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# A review of the design and modification of lactoferricins and their derivatives

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**Abstract** Lactoferricin (Lfcin), a multifunction short peptide with a length of 25 residues, is derived from the whey protein lactoferrin by acidic pepsin hydrolysis. It has potent nutritional enhancement, antimicrobial, anticancer, antiviral, antiparasitic, and anti-inflammatory activities. This review describes the research advantages of the above biological functions, with attention to the molecular design and modification of Lfcin. In this examination of design and modification studies, research on the identification of Lfcin active derivatives and crucial amino acid residues is also reviewed. Many strategies for Lfcin optimization have been studied in recent decades, but we mainly introduce chemical modification, cyclization, chimera and polymerization of this peptide. Modifications such as incorporation of D-amino acids, acetylation and/or amidation could effectively improve the activity and stability of these compounds. Due to their wide array of bio-functions and applications, Lfcins have great potential to be developed as biological agents with multiple functions involved with nutritional enhancement, as well as disease preventive and therapeutic effects.

**Keywords** Lactoferricin · Modification · Nutritional enhancement · Preventive and therapeutic activity · Biological agent

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## Introduction

Lactoferrin (Lf), a nonheme iron-binding glycoprotein, is secreted by the epithelial cells of the mammalian mucosa. It exists in exocrine secretions including tears, saliva, sweat (Park et al. 2011), vaginal fluids (Masson et al. 1968), semen, nasal fluids, bile, urine, bronchial secretions (Masson et al. 1965), gastrointestinal fluids, milk and colostrum. The content of Lf in human colostrum (6–8 mg/mL) and breast milk (2–4 mg/mL), which helps to regulate iron absorption and protect the newborn against gastrointestinal infections (Brock 1980), is much higher than the amount in other body fluids and in fluids of other mammals (Lonnerdal and Iyer 1995). In addition, Lf

released from neutrophils plays a determinant role in immune and inflammatory responses (Van Snick et al. 1974). As we know, Lf has been reported in many various physiological functions such as antimicrobial, antiviral, antiparasitic (Leboffe 2009), anticancer, antioxidant (Burrow et al. 2011) and anti-allergic (Kruzel et al. 2006) activities, in addition to the abovementioned iron absorption and other nutritional functions (Brock 1980). Therefore, it has attracted the focus of intensive research for many years mainly due to the strong safety guarantee of this protein from mammary animals.

Lactoferricin (Lfcin) is located in the *N*-terminal lobe of Lf and is released from the polypeptide chain by pepsin hydrolysis (Bellamy et al. 1992). It is supposed that Lfcins contribute to the normal physiological functions of Lf in vivo and in vitro (Sinha et al. 2013). Particularly, it has a potent antimicrobial activity. Lfcin is highly basic, containing many positively charged Arg and Lys residues. Trp residues in the sequence are important for its antimicrobial activity (Strøm et al. 2002). Lf molecules are highly conserved with homologous structures in various species but release different Lfcins with various lengths, and they exhibit different antimicrobial activities. The two Lfcins studied most extensively are from the native bovine Lf (LfcinB) and human Lf (LfcinH). LfcinB is composed of 25 residues, which form a somewhat distorted antiparallel β-sheet by an intra-molecular disulfide bond (Bellamy et al. 1992). LfcinH consists of the 49 *N*-terminal residues of Lf including the two pairs of disulfide bonds, and it exhibits a helical conformation instead of the β-sheet structure (Hunter et al. 2005), despite that both LfcinH and LfcinB share an amphipathic α-helical structure. Interestingly, LfcinB was more effective at killing bacterial and fungal organisms when compared with LfcinH in vitro. This difference could be explained in terms of the hydrophobicity, cationicity, secondary structure, amino acid composition and disulfide bonds of the proteins (Fjell et al. 2011). Thus, changing these properties is a common and effective tool to improve the antimicrobial activity of Lfcins. Generally, as nutritional, preventive and therapeutic agents, the stability, cytotoxicity, multidrug resistance and adsorption of Lfcins in vivo should be paid high attention. However, the basic safety baseline and support of lactoferricins and their derivatives from native milk proteins in mammary glands encouraged us to better track their properties for wider applications in the health of animals and humans. Therefore, many modifications such as unnatural amino acid substitutions, cyclization, multimerization and chimera have been studied. Next, we will briefly review study cases and progress of the above modifications.

