” with the central β -LG cavity through hydrophobic interactions has been described, which is accompanied by modifications of the secondary and tertiary structures of β -LG (Al-Shabib et al., 2020). There are several types of catechins: epicatechin, epicatechin-3-gallate, epigallocatechin-3-gallate, and elagic acid (Naghma and Hasan, 2019). All these polyphenols possess anticancer properties and are known as important chemoprophylactic products, the consumption of which was also associated with a reduced risk of stroke, coronary heart disease (Shah et al., 2010; Du et al., 2013), and are also powerful antioxidants (Mao et al., 2019).

When comparing the affinity of a yellow-orange pigment from the group of carotenoids beta-carotene (β -C) with whey proteins, the order of affinity of whey proteins to (β -C) was established. Thus, the β -LG can serve as a suitable carrier for β -C (Allahdad et al., 2019).

It is also known that β -LG interacts with various vitamins. It has been shown that binding and transferring vitamin A, which plays an important role in a number of physiological functions, is necessary for stimulating the growth and proper development of tissues, for the normal differentiation of epithelial tissue, etc. It was demonstrated that the binding of retinol by β -Lg quenches the fluorescence of Trp 194 positioned at the bottom of the calyx (Mensi et al., 2013). Studies of the interactions of β -LG with ascorbic, folic acids and vitamin K3 (a synthetic form of fat-soluble vitamin K) using spectroscopic methods together with molecular docking techniques revealed that these substances significantly damped the fluorescence of β -Lg in static (ascorbic and folic acids) or in the combination of static-dynamic combined quenching (VK3) mode. Binding of β -Lg to these three compounds causes its conformational changes. The results of thermodynamic studies revealed that folic acid interacts with β -Lg mainly through hydrophobic interactions, while the other two combinations are due to hydrogen bonds and Van der Waals interactions (Shahraki et al., 2018). Another equally important investigation was the study of the complex of β -Lg with vitamin D3, the main biological function of which is to maintain the concentration of calcium and phosphorus in the blood serum. Some studies have shown that high serum vitamin D3 concentrations are associated with a decrease in the levels of breast, ovarian, prostate and colorectal cancer, as well as a reduced risk of developing multiple sclerosis (Vieth, 2001). It has been found that at pH = 2.5 approximately one molecule of vitamin D3 binds to the monomeric form of β -Lg, this interaction is rather weak, while with a decrease in ionic strength the binding strength increased to two D3 molecules per monomer, most likely by expanding access to the ligand (Forrest et al., 2005). At pH = 6.6 and 8.0, one molecule of vitamin D3 binds to β -Lg in the central cavity, and the other molecule is densely distributed between the monomers and is connected with them by hydrophobic interactions. Near-UV CD spectra suggested that vitamin D3 was positioned in close proximity to a phenylalanine residue (Phe 82, 105, 136, or 151), binding is believed to have occurred near Phe105 located in the interior of the calyx (Zsila et al., 2002). Recently, a study of the interaction of this serum protein with water-soluble vitamin B12 has been presented, which does not affect the secondary and tertiary structures of β -LG (Swain et al., 2020).

General data characterizing the β -LG as a nanotransporter of some ligands are presented in Table 1.

Table 1

Characteristics of beta-lactoglobulin ligands and advantages of beta-lactoglobulin as a nanotransporter.

Ligand name	Mechanism of action of the ligand	Ligand disadvantages	The effect of interacting with β -lactoglobulin
Irinotecan (Camptosar)	It is camptothecin cytotoxic drugs, which activates by hydrolysis to SN-38. It is an inhibitor of topoisomerase I. It contributes to damage to a double strand of DNA formed during its synthesis	pH-dependent rapid and reversible hydrolysis	Maximum degree of irinotecan release in colon. The treatment of colorectal cancer, ovarian cancer, and small cell lung cancer is increasing
3,3'-diindolylmethane (DIM)	It is a compound derived from the digestion of indole-3-carbinol, found in cruciferous vegetables. It is capable of inducing the expression of the CYP1A1 gene	Low stability and poor oral bioavailability	Increase stability and oral bioavailability of DIM, significantly inhibits the proliferation of breast cancer cells
Glabridin	It is a bio-available isoflavan isolated from licorice (<i>Glycyrrhiza glabra</i> L.) root extract. It has various biological activities including strong antioxidant activity, antioxidant against LDL oxidation, anti- <i>Helicobacter pylori</i> properties, estrogen-like activity and antinephritic and radical scavenging activities	Low solubility in water, low bioavailability and incomprehensible stability	Facilitate transport and preservation of bioactivity of glabridin, as well as the ability of this complex to absorb DPPH radicals
Astaxanthin (ATX) and 5-fluorouracil (5-FU) complex	ATX is one of the strongest antioxidants, it increases the stability of cell membranes, preventing the penetration of substances that promote lipid peroxidation through the lipid layer, and can also provide additional protection against damage caused by free radicals. 5-FU is antitumor drug from the group of antimetabolites, pyrimidine antagonists. It inhibits the process of cell division by blocking DNA synthesis (due to inhibition of the activity of the enzyme thymidylate synthetase) and the formation of structurally imperfect RNA (due to the introduction of fluorouracil into its structure)	High toxicity of the 5-FU drug, the complexity of joint simultaneous delivery of ATX and 5-FU	Reducing the toxicity of the 5-FU and side effects of the drug, targeted delivery of the ATX and 5-FU complex to the tumor at the same time, maintaining the effective concentration of the drug
Dendritic mesoporous silica nanoparticles (DMSNs) and insulin complex	Insulin hormone of protein nature, is formed in the beta cells of the islets of the Langerhans of the pancreas. It regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of glucose from the blood into liver, fat and skeletal muscle cells	Degradation at low pH	Insulin protection from early release/degradation in the stomach at low pH, targeted delivery of insulin to the intestine and its full release
DAB-1-small aminobenzoic acid derivative	It has anti-inflammatory and anticancer activities. It efficiently inhibits cytokine-induced expression of pro-inflammatory genes and motility in human M1 macrophage and inhibits the production of NO in murine urothelial bladder carcinoma cells and macrophages	Instability and lack of targeted delivery	Improving targeting while decreasing the side effects of drugs
Catechin– Epigallocatechin gallate (EGCG) and Chitosan nanoparticles (CS NPs) complex and other polyphenols	It is the ester of epigallocatechin and gallic acid, and is a type of catechin. It has antioxidant, anti-inflammatory, antitumor properties. Chitosan is considered as a harmless material as it is natural polymer that possesses biodegradable and biocompatible properties.	Instability and biodegradation in the gastrointestinal environment, low bioavailability	The level of release of EGCG in the stomach decreases by approximately 60–75% and stability and antioxidant activity of EGCG increase
Selenium	Is an essential micronutrient, which exerts its biological functions through selenoproteins. Se, selenium-containing compounds of organic and inorganic nature, as well as selenoproteins, have pronounced antioxidant and antitumor activities	Although selenium is an essential trace element, it is toxic. Concentrations of selenium causing apoptosis of cancer cells are often fatal to normal cells.	An increase in the cytotoxicity of cancer cells compared with selenium-containing agents without β -LG, while inhibition of normal cell proliferation was not observed. The targeted delivery of selenium to the tumor is carried out
Palladium (II) (oxali-palladium complex)	Palladium complexes are capable of interacting with important biological molecules, for example, with sulfur-containing peptides, and possess chemotherapeutic and antitumor potential	Some palladium (II) complexes exhibit marked in vitro cytotoxicity	Targeted delivery of palladium-based drugs, reducing its cytotoxicity for normal cells
Catecholamines: arachidonoyl adrenalin (AA-AD) and arachidonoyl noradrenalin (AA-NOR)	AA-AD and AA-NOR perform a number of important physiological functions: control of the excitability of the heart, control of blood pressure, vasodilation, respiration, etc. Arachidonoyl adrenalin (AA-AD) and arachidonoyl noradrenalin (AA-NOR) are closely related to endocannabinoid/endovanilloid N-arachidonoyl dopamine.	Circulating catecholamines are oxidized to aminochromes, which causes a number of disorders: coronary spasm, myocardial cell damage, depletion of high energy reserves and ventricles arrhythmias, which can lead to sudden cardiac death.	Protection of catecholamines from oxidation and improving their bioavailability
Naringin	It is a plant citrus flavonoid. It inhibits some drug-metabolizing cytochrome P450 enzymes, including CYP3A4 and CYP1A2, which may result in drug-drug interactions. This flavonoid	Low bioavailability	Increased bioavailability and retention of this flavonoid in the gastrointestinal tract