## Biological function of Lfcins

Native Lf plays a role in the host defense against microbial infectious diseases in addition to iron transfer and other nutritional functions (Brock 1980). Lfcins, as a part of Lf, are released by proteolysis enzymes. It is known that Lfcins have the better antimicrobial activities than their parent Lf forms (Arias et al. 2014; Yamauchi et al. 1993). Both LfcinB and LfcinH showed antibacterial activity toward a wide variety of Gram-negative bacteria, Gram-positive bacteria and fungi, but LfcinB exhibited more bactericidal activity while LfcinH showed bacteriostatic activity (Gifford et al. 2005). Moreover, LfcinB is considered as the most effective among the Lfcins from many species including cows, mice and goats (Vorland et al. 1998). When a host is infected with bacteria, a series of toxic substances are released such as exotoxin and unmethylated CpG-containing oligonucleotides, inducing an immune response (Krieg et al. 1995). Lfcins can regulate the secretion of inflammatory factors to neutralize these toxins, indicating an immunomodulatory function (Mattsby-baltzer et al. 1996; Shinoda et al. 1996).

In addition, Lfcins exert antiviral activity against human cytomegalovirus (HCMV) (Andersen et al. 2001), herpes simplex virus (HSV) (Andersen et al. 2003), human papilloma virus (HPV) (Mistry et al. 2007), and human immunodeficiency virus (HIV) (Berkhout et al. 2002). The parent protein Lf possesses a much higher antiviral activity than do Lfcins in vitro; however, Shestakov et al. (2012) reported that Lfcin instead of Lf could block viruses in mice, indicating its role as a potent antiviral agent in vivo. Lfcins are highly positively charged peptides, an attribute which is critical to the affinity of heparan sulfate (HS, highly negatively charged) which blocks the initial viral entry, thus inhibiting cell-to-cell spreading of an ongoing infection (Andersen et al. 2001; Jenssen et al. 2008). In addition, structural features such as hydrophobicity, molecular size, and the secondary

structure seem to be related to the antiviral activity (Jenssen et al. 2004). Binding with the viral cell membrane as a part of the antiviral mechanisms, Lfcins can also directly interact with the virus or interfere with viral trafficking, consequently delaying coat protein synthesis and viral replication (Marr et al. 2009).

In regard to antitumor activities, Lfcins have been found to exert very potent antitumor effects on various cancer cell lines. LfcinB can induce human monocyte leukemia cell (THP-1) apoptosis by the production of intracellular reactive oxygen species (ROS) and activation of $Ca^{2+}$/$Mg^{2+}$-dependent endonucleases in the early stage (20 min) (Yoo et al. 1997a). Lfcins also act against gastric cancer cells and human colorectal cancer cells, with both of these types being the most common causes of cancer death in the world (Jiang and Lönnerdal 2017; Pan et al. 2013). Lfcins target the tumor cell membranous phosphatidylserine, performing highly selective cytotoxicity against tumor cells by activating diverse signaling pathways and preventing their metabolism, eventually inducing cell apoptosis (Khan and Taneja 2015; Pan et al. 2013; Riedl et al. 2011). When tested in vivo, iron-free bovine lactoferrin (LfB) significantly suppressed tumor growth and metastasis, indicating that multifunctional LfB and LfcinB may act in host defense (Yoo et al. 1997b). The applications of Lfcins in many aspects such as nutritional supplementation, immunomodulation, antimicrobial activity, antiviral activity, antiparasitic activity, and antitumor activity are summarized in Table 1. However, the research and development of antimicrobial Lfcins as therapeutic agents for animals and humans through modification will be focused on in this review.