(continued on next page)

Table 1 (continued)

Ligand name	Mechanism of action of the ligand	Ligand disadvantages	The effect of interacting with β -lactoglobulin
Vitamins: vitamin A vitamin D3 vitamin K3 and others	helps strengthen the immune system by increasing the activity of catalase, superoxide dismutase, paraoxonase and other antioxidants Vitamin A performs many biochemically important functions in humans and animals. Retinal is a component of rhodopsin - the main visual pigment. In the form of retinoic acid, the vitamin stimulates growth and development. Retinol is a structural component of cell membranes, provides antioxidant protection of the body Vitamin D3-is organic compound, fat soluble. It participates in the regulation of calcium-phosphorus metabolism, enhances the absorption of calcium in the intestines and phosphorus reabsorption in the renal tubules, normalizes the formation of the bone skeleton and teeth in children, and helps to preserve the structure of bones. Vitamin K3-is polycyclic aromatic ketone, promotes the synthesis of prothrombin and proconvertin, increases blood coagulability due to increased synthesis of II, VII, IX, X coagulation factors, has a hemostatic effect, stimulates substrate K-vitamin reductase, activating vitamin K and ensuring its participation in the hepatic synthesis of K-vitamin-dependent plasma hemostasis factors	Low bioavailability and stability,	Improving the bioavailability of vitamins, their stability, targeted hinge

3. β -LG—the main protein of milk plasma and the strongest allergen

β -LG—sulfur-containing protein, which was first isolated from cow's milk in crystalline form in 1934 (Palmer, 1934). Currently, 11 β -LG alleles are known, designated A, B, C, D, E, F, G, H, X, Dr. and W of which A and B are dominant. Various studies have shown that A and B variants of β -LG can affect the composition and properties of milk. However, many researchers do not agree on which of the β -LG alleles (A or B) unambiguously affects the biochemical and technological characteristics of milk. Cows with genotype AA of β -LG were slightly superior to cows with genotypes AB and BB in milk yield and milk composition, with the exception of high protein content. The yield of protein, fat, and solids was also higher in cows with the AA genotype, which was confirmed by previously obtained data (Padilla et al., 2018; Kyselová et al., 2019). To determine the polymorphism of β -LG genes and to evaluate economically useful traits in animals with different β -LG genotypes, we previously optimized the PCR-RFLP protocol and found that milk of cows carrying the β -LG gene in the B allele in their genome had the highest yield of the desired dense rennet clot (80%) and the shortest coagulation duration (27.3 min), while milk of cows with the β -LG allele in its genome has the greatest ability to withstand high-temperature exposure (58.9 min) (Valiullina et al., 2007; Ahmetov et al., 2010).

The presence of different forms of bovine is the result of missense polymorphisms occurring in one gene, whereas in a horse, a donkey, and a dog, two different forms of β -LG are encoded by two paralogous genes LGB1 and LGB2 (Brinkmann et al., 2016), which are located on the 25th chromosome. The mean level of β -LG in mare's milk is 2.55 g kg⁻¹, which is generally lower than in cow's milk (~ 3.2 g kg⁻¹) (Uniacke-Lowe et al., 2010). The content of β -LG in the milk of other farm animals is: goats – 3.02 g/l, sheep – 4.78 g/l, in mare's milk – 2.6 g/l and sheep – 4.13 g/l. Camel milk, according to the studied criterion, is close to human milk and also does not contain β -LG (Pastuszka et al., 2016). At the same time, the content of β -LG can vary greatly within a species between cattle breeds. The most voluminous studies on this issue were carried out on cattle, which is associated with

its maximum prevalence. So in the most numerous breed—Holstein black-and-white, this indicator is 3.2 g / l, which is close to the Jersey breed with 3.25 g/l, (Litwińczuk et al., 2011) but is much inferior to red cattle breeds with an indicator of 5.9 g/l and simmental cow—4.01 g/l (Maurmayr et al., 2018). When screening polymorphisms in the structural parts (exons and introns) and 5'-flanking regions of the horse LGB1 and LGB2 genes, 83 polymorphic regions were revealed, 47 of which were localized in the LGB1 gene, but all missense SNPs were found exclusively in the sequence encoding the gene LGB2 (Wodas et al., 2020).

Among serum proteins, β -LG is the only polymorphic protein. It is believed that this protein, like other protein fractions of serum, is not amenable to coagulation, and therefore they are absent in the cheese mass. However, the genetic types of this protein can influence the process of isolating whey from a casein clot and thereby improve the quality of the cheese mass. Previously, we found that the cow genotype for the β -LG gene has a certain effect on both the state of the casein clot and the duration of milk coagulation. In all groups of cows, a negative dependence of the technological properties of milk on the β -LG allele A was revealed. From milk of 42.5–48.0% of cows with genotype AB and AA, a casein clot of a loose and flabby state was obtained. In addition, our studies showed that cows with the BB genotype had the best cheese-making properties of milk, they have the highest yield of a desirable dense rennet and the shortest milk coagulation time. According to these indicators, they exceeded animals with the AA genotype. Cows carrying the allelic variant A of the β -LG were superior to analogues with the homozygous β -LG BB genotype in thermal stability of milk (Valiullina et al., 2007; Ahmetov et al., 2010). As is known, thermostability of milk is an important technological property, showing its suitability for high-temperature processing, and reflects its ability to maintain its original colloidal properties when exposed to high temperatures.