## Design and modification of Lfcins

The therapeutic and healthy application of peptides in animals and humans usually suffers from problems such as cell toxicity, poor stability, high cost, and low selectivity. Based on the active sites and sequences, key bonds and amino acids of Lfcin, many molecular design strategies have been employed in order to optimize the antimicrobial activity and selectivity and develop novel functions of the peptide, including modifications through insertion of large hydrophobic side-chains, D-amino acid substitution, methylation, acetalization, amidation, cyclization, and chimera and polymerization of the peptide, as well as their cross utilization and combination.

## Active site of Lfcins

The amino acid composition of Lfcin derived from different mammalian species is found in the 17–41 amino acid sequence of the *N*-terminal of Lf with a high homology, and most Lfcins contain the structure characteristic of bacteriostasis. The activities of Lfcins from different species such as cows, mice and goats shows that LfcinB is a potent candidate for development of new antimicrobial agents (Bruni et al. 2016). Many studies of the amino acid sequences and residues have revealed the active center and important residues of Lfcin. The function and structural features of the active sequences are studied and compared in detail.

Using truncation, shorter derived sequences were designed. Furthermore, it was found that most of these truncated sequences retained biological effects. The 11-residue peptide LfcinB20–30 was essential for bacterial membrane interaction and played an important role in antimicrobial activity with low hemolytic activity (Kang et al. 1996). The shorter versions of LfcinB containing amino acids 17–31 (LfcinB17–31) and 17–27 (LfcinB17-27) from the *N*-terminal of Lf were found to have a slightly lower antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* than did LfcinB17–41 (Rekdal et al. 1999; Strøm et al. 2002; Liu et al. 2011). Later, the sequence RRWQWR (LfcinB20–25) was identified as being the antimicrobial center of LfcinB (Tomita et al. 1994; Schibli et al. 1999). Peptides LfcinH21–36, corresponding to the loop region of the *N*-terminal of LfcinH (FQWQRNMRKVRGPPVS), and LfcinH21–31, corresponding to its charged portion, exerted significant antibacterial effects against *E. coli* (Odell et al. 1996). It was later found that the multiple antigen peptides (MAP) of LfcinH21–31 exerted significant antibacterial effects against a broad spectrum of bacteria including methicillin-resistant *S. aureus*, and the 11-residue peptide (FQWQRNMRKVR) at the *N*-terminal of LfcinH was thought to account for all of the activity of the larger peptides (Azuma et al. 1999). Truncated sequence from LfcinH14–31, the residue fragment LfcinH19–31 was identified as the optimal

**Table 1** Applications of Lfcins

| Field | Actual question | Target | Object | References |
|---|---|---|---|---|
| Preclinical and clinical | – | *Pseudomonas*, *Staphylococcus* | Biomaterial surface | Chen et al. (2017) |
| | Skin cancer | – | Mouse | Khan et al. (2016) |
| | Parasitic infection | *Toxoplasma gondii*, *Eimeria stiedai* | Mouse, rabbit | Omata et al. (2001) |
| | Keratitis | *Pseudomonas aeruginosa, Staphylococcus aureus* | Mouse | Oo et al. (2010) |
| | Dermatophytosis | *Trichophyton mentagrophytes* | Guinea pig | Wakabayashi et al. (2000) |
| | Herpes simplex virus | HSV-2 line | Mouse | Shestakov et al. (2012) |
| Veterinary | Atopic dermatitis | – | Dog | (Biasibetti et al. 2017) |
| | Otitis externa | Bacteria and yeast | Dog | Vercelli et al. (2015) |
| | Bovine mastitis | *Staphylococci, Streptococci*, *E. coli* | Cow | Kawai et al. (2003) |
| | Dietary supplementation | Antibiotic alternative | Weaned piglet | Tang et al. (2009, 2011) |
| | Dietary supplementation | *E. coli* | Weaned piglet | Hou et al. (2011) |
| Food | Spoilage | *Pseudomonas fluorescens* | Ground beef | Del et al. (2009) |
| | Spoilage | *Pseudomonas* spp | Vegetable | Federico et al. (2015) |
| | Spoilage | *Penicillium digitatum* | Mandarins | Muñoz and Marcos (2006) |
| | Spoilage | Lactic acid bacteria, *Dekkera bruxellensis* | Wine | Enrique et al. (2008, 2009) |
| | Spoilage | *Pseudomonas* spp, *E. coli* | Cheese | Caputo et al. (2015), Quintieri et al. (2012, 2013) |
| GMO | | *Pseudomonas syringae*, *Botrytis cinerea* | Tobacco | Fukuta et al. (2012) |
| | | *E. coli*, *Edwardsiella tarda*, *Aeromonas hydrophila* | Zebrafish | Lin et al. (2010) |

length for antimicrobial activity. It was noted that LfcinH19–31 could bind to lipid A/lipopolysaccharide, as shown by neutralizing endotoxic activity in a *Limulus* assay (Håversen et al. 2010). Therefore, these sequences are intensively studied due to their activity against different targets.

A series of experiments examining each of the residues in LfcinB17–31 were performed to identify the specific amino acids that were important for the antibacterial activity. The result showed that neither of the two Trp residues at positions 6 and 8 in the LfcinB17–31 derivatives could be replaced by Ala without a severe loss of antibacterial activity (Strøm et al. 2000). This "Trp-effect" was further explored in the derivatives of LfcinB17–31 wherein one, two or three residues in the native sequence were replaced by Trp. A higher antibacterial activity was found with the Trp-rich LfcinB17–31 (Strøm et al. 2002). The same result was indicated with porcine lactoferricin (LFP-20), in which the antibacterial activity increased with an increasing content of Trp (Han et al. 2013). Another crucial amino acid is Arg. In the peptide LfcinB20–30, using lysine to replace Arg or using Gln to replace all Arg and Lys residues resulted in a decrease of the antibacterial activity. However, using Arg to replace Lys rendered stronger activity in this peptide (Kang et al. 1996). Similarly, when all Arg residues in the LfcinB17–30 and LfcinH1–11 were replaced by Lys, the activity of all-K variants against mycobacterial infections was significantly lower than that of the corresponding original peptide (Silva et al. 2014). Hence, the peptide, which is rich in Arg and Trp, is

often used as a good original master/sequence for the design of novel antimicrobial peptides. Amino acid substitutions affect the function and activity of peptides in essence by altering the number of positive net charges, the ratio of hydrophilic and hydrophobic amino acids, and the size of amino acid side chains.

## Chemical modification of Lfcins

To determine how Trp is pivotal for the biological activity of LfcinB17–31, peptides wherein Trp was replaced by Bal, termed 1-Nal and 2-Nal, with different properties were prepared and tested for antibacterial activity against *S. aureus* and *E. coli*. The derivatives containing aromatic hydrocarbon residues had higher antibacterial activities. It was shown that neither the hydrogen bonding ability nor the amphipathicity of Trp were essential properties for the antimicrobial effect. However, the results indicated that the shape, size and aromatic properties of Trp seem to be the most important features for the biological activity (Haug and Svendsen 2001). In a further study, Trp residues in LfcinB17–31 were replaced with the bulky aromatic amino acids Phe, Tbt, Ath, Dip and Bip to elucidate and confirm the importance of size and shape. The largest aromatic amino acids employed resulted in the most active peptides. The bacterial selectivity could be altered to some degree by utilizing aromatic residue substitution (Haug et al. 2001; Strøm et al. 2002). The presence of the bulky group on the side chain enabled the peptides to more effectively interact with the membrane.

Using unnatural amino acids such as D-amino acids is a common way to increase the stability and prolong the duration of action of peptides and protect them from digestion in vivo. D-amino acid-based derivatives of the sequence RRWQWRMKK in LfcinB resulted in higher antimicrobial activities against bacteria than those observed with other LfcinB (Wakabayashi et al. 1999). Comparing with the bactericidal effects of LfcinB17–31 and its all-D-amino acid counterpart against *E. coli* and *S. aureus*, D-LfcinB17–31 showed a significantly better efficacy (Ulvatne and Vorland 2001). Similarly, D-LfcinB17–30 displayed the highest antimycobacterial activity among Lfcin-based peptides (Silva et al. 2014).