However, β -LG can cause allergies in infants. So, according to the list of the official World Health Organization, 11 bovine milk allergens are classified (Ganguly et al., 1980), among them caseins (Bos d8) and β -LG (Bos d5) are the strongest allergens. It is known that β -LG occurs in a dimeric form, represented by two chains A and B, with a total

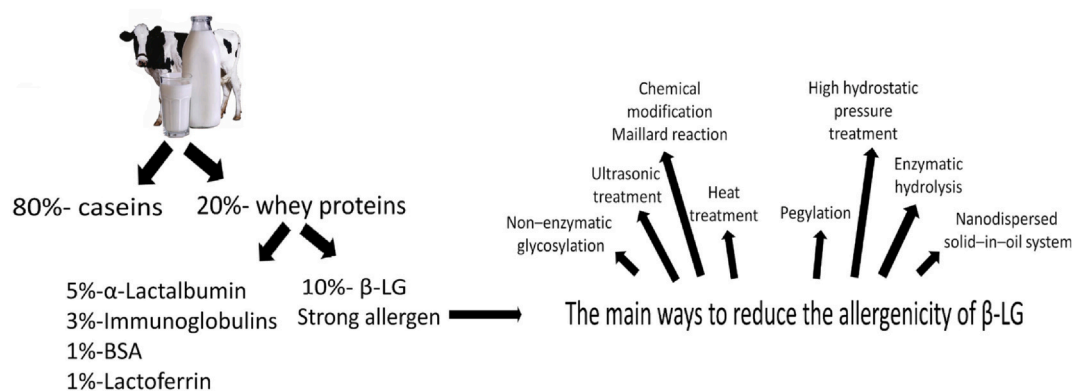


Fig. 1. The main ways to reduce the allergenicity of beta-lactoglobulin

molecular weight of 36 kDa.

4. Ways to reduce the allergenicity of β-lactoglobulin

The molecular basis for allergenic changes the potential of proteins is the destruction of antigenic epitope regions or the formation of new ones, as well as facilitating access to previously hidden epitopes as a result of denaturation native allergen. The main approaches to reduce the allergenicity of milk-based products are: heating, high pressure treatment, chemical modification (Maillard reaction), enzymatic hydrolysis. The combination of physical effects, in particular thermal denaturation, and proteolysis seems to be the most acceptable way to reduce the antigenic properties of serum protein. The main mechanisms and ways to reduce the allergenicity of β-LG are shown in Fig. 1.

It has been shown that dimeric β-LG reversibly dissociates into monomers at 30–55 °C, with a change in protein conformation, namely, the position of the EF loop (Asp85-Asn90) changes. This is accompanied by exposure of latent carboxyl groups (Glu79 and Tyr42) and free cysteine (Cys121) is able to enter into intermolecular reactions (Oliveira et al., 2001). Partially irreversible thermal denaturation of β-LG proceeds in two phases and depends on the concentration of β-LG, salts, sugars, pH and ionic strength of the solution. The first phase of denaturation established at 58–60 °C (pH 6.4) and mainly affects α-helices. Reversible structural changes on the surface of the globule in the region of Lys8-Tyr19 (β-folded layer) are shifted to Thr125-Lys135 (α-helix) (Casal et al., 1988).

Modifications of proteins caused by high hydrostatic pressure are based on the compressibility of molecules and a change in their volume, which leads to an equilibrium shift in favor of the state with the lowest total volume. The pressure has a destructive effect on the quaternary (< 150 MPa) and the tertiary (> 200 MPa) structure of most globular proteins, but causes a relatively small effect on the secondary structure (> 300–700 MPa). Protein denaturation involves the dissociation of oligomeric proteins, unfolding and aggregation, while the covalent bonds of the protein remain unaffected (Jonas, 2002; Lullien-Pellerin and Balny, 2002; López-Fandiño, 2006). According to fluorescence analysis as a result of exposure to pressure 100–200 MPa in the β-LG molecule important structural changes occur; in the same time a further increase in pressure does not affect the quantum yield of fluorescence. Pressure-induced β-LG unfolding causes an increase in the surface hydrophobicity of the protein globule, which leads to aggregation monomers, and the unfolding occurs more intensively and irreversibly at higher pH values (Stapelheldt et al., 1996; Ikeuchi et al., 2001).

The chemical modification of the β-LG in the Maillard reaction is accompanied by the formation of a covalent bond between the sugars and the γ-amino group of the Lys residues of the protein, after which condensation reactions occur and polymerizations leading to the formation of melanoidins—brown pigments. Features of IgE binding from sera of patients with allergies to cow milk with glycosylated β-LG. It

was shown that heat treatment β-LG, leading to the loss of the secondary and tertiary structure, causes a decrease in the IgE binding capacity of whey protein. A high degree of glycosylation has a pronounced masking effect in the interaction of antibodies with protein, which indicates the importance of Lys γ-amino groups in the recognition of antigenic epitopes by immunoglobulins.

One of the modern approaches to reduce the allergenicity of β-LG is its pegylation—covalent modification with fragments of polyethylene glycol (PEG). The specific conjugation of β-LG with PEG for glycine residues in two positions (Gln155 and Gln159) reduced the antigenicity of β-LG from 72.2 µg/ml to 22.7 µg/ml, which can be caused by structural changes and steric hindrance effect of PEG. However, according to the results of circular dichroism and fluorescence analysis, no conformational changes in β-LG were observed. Therefore, it is most likely that the decrease in protein antigenicity is caused by the steric hindrance effect of PEG, i.e. the flexible and bulky PEG chain interfered with the binding of β-LG to specific antibodies. An advantage of this approach as compared, for example, with glycation or random pegylation, is a significant decrease in the antigenicity of the protein without significant changes in its conformation (Luo et al., 2019).

Protein glycation (non-enzymatic glycosylation) is a reaction between reducing carbohydrates and free amino groups of proteins without enzymes. It was shown that the use of the modern high-energy c-trap dissociation (HCD) method allows accurate identification of glycosylated sites and the degree of glycation of each point. The determination of glycosylated adducts and changes in IgG / IgE binding ability using this approach was also applied to β-LG (Yang et al., 2020). It was shown that this spray drying technology at 120 °C contributes to minor changes in the structure of β-LG, but at the same time it did not affect the IgG/IgE binding ability. Spray drying at a higher temperature (180 °C) contributed to a decrease in binding to IgG/IgE, possibly due to the aggregation of protein molecules, which is most likely due to the reduced availability of some epitopes initially located on the surface of this protein. Under such conditions, seven glycation sites are found in β-LG.