In many cases, *N*-terminus acetylation and/or *C*-terminus amidation are ways to improve the activity of peptides. Based on the sequence of LFcinB20–28, *N*-acylated derivative peptides having a carbon-chain length of six to eleven carbons showed similar or higher antimicrobial activities compared with those of LfcinB (Wakabayashi et al. 1999). A series of mutant sequences were designed based on LfcinH21–31, which included strengthening of the hydrophobic *N*-terminal end by adding *N*-acylation in the derivative peptides and resulted in an enhanced antimicrobial activity against *E. coli*. These *N*-acylated peptides perturbed the lateral packing of neutral lipids and exhibited better permeability into *E. coli* lipid vesicles (Zweytick et al. 2011). Further study revealed that the *N*-acylated peptides perturb the organization of cardiolipin and phosphatidylethanolamine in cell membranes and induce defects in *E. coli* cell division (Zweytick et al. 2014). LfcinB20–25 led to an 8-fold decrease of the MIC against *E. coli* and *S. aureus* due to the *C*-terminal amidation (Tomita et al. 1994). Another example showed that *C*-terminal amidation improved LfcinB20–25 antibacterial activity. In terms of proteolytic stability, protection through amidation at the *C*-terminal had a slightly lesser effect. However, *N*-terminal acetylation decreased antimicrobial activity but conferred notable resistance against serum aminopeptidases. Meanwhile, the combination of the two modifications produced even greater antibacterial activity and stronger proteolytic stability of the peptide (Nguyen et al. 2010).

It was reported that methylation alone in C0-RRWQ[1-Me-W]R-NH2 resulted in a 3 to 4-fold increase in antimicrobial activity against *E. coli* (Greathouse et al. 2008). Later, a heptapeptide produced with 2 methylated Trp residues (RRMeWQ-MeWRR-NH2; MeTrp-LfB7) exhibited enhanced activity against Gram-negative and Gram-positive bacteria. To determine whether Trp methylation was essential for the increase of efficacy, the peptide was synthesized and characterized without methylated Trp (LfB7: RRWQWRR-NH2). Meanwhile, two peptides (LfB7-MeTrp3,5 Ala4: RRMeWAMeWRR-NH2 and LfB7-Ala4: RRWAWRR-NH2) in which Gln was replaced by Ala were also prepared. Results indicated that LfB7-Ala4 has a 5-fold reduced activity against *E. coli*, while LfB7MeTrp3,5 exhibited the highest activity, with a 4- and 6-fold increase compared to

LfB7 against *E. coli* and *S. aureus*, respectively (Kim and Greathouse 2016).

## Cyclization of Lfcins

Cyclization (backbone cyclization or disulfide-bridged cyclization) of peptides has been used successfully in some cases as a viable strategy to improve antimicrobial activity while maintaining the structural stability of the peptides. To cyclize LfcinB20–30 chemically, Nguyen et al. added cysteine residues to each terminal end of the peptide to form a disulfide bond. The cyclic peptide was found to have stronger antimicrobial potency against both *E. coli* and *S. aureus* than its linear counterpart and to be embedded deeper into the membrane (Nguyen et al. 2005). The RW-rich hexapeptides RRWWRF (Lfcin-RW) were derived from screening of a synthetic combinatorial library (Blondelle et al. 1995), and many studies have shown that the backbone-cyclization of Lfcin-RW has a pronounced activity-improving and bacterial selectivity-enhancing effect (Wessolowski et al. 2004; Dathe et al. 2004; Appelt et al. 2005). The hexameric peptides LfcinB6 and Lfcin-RW were cyclized by both head-to-tail linking and disulfide crosslinking of the peptide backbone for comparison. The two cyclic hexapeptides were found to be highly effective in both antimicrobial activity and serum stability. However, the two cyclization methods produced different effects—disulfide-bond cyclization was clearly more advantageous for antimicrobial activity while backbone cyclization resulted in more proteolytically stable peptides. However, the benefit of backbone cyclization did not extend to the longer LfcinB11 (Nguyen et al. 2010).