Another method for reducing the allergenicity of β-LG is the nanodispersed solid-in-oil (S/O) system (Kitaoka et al., 2020). The S/O nanodispersion system contains protein nanoparticles coated with surfactant molecules with a low hydrophilic-lipophilic balance (HLB) (< 2) and an oil carrier compatible with ligands in the surface layer of the skin (Kitaoka et al., 2014). The S/O nanodispersion system containing β-LG and obtained using sucrose laurate L-195 had HLB = 1 (Kitaoka et al., 2020). After the treatment of whey-sensitized mice with S/O nanodispersions carrying the antigen β-LG, the level of total IgE was reduced compared to the control, the skewing of the immune reaction towards Th1-type immunity and a decrease in ear swelling were observed in such mice. This method is quite simple to use and is a promising approach for immunotherapy.

5. Discussion

Among genes that control milk productivity and its quality, one can single out a group of major genes that contribute most to the formation and functioning of these quantitative traits. These genes include milk protein genes: beta-lactoglobulin and kappa-casein.

The β -LG gene is responsible for milk protein, as well as for a property such as the biological value of milk. There are several genetic variants of β -LG, the most common of which are the A and B variants, which differ from each other in two amino acids at positions 64 (the remainder of aspartic acid in variant A, glycine in variant B) and 118 (the residue of valine in variant A and Alanine in option B). It is known that milk from cows with genotype BB of the β -LG gene contained less protein than milk from cows with genotype AA and AB, and the yield of milk fat from milk from cows with genotype BB was less than from milk from cows with genotypes AA and AB. Milk of cows carrying the B allele of β -LG gene in their genome has the highest yield of the desired dense rennet and the shortest coagulation time. At the same time, the milk of cows with the A-allele in their genome has the greatest ability to withstand high-temperature effects.

At the same time, this protein is the strongest allergen, currently there are a number of approaches to reduce its allergenicity. The most common of them are: pegylation-covalent modification with polyethylene glycol (PEG) fragments (Luo et al., 2019), glycation or non-enzymatic glycosylation (Yang et al., 2020), use of nanodispersed systems (Kitaoka et al., 2014; Kitaoka et al., 2020).

However, in recent years, this protein has become increasingly interesting as a nanotransporter of various nature ligands, capable of delivering them with the least loss to the intestinal cavity, bypassing the acidic environment of the stomach, which is harmful for many of them. This is a globular protein (18.4 kDa, 162 aa), belonging to the family of lipocalins, a group of transport proteins with a characteristic secondary structure, containing eight antiparallel peptide sequences in the β -folded structure that form the cylinder (Flower, 1996; Albani et al., 2014). Such a cylinder contains an inside ligand-binding site.

It has been shown that β -LG can deliver directly to the intestine the widespread drug irinotecan with high antitumor activity, especially in the treatment of intestinal cancer (Potmesil, 1994; Le et al., 2012; Bijari et al., 2019), 3,3'-diindolylmethane (DIM)-bioactive substance related to indoles capable of inducing the expression of the CYP1A1 gene (Cytochrome P450, family1, subfamily A, popypeptide1), which significantly inhibits the proliferation of breast cancer cells (Reed et al., 2008; Wang et al., 2019), various fatty acids (Frapin et al., 1993; Beneventi et al., 2001; Collini et al., 2003; Lide, 2003; Loch et al., 2012; Loch et al., 2013), polyphenols (Kanakakis et al., 2011; Xie and Zhou, 2017; Yeh et al., 2017; Xu et al., 2019a, 2019b), vitamins (Vieth, 2001; Mensi et al., 2013; Shahraki et al., 2018), selenium-containing compound (Ji et al., 2019; Xu et al., 2019a, 2019b; Zhao et al., 2019), and others.

Thus, this review allows us to understand that beta-lactoglobulin is not just an important protein of milk, but due to its physicochemical properties and structure, it can be considered as it as a reliable carrier of drugs directly into the intestine, which opens up the prospects for its practical applications in medicine and pharmaceuticals.

Acknowledgements

The article was written on the subject of state assignment No. 075-01250-20-01 and with the support of a grant of the Presidium of the Russian Academy of Sciences "Postgenomic technologies and promising solutions in biomedicine".

References

Ahmetov, T.M., Tjulkin, S.V., Zaripov, O.G., 2010. Beta-Lactoglobulin Gene Polymorphism in Livestock Herds. Scientific Notes of Kazan State Academy of