In recent years, along with the combination of chemistry and biology, olefin ring-closing metathesis reactions and click chemistry reactions have been applied to the cyclization of antimicrobial peptides and to the design of peptide-based drugs to various degrees. LfcinB-CLICK was generated using "click chemistry" (a $Cu^{I}$-promoted alkyne/azide cycloaddition reaction) in order to replace the disulfide bridge with a triazole linkage that allowed the peptide to retain the cyclic hairpin characteristics of the original peptide, LfcinB. The LfcinB-CLICK peptide inhibited the growth of *E. coli*, and there was no difference in antimicrobial activity when compared with the original peptide. Furthermore, the antimicrobial mechanism indicated that LfcinB-CLICK exhibited aggregation of vesicles and permeabilization into *E. coli* (Arias et al. 2014). Similar results had been reported from the comparison of LfcinB-CLICK to LfcinB indicating an improved anticancer activity (Arias et al. 2016). Another interesting modification method relies on the use of the *S. aureus* enzyme sortase A. Arias et al. showed that the use of sortase A provided an alternative way to form cyclic LFcinB, preserving the antimicrobial activity of the original peptide by inducing vesicle aggregation and permeabilization into the inner membrane of *E. coli* (Arias et al. 2014).

## Chimera of Lfcins

To test whether two Lf-derivative peptides together can form a new functional unit, a chimeric construct (LFchimera) was designed by linking the two peptides into one molecule. The short peptide LfcinH1–11, corresponding to amino acids 1–11 of LfcinH at the N-end, can act as a cell penetrating peptide (CPP). LfcinB20–25 produces cytotoxic activities towards cancer cells. Therefore, two new chimera peptides were created by combining LfcinH1–11 and LfcinB20–25 through either a Pro or Gly–Gly linker. The results supported that the new adducts exhibited interesting anticancer activities with considerable special selectivity for cancerous cells (Arias et al. 2016).

Lactoferrampin (Lfampin) was discovered by a Dutch work group in 2004. LfampinB, encompassing the sequence of amino acids 265–284 in the N1-terminal of bovine Lf, was found to exert potent antibacterial and antifungal effects (van der Kraan et al. 2004). The same region of human lactoferrin had no detectable antibacterial activity, but it could be mutated to increase its potency (Haney et al. 2009). Recent studies illustrated that this chimeric structure containing LfcinB17–30 and LfampinB265–284 exerted stronger antimicrobial effects than those of the constituent peptides, and it has been used in various animal-model tests. Previous reports concerning LFchimera (LfampinB265–284-K-LfcinB17–30) showed not only bactericidal activity towards both Gram-positive and Gram-negative bacteria but also absence of the sensitivity to ionic strength (Bolscher

et al. 2009). LFchimera (LfcinB17–30-K-Lfampin-B265–284) was very potent agent against *Burkholderia pseudomallei* (Puknun et al. 2013) and showed better therapeutic effects against enterohaemorrhagic *E. coli* O157:H7 in a mouse model (Flores-Villaseñor et al. 2012).

## Polymerization of Lfcins

It has been shown that the polymerization of Lfcin is of notable significance to structural stability and transmembrane characteristics (Johnson et al. 1995; Lemmon et al. 1992). A tetrameric peptide, LfcinB $(20–25)_4$ (Fig. 1), based on the core sequence RRWQWR of bovine lactoferricin, exhibited selective inhibition of oral squamous-cell carcinoma (OSCC) at low doses but no lytic effect in normal red blood cells. This peptide induced a higher degree of tumor apoptosis, higher levels of IgG antibodies, and a lower inflammatory response relative to its monomer LfcinB (20–25) (Solarte et al. 2015, 2017). In addition, LfcinB $(20–25)_2$ and LfcinB $(20–25)_4$ also exhibited effective activity against the Gram-negative bacteria *E. coli*, *S. enteritidis*, and *S. maltophilia*, and the Gram-positive bacteria *S. aureus* and *E. faecalis* (Huertas Mendez et al. 2017; Huertas et al. 2017; Leon-Calvijo et al. 2015).

Through predictive analysis of the physical and chemical properties of the designed polymer, constructs were designed to increase the hydrophobicity and the positive charges. When the net charge of tetrameric LfcinB $(20–25)_4$ was shifted from + 3 of the monomer LfcinB (20–25) up to +16, a net charge of + 4 was necessary for antimicrobial activity, and at least + 7 or higher is essential for antitumoral activity (Gifford et al. 2005). Due to the overexpression of anionic molecules on tumor surfaces, the positive charge also plays a crucial role in the electrostatic interactions that occur between peptide and tumor. This indicates that the selective cytotoxicity is closely associated with the positive charge. (Solarte et al. 2017). On the other hand, peptide hydrophobicity also has an important role in membrane selectivity and insertion, thus enhancing antimicrobial activity (Chen et al. 2007). However, the hydrophobicity of the peptide has a "threshold", and once beyond it, the activity of the peptide declines and toxicity to mammalian cells increases (Glukhov et al. 2008).

## Conclusion

In recent years, the design and modification of Lfcins have been studied intensively. In particular, the basic safety and support of Lfcins and their derivatives from native milk proteins found in mammary glands encouraged researchers to improve and screen their properties and functions for wider applications in the health of animals and humans. Despite these characteristics, the third safety evaluation cannot be waived for modified Lfcins. Molecular modification is no longer limited to site substitution of residues. New and better tools have been developed, including chemical modification, cyclization, chimera and polymerization, as well as their combination or cross utilization

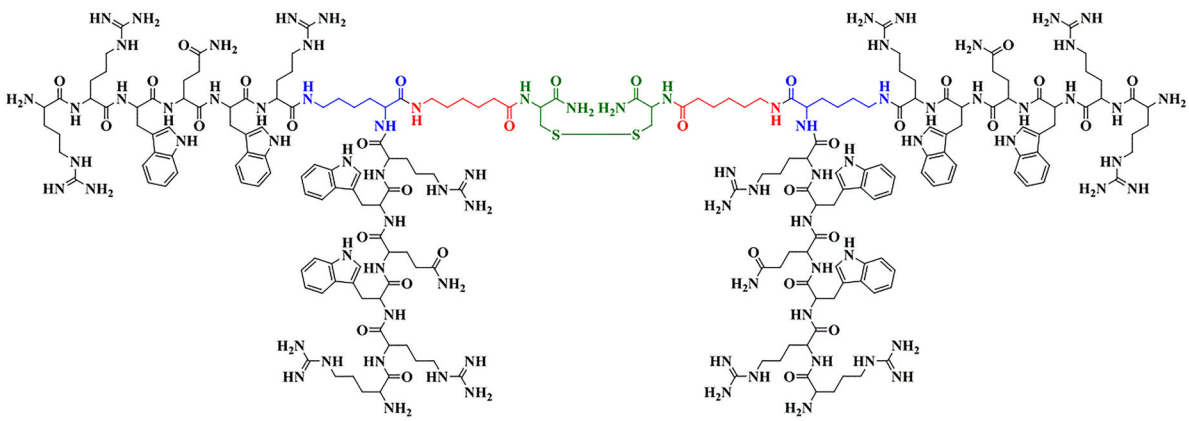

**Fig. 1** LfcinB$(20–25)_4$ tetrameric peptide was obtained by oxidation of dimer molecule. Reproduced with permission from Huertas Mendez et al. (2017)

depending on requirements. Additionally, these strategies effectively expand the application scope of Lfcins and their derivatives in various fields such as nutritional enhancement and antimicrobial, anticancer, antiviral, antiparasitic, and anti-inflammatory activities. However, antimicrobial activity is not the only factor to be considered. Rather, attention should also be paid to obtaining a good balance among optimum hydrophobicity, positive charges and their distribution, drug resistance, stability, toxicity and convenience of production. Currently, most modified peptides have some shortcomings that limit their application. Therefore, future studies should obtain deeper insight into the design and optimization of Lfcins as a whole as well as the effects of the above factors to develop better nutrition-enhancing, preventive and therapeutic peptides.

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