- Veterinary Medicine Named after N.E. Bauman. vol. 202. pp. 36–41.
- Albani, J.R., Vogelaer, J., Bretschke, L., Kmiecik, D., 2014. Tryptophan 19 residue is the origin of bovine β -lactoglobulin fluorescence. J. Pharm. Biomed. Anal. 91, 144–150.
- Allahdad, Z., Varidi, M., Zadarmad, R., Saboury, A.A., Haertle, T., 2019. Binding of β -carotene to whey proteins: multi-spectroscopic techniques and docking studies. Food Chem. 277, 96–106.
- Al-Shabib, N.A., Khan, J.M., Malik, A., Tabish Rehman, M., AlAjmi, M.F., Husain, F.M., Hisamuddin, M., Altwaijry, N., 2020. Molecular interaction of tea catechin with bovine β -lactoglobulin: a spectroscopic and in silico studies. Saudi Pharm. J. 28, 238–245.
- Bach, S.B.H., Sepeda, T.G., Merrill, G.N., Walmsley, J.A., 2005. Complexes of dibromo (ethylenediamine)-palladium(II) observed from aqueous solutions by electrospray mass spectrometry. J. Am. Soc. Mass Spectrom. (9), 1461–1469.
- Belinky, P.A., Aviram, M., Fuhrman, B., Rosenblat, M., Vaya, J., 1998. The antioxidative effects of the isoflavan glabridin on endogenous constituents of LDL during its oxidation. Atherosclerosis 137, 49–61.
- Beneventi, D., Carré, B., Gandini, A., 2001. Precipitation and solubility of calcium soaps in basic aqueous media. J. Colloid Interface Sci. 231, 142–144.
- Bijari, N., Ghobadi, S., Derakhshandeh, K., 2019. β -lactoglobulin-irinotecan inclusion complex as a new targeted nanocarrier for colorectal cancer cells. Res. Pharm. Sci. 14, 216–227.
- Bing, H., Yuwen, T., Xiaoxiong, Z., Qingrong, H., 2013. Bioactive peptides/chitosan nanoparticles enhance cellular antioxidant activity of (–)-epigallocatechin-3-gallate. J. Agric. Food Chem. 61, 875–881.
- Bouhallab, S., Henry, G., Caussin, F., Croguennec, T., Fauquant, J., Mollé, D., 2004. Copper-catalyzed formation of disulfide-linked dimer of bovine β -lactoglobulin. Lait 84, 517–525.
- Boye, J.I., Ma, C.Y., Ismail, A., 2004. Thermal stability of β -lactoglobulins a and B: effect of SDS, urea, cysteine and N-ethylmaleimide. J. Dairy Res. 71, 207–215.
- Brinkmann, J., Jagannathan, V., Drögemüller, C., Rieder, S., Leeb, T., Thaller, G., Tetens, J., 2016. DNA-based analysis of protein variants reveals different genetic variability of the paralogous equine β -lactoglobulin genes LGB1 and LGB2. Livest. Sci. 187, 181–185.
- Brownlow, S., Cabral, J.H.M., Cooper, R., Flower, D.R., Yewdall, S.J., Polikarpov, I., North, A.C., Sawyer, L., 1997. Bovine beta-lactoglobulin at 1.8 angstrom resolution—still an enigmatic lipocalin. Structure 5, 481–495.
- Burova, T.V., Choiset, Y., Tran, V., Haertle, T., 1998. Role of free Cys121 in stabilization of bovine beta-lactoglobulin B. Protein Eng. 11, 1065–1073.
- Cai, X., Wang, C., Yu, W., Fan, W., Wang, S., Shen, N., Wu, P., Li, X., Wang, F., 2016. Selenium exposure and cancer risk: an updated meta-analysis and meta-regression. Sci. Rep. 20, 19213.
- Casal, H.L., Köhler, U., Mantsch, H.H., 1988. Structural and conformational changes of beta-lactoglobulin B: an infrared spectroscopic study of the effect of pH and temperature. Biochim. Biophys. Acta 957, 11–20.
- Chanphai, P., Cloutier, F., Oufqir, Y., Leclerc, M.F., Eiján, A.M., Reyes-Moreno, C., 2020. Biomolecular study and conjugation of two paraaminobenzoic acid derivatives with serumproteins: drug binding efficacy and protein structural analysis. J. Biomol. Struct. Dyn. 3, 1–12.
- Collini, M., D'Alfonso, L., Molinari, H., Ragona, L., Catalano, M., Baldini, G., 2003. Competitive binding of fatty acids and the fluorescent probe 1–8-anilinoanthracene sulfonate to bovine beta-lactoglobulin. Protein Sci. 12, 1596–1603.
- Dai, W., Ruan, C., Sun, Y., Gao, X., Liang, J., 2020. Controlled release and antioxidant activity of chitosan and β lactoglobulin complex nanoparticles loaded with epigallocatechin gallate. Colloids Surf. B: Biointerfaces 188, 110802.
- Derakhshandeh, K., Erfan, M., Dadashzade, S., 2007. Encapsulation of 9-nitrocamptothecin, a novel anticancer drug, in biodegradable nanoparticles: factorial design, characterization and release kinetics. Eur. J. Pharm. Biopharm. 66, 34–41.
- Drengler, R.L., Kuhn, J.G., Schaaf, L.J., Rodriguez, G.I., Villalona-Calero, M.A., Hammond, L.A., Stephenson Jr., J.A., Hodges, S., Kraynak, M.A., Staton, B.A., et al., 1999. Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. J. Clin. Oncol. 17, 685–696.
- Du, G.J., Wang, C.Z., Qi, L.W., 2013. The synergistic apoptotic interaction of panaxadiol and epigallocatechingallate in human colorectal cancer cells. Phytother. Res. 27, 272–277.
- Euston, S.R., 2013. Molecular dynamics simulation of the effect of heat on the conformation of bovine β -lactoglobulin a: a comparison of conventional and accelerated methods. Food Hydrocoll. 30, 519–530.
- Fassberg, J., Stella, V.J., 1992. A kinetic and mechanistic study of the hydrolysis of camptothecin and some analogues. J. Pharm. Sci. 81, 676–684.
- Feng, T., Wang, K., Liu, F., Ye, R., Zhu, X., Zhuang, H., Xu, Z., 2017. Structural characterization and bioavailability of ternary nanoparticles consisting of amylose, α -linoleic acid and β -lactoglobulin complexed with naringin. Int. J. Biol. Macromol. 99, 365–374.
- Flower, D.R., 1996. The lipocalin protein family: structure and function. Biochem. J. 318, 1–14.
- Forrest, S.A., Yada, R.Y., Rousseau, D., 2005. Interactions of vitamin D3 with bovine beta-lactoglobulin a and beta-casein. J. Agric. Food Chem. 53, 8003–8009.
- Frapin, D., Dufour, E., Haertle, T., 1993. Probing the fatty acid binding site of beta-lactoglobulins. J. Protein Chem. 12, 443–449.
- Fukai, T., Satoh, K., Nomura, T., Sakagami, H., 2003. Preliminary evaluation of antinephritis and radical scavenging activities of glabridin from Glycyrrhiza glabra. Fitoterapia 74, 624–629.
- Ghalandari, B., Divsalar, A., Saboury, A., Haertle, T., Parivar, K., Bazl, R., Eslami-Moghadam, M., Amanlou, M., 2014. Spectroscopic and theoretical investigation of oxali-palladium interactions with b-lactoglobulin. Spectrochim. Acta A Mol. Biomol. Spectrosc. 118, 1038–1046.

- Gholami, S., Bordbar, A.K., Akvan, N., Parastar, H., Fani, N., Gretskeya, N.M., Bezuglov, V.V., Haertlé, T., 2015. Binding assessment of two arachidonic-based synthetic derivatives of adrenalin with β -lactoglobulin: molecular modeling and chemometrics approach. *Biophys. Chem.* 207, 97–106.
- Gholami, H., Divsalar, A., Abbasalipourkabir, R., Ziamajidi, N., Saeidifar, M., 2020. The simultaneous carrier ability of natural antioxidant of astaxanthin and chemotherapeutic drug of 5-fluorouracil by whey protein of β -lactoglobulin: spectroscopic and molecular docking study. *J. Biomol. Struct. Dyn.* 5, 1–13.
- Godovac-Zimmermann, J., Krause, I., Baranyi, M., Fischer-Frühholz, S., Suszczak, J., Erhardt, G., Buchberger, J., Klostermeyer, H., 1996. Isolation and rapid sequence characterization of two novel bovine β -lactoglobulins I and J. *J. Protein Chem.* 15, 743–750.
- Halder, U.C., Chakraborty, J., Das, N., Bose, S., 2012. Tryptophan dynamics in the exploration of micro-conformational changes of refolded β -lactoglobulin after thermal exposure: a steady state and time-resolved fluorescence approach. *J. Photochem. Photobiol. B Biol.* 109, 50–57.
- Hamelin-Morrisette, J., Cloutier, S., Girouard, J., Belgorosky, D., Eijian, A.-M., Legault, J., Reyes Moreno, C., Bérubé, G., 2015. Identification of an anti-inflammatory derivative with anti-cancer potential: the impact of each of its structural components on inflammatory responses in macrophages and bladder cancer cells. *Eur. J. Med. Chem.* 96, 259–268.
- Houghton, P.J., Stewart, C.F., Zamboni, W.C., Thompson, J., Luo, X., Danks, M.K., 1996. Schedule-dependent efficacy of camptothecins in models of human cancer. *Ann. N. Y. Acad. Sci.* 803, 188–201.
- Ikeuchi, Y., Nakagawa, K., Endo, T., Suzuki, A., Hayashi, T., Ito, T., 2001. Pressure-induced denaturation of monomer β -lactoglobulin is partially reversible: comparison of monomer form (highly acidic pH) with dimer form (neutral pH). *J. Agric. Food Chem.* 49, 4052–4059.
- Jellinck, P.H., Makin, H.L., Sepkovic, D.W., Bradlow, H.L., 1993. Influence of indole carbinols and growth hormone on the metabolism of 4-androstenedione by rat liver microsomes. *J. Steroid Biochem. Mol. Biol.* 46, 791–798.
- Ji, H.Y., Yu, J., Liu, A.J., 2019. Seleno- β -lactoglobulin (Se- β -Lg) induces mitochondria-dependent apoptosis in HepG2 cells. *Mol. Biol. Rep.* 46, 5025–5031.
- Jonas, J., 2002. High-resolution nuclear magnetic resonance studies of proteins. *Biochim. Biophys. Acta* 1595, 145–159.
- Juère, E., Caillard, R., Marko, D., Del Favero, G., Kleitz, F., 2020. Smart protein-based formulation of dendritic mesoporous silica nanoparticles: toward oral delivery of insulin. *Chemistry* 26, 5195–5199.
- Kanakis, C.D., Hasni, I., Bourassa, P., Tarantilis, P.A., Polissiou, M.G., Tajmir-Riahi, H.-A., 2011. Milk β -lactoglobulin complexes with tea polyphenols. *Food Chem.* 127, 1046–1055.
- Kepler, J.K., Sonnichsen, F.D., Lorenzen, P.C., Schwarz, K., 2014. Differences in heat stability and ligand binding among β -lactoglobulin genetic variants A, B and C using (1) H NMR and fluorescence quenching. *Biochim. Biophys. Acta* 1844, 1083–1093.
- Kitaoka, M., Imamura, K., Hirakawa, Y., Tahara, Y., Kamiya, N., Goto, M., 2014. Sucrose laurate-enhanced transcutaneous immunization with a solid-in-oil nanodispersion. *Med. Chem. Commun.* 5, 20–24.
- Kitaoka, M., Xiao, W., Kong, Q., Tahara, Y., Kamiya, N., Goto, M., 2020. A solid-in oil Nanodispersion system for transcutaneous immunotherapy of Cow's Milk allergies. *Pharmaceutics* 12, E205.
- Kuznetsova, Y.P., Goltyshev, M.V., Gorbacheva, O.S., Novoselov, S.V., Varlamova, E.G., Fesenko, E.E., 2018. Influence of sodium selenite on the mRNA expression of the mammalian Selenocysteine-containing protein genes in testicle and prostate Cancer cells. *Dokl. Biochem. Biophys.* 480, 131–134.
- Kyselová, J., Ječmínková, K., Matějčková, J., Hanuš, O., Kott, T., Štípková, M., Krejčová, M., 2019. Physicochemical characteristics and fermentation ability of milk from Czech Fleckvieh cows are related to genetic polymorphisms of β -casein, κ -casein, and β -lactoglobulin. *Asian-Australas J. Anim. Sci.* 32, 14–22.
- Le Maux, S., Bouhallab, S., Giblin, L., Brodtkorb, A., Croguennec, T., 2014. Bovine β -lactoglobulin/fatty acid complexes: binding, structural, and biological properties. *Dairy Sci. Technol.* 94, 409–426.
- Le, M.S., Giblin, L., Croguennec, T., Bouhallab, S., Brodtkorb, A., 2012. β -Lactoglobulin as a molecular carrier of linoleate: characterization and effects on intestinal epithelial cells in vitro. *J. Agric. Food Chem.* 60, 9476–9483.
- Liang, J., Yan, H., Zhang, J., Dai, W., Gao, X., Zhou, Y., Wan, X., Puligundla, P., 2017. Preparation and characterization of antioxidant edible chitosan films incorporated with epigallocatechin gallate nanocapsules. *Carbohydr. Polym.* 171, 300–306.
- Lide, D., 2003. *Handbook of Chemistry and Physics*, 84th edition. CRC, London.
- Lipinski, B., 2016. Sodium selenite as an anti-cancer agent. *Anti Cancer Agents Med. Chem.* 17, 658–661.
- Litwińczuk, Z., Król, J., Brodziak, A., Barłowska, J., 2011. Changes of protein content and its fractions in bovine milk from different breeds subject to somatic cell coun. *J. Dairy Sci.* 94, 684–691.
- Loch, J., Polit, A., Gerocki, A., Bonarek, P., Kurpiewska, K., Dziedzicka-Wasylewska, Lewiński, K.M., 2011. Two modes of fatty acid binding to bovine β -lactoglobulin—crystallographic and spectroscopic studies. *J. Mol. Recognit.* 24, 341–349.
- Loch, J., Polit, A., Bonarek, P., Olszewska, D., Kurpiewska, K., Dziedzicka-Wasylewska, M., Lewiński, K., 2012. Structural and thermodynamic studies of binding saturated fatty acids to bovine β -lactoglobulin. *Int. J. Biol. Macromol.* 50, 1095–1102.
- Loch, J.I., Bonarek, P., Polit, A., Riès, D., Dziedzicka-Wasylewska, M., Lewiński, K., 2013. Binding of 18-carbonunsaturated fatty acids to bovine β -lactoglobulin—structural and thermodynamic studies. *Int. J. Biol. Macromol.* 57, 226–231.
- Loch, J.I., Molenda, M., Kopeć, M., Świątek, S., Lewiński, K., 2014. Structure of two crystal forms of sheep β -lactoglobulin with EF-loop in closed conformation. *Biopolymers* 101, 886–894.
- Loch, J., Bonarek, P., Lewiński, K., 2019. Conformational flexibility and ligand binding properties of ovine β -lactoglobulin. *Acta Biochim. Pol.* 66, 577–584.
- López-Fandiño, R., 2006. Functional improvement of Milk whey proteins induced by high hydrostatic pressure. *Crit. Rev. Food Sci. Nutr.* 46, 351–363.
- Lü, J., Zhang, J., Jiang, C., Deng, Y., Ötzen, N., Bosland, M.C., 2016. Cancer chemoprevention research with selenium in the post-SELECT era: promises and challenges. *Nutr. Cancer* 68, 1–17.
- Lullien-Pellerin, Balny, C., 2002. High pressure as a tool to study some proteins' properties: conformational modification, activity and oligomeric dissociation. *Innov. Food Sci. Emerg. Technol.* 3, 209–221.
- Luo, S., Lu, X., Liu, C., Zhong, J., Zhou, L., Chen, T., 2019. Site-specific PEGylation of β -lactoglobulin glutamine residues and its influence on conformation and antigenicity. *Food Res. Int.* 123, 623–630.
- Mamdouh, M.A., Monira, A., 2004. The influence of naringin on the oxidative state of rats with streptozotocin-induced acute hyperglycaemia. *Z. Naturforsch. C* 59, 726–733.
- Manderson, G.A., Hardman, M.J., Creamer, L.K., 1999. Effect of heat treatment on bovine β -lactoglobulin A, B, and C explored using thiol availability and fluorescence. *J. Agric. Food Chem.* 47, 3617–3627.
- Mao, X., Xiao, X., Chen, D., 2019. Tea and its components prevent cancer: a review of the redox-related mechanism. *Int. J. Mol. Sci.* 20, 5249.
- Maurmayr, A., Pegolo, S., Malchiodi, F., Bittante, G., Cecchinato, A., 2018. Milk protein composition in purebred Holsteins and in first/second-generation crossbred cows from Swedish Red, Montbeliarde and Brown Swiss bulls. *Animals* 12, 2214–2220.
- Mensi, A., Choiset, Y., Rabesona, H., Haertlé, T., Borel, P., Chobert, J.M., 2013. Interactions of β -lactoglobulin variants A and B with Vitamin A. Competitive binding of retinoids and carotenoids. *J. Agric. Food Chem.* 61, 4114–4119.
- Mercadante, D., Melton, L.D., Norris, G.E., Loo, T.S., Williams, M.A.K., Dobson, R.C.J., Jameson, G.B., 2012. Bovine β -lactoglobulin is dimeric under imitative physiological conditions: dissociation equilibrium and rate constants over the pH range of 2.5–7.5. *Biophys. J.* 103, 303–312.
- Naghma, K., Hasan, M., 2019. Tea polyphenols in promotion of human health. *Nutrients* 11, 39.
- Nerya, O., Vaya, J., Musa, R., Izrael, S., Ben-Arie, R., Tamir, S., 2003. Glabrene and isoliquiritigenin as tyrosinase inhibitors from licorice roots. *J. Agric. Food Chem.* 51, 1201–1207.
- O'Kennedy, B.T., Mounsey, J.S., Murphy, F., Pesquera, L., Mehra, R., 2006. Preferential heat-induced denaturation of bovine β -lactoglobulin variants as influenced by pH. *Milchwissenschaft-Milk Sci Int* 61, 366–369.
- Oliveira, K.M., Valente-Mesquita, V.L., Botelho, M.M., Sawyer, L., Ferreira, S.T., Polikarpov, I., 2001. Crystal structures of bovine β -lactoglobulin in the orthorhombic space group C222 (1). Structural differences between genetic variants A and B and features of the Tanford transition. *Eur. J. Biochem.* 268, 477–483.
- Padilla, P., Izquierdo, M., Martínez-Trancón, M., Parejo, J.C., Rabasco, A., Salazar, J., Padilla, J.A., 2018. Polymorphisms of α -lactalbumin, β -lactoglobulin and prolactin genes are highly associated with milk composition traits in Spanish Merino sheep. *Livest. Sci.* 217, 26–29.
- Palmer, A., 1934. The preparation of crystalline globulin from the albumin fraction of cow's milk. *J. Biol. Chem.* 104, 359.
- Pastuszka, R., Barłowska, J., Litwińczuk, Z., 2016. Allergenicity of milk of different animal species in relation to human milk. *Postępy Hig. Med. Dosw.* 70, 1451–1459.
- Potmesil, M., 1994. Camptothecins: from bench research to hospital wards. *Cancer Res.* 54, 1431–1439.
- Qin, B.Y., Bewley, M.C., Creamer, L.K., Baker, H.M., Baker, E.N., Jameson, G.B., 1998. Structural basis of the Tanford transition of bovine β -lactoglobulin. *Biochemistry* 37, 14014–14023.
- Rady, I., Mohamed, H., Rady, M., Siddiqui, I.A., Mukhtar, H., 2018. Cancer preventive and therapeutic effects of EGCG, the major polyphenol in green tea. *Egypt. J. Basic Appl. Sci.* 5, 1–23.
- Ramoutar, R.R., Brumaghim, J.L., 2010. Antioxidant and anticancer properties and mechanisms of inorganic selenium, Oxo-Sulfur, and Oxo-selenium compounds. *Cell Biochem. Biophys.* 58, 1–23.
- Reed, G.A., Sunega, J.M., Sullivan, D.K., Gray, J.C., Mayo, M.S., Crowell, J.A., Hurwitz, A., 2008. Single-dose pharmacokinetics and tolerability of absorption-enhanced 3,3'-diindolylmethane in healthy subjects. *Cancer Epidemiol. Biomark. Prev.* 17, 2619–2624.
- Sakurai, K., Goto, Y., 2006. Dynamics and mechanism of the Tanford transition of bovine β -lactoglobulin studied using heteronuclear NMR spectroscopy. *J. Mol. Biol.* 35, 6483–6496.
- Sakurai, K., Konuma, T., Yagi, M., Goto, Y., 2009. Structural dynamics and folding of β -lactoglobulin probed by heteronuclear NMR. *Biochim. Biophys. Acta* 1790, 527–537.
- Scalbert, A., Williamson, G., 2000. Dietary intake and bioavailability of polyphenols. *J. Nutr.* 130 2073S–85S.
- Schütz, C.A., Lucienne, J.J., Peter, K.U., Christine, W., 2011. Cell response to the exposure to chitosan-TPP//alginate nanogels. *Biomacromolecules* 12, 4153–4161.
- Shafaei, Z., Ghalandari, B., Vaseghi, A., Divsalar, A., Haertle, T., Saboury, A.A., Sawyer, L., 2017. β -Lactoglobulin: an efficient nanocarrier for advanced delivery systems. *Nanomedicine* 13, 1685–1692.
- Shah, Z.A., Li, R.C., Ahmad, A.S., 2010. The flavanol (–)-epicatechin prevents stroke damage through the Nrf2/HO1 pathway. *J. Cereb. Blood Flow Metab.* 30, 1951–1961.
- Shahraki, S., Heydari, A., Saeidifar, M., Gomroki, M., 2018. Biophysical and computational comparison on the binding affinity of three important nutrients to β -lactoglobulin: folic acid, ascorbic acid and vitamin K3. *J. Biomol. Struct. Dyn.* 36, 3651–3665.
- Shigemitsu, Z., Manabe, K., Hara, N., Baba, Y., Hosokawa, K., Kagawa, H., Watanabe, T., Fujimuro, M., 2017. Methylseleninic acid and sodium selenite induce severe

- ER–stress and subsequent apoptosis through UPR activation in PEL cells. *Chem. Biol. Interact.* 266, 28–37.
- Shin, Y.S., Kang, S.U., Ju, K.P., Yang, E.K., Kim, Y.S., Baek, S.J., Lee, S.H., Kim, C.H., 2016. Anti-cancer effect of (–)-epigallocatechin-3-gallate (EGCG) in head and neck cancer through repression of transactivation and enhanced degradation of β -catenin. *Phytomed. Int. J. Phytother. Phytopharmacol.* 23, 1344–1355.
- Stapelfeldt, H., Petersen, P.H., Kristiansen, K.R., Qvist, K.B., Skibsted, L.H., 1996. Effect of high hydrostatic pressure on the enzymic hydrolysis of β -lactoglobulin B by trypsin, thermolysin and pepsin. *J. Dairy Res.* 63, 111–118.
- Swain, B.C., Subadini, S., Rout, J., Sakshi, Mishra, P.P., Sahoo, H., Tripathy, U., 2020. Biophysical study on complex formation between β -Lactoglobulin and vitamin B12. *Food Chem.* 312, 126064.
- Tanford, C., Bunville, L.G., Nozaki, Y., 1959. The reversible transformation of β -Lactoglobulin at pH 7.5. *J. Am. Chem. Soc.* 81, 814032–814036.
- Uniacke, T., Fox, P.F., 2011. Milk. *Milk of Primates*. In: *Encyclopedia of Dairy Sciences*, pp. 613–631.
- Uniacke-Lowe, T., Huppertz, T., Fox, P.F., 2010. Equine milk proteins: chemistry, structure and nutritional significance. *Int. Dairy J.* 20, 609–629.
- Valiullina, E.F., Zaripov, O.G., Tjulkun, S.V., Ahmetov, T.M., Vafin, R.R., 2007. Characterization of bull-producers with different combinations of kappa-casein & beta-lactoglobulin genotypes by milk production their mothers. *Aust. Vet. Pract.* 4, 59–63.
- Varlamova, E.G., 2018. Participation of selenoproteins localized in the ER in the processes occurring in this organelle and in the regulation of carcinogenesis-associated processes. *J. Trace Elem. Med. Biol.* 48, 172–180.
- Varlamova, E.G., Cheremushkina, I.V., 2017. Contribution of mammalian selenocysteine-containing proteins to carcinogenesis. *J. Trace Elem. Med. Biol.* 39, 76–85.
- Varlamova, E.G., Goltyaev, M.V., 2018. The role of sodium selenite in regulation of gene expression of endoplasmic reticulum resident Selenoproteins as in the case of human fibrosarcoma. *Biophysics (Mosk).* 63, 880–887.
- Varlamova, E.G., Goltyaev, M.V., Fesenko, E.E., 2019. Protein–partners of selenoprotein SELM and the role of selenium compounds in regulation of its expression in human cancer cells. *Dokl. Biochem. Biophys.* 488, 300–303.
- Vieth, R., 2001. Vitamin D nutrition and its potential health benefits for bone, cancer and other conditions. *J. Nutr. Environ. Med.* 11, 275–291.
- Wang, C., Zhou, X., Wang, H., Sun, X., Guo, M., 2019. Interactions between β -lactoglobulin and 3,3'-diindolylmethane in model system. *Molecules* 24, E2151.
- Wei, Y., Vriesekoop, F., Yuan, Q., Liang, H., 2018. β -Lactoglobulin as a nanotransporter for glabridin: exploring the binding properties and bioactivity influences. *ACS Omega* 3, 12246–12252.
- Wodas, L., Mackowski, M., Borowska, A., Puppel, K., Kuczynska, B., Cieslak, J., 2020. Genes encoding equine β -lactoglobulin (LGB1 and LGB2): polymorphism, expression, and impact on milk composition. *PLoS One* 15, e0232066.
- Xie, S., Zhou, J., 2017. Harnessing plant biodiversity for the discovery of novel anticancer drugs targeting microtubules. *Front. Plant Sci.* 8, 720.
- Xu, J., Hao, M., Sun, Q., Tang, L., 2019a. Comparative studies of interaction of β -lactoglobulin with three polyphenols. *Int. J. Biol. Macromol.* 136, 804–812.
- Xu, X., Feng, Y., Chen, X., Wang, Q., Meng, T., Liu, A., 2019b. Antitumor effects of seleno- β -lactoglobulin on human breast cancer MCF-7 and MDA-MB-231 cells in vitro. *Toxicol. in Vitro* 61, 104607.
- Yan, Y., Seeman, D., Zheng, B., Kizilay, E., Xu, Y., Dubin, P.L., 2013. pH-dependent aggregation and disaggregation of native β -lactoglobulin in low salt. *Langmuir* 29, 4584–4593.
- Yang, R., Sun, G., Zhang, M., Zhou, Z., Li, Q., Strappe, P., Blanchard, C., 2016. Epigallocatechin gallate (EGCG) decorating soybean seed ferritin as a rutin nano-carrier with prolonged release property in the gastrointestinal tract. *Plant Foods Hum. Nutr.* 71, 277–285.
- Yang, S., Tu, Z.C., Wang, H., Huang, T., 2020. The reduction in the immunoglobulin G and immunoglobulin E binding capacity of β -lactoglobulin via spray-drying technology. *J. Dairy Sci.* 103, 2993–3001.
- Yeh, W.-J., Hsia, S.-M., Lee, W.-H., Wu, C.-H., 2017. Polyphenols with antiglycation activity and mechanisms of action: a review of recent findings. *J. Food Drug Anal.* 25, 84–92.
- Yehuda, I., Madar, Z., Leikin-Frenkel, A., Tamir, S., 2015. Glabridin, an isoflavon from licorice root, downregulates iNOS expression and activity under high-glucose stress and inflammation. *Mol. Nutr. Food Res.* 59, 1041–1052.
- Yokota, T., Nishio, H., Kubota, Y., Mizoguchi, M., 1998. The inhibitory effect of glabridin from licorice extracts on melanogenesis and inflammation. *Pigment Cell Res.* 11, 355–361.
- Zhao, Y., Sun, N., Gao, J., Wu, D., Liu, A., 2019. Antitumor activity of selenium modification of the bovine milk component β -Lg (se- β -Lg) on H22 cells. *Food Funct.* 10, 3626–3636.
- Zheng, G., Liu, H., Zhu, Z., Zheng, J., Liu, A., 2016. Selenium modification of β -lactoglobulin (β -Lg) and its biological activity. *Food Chem.* 204, 246–251.
- Zsila, F., Bikadi, Z., Simonyi, M., 2002. Retinoic acid binding properties of the lipocalin member α -lactoglobulin studied by circular dichroism, electronic absorption spectroscopy and molecular modeling methods. *Biochem. Pharmacol.* 64, 1651–1660.