

Nutraceutical Peptides from Lactoferrin

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Abstract

Lactoferrin belongs to the transferrin protein superfamily and is a major component of the mammalian innate immune system. Proteolytic cleavage of either human or bovine lactoferrin results in the generation of several peptides, most of which manifest the protective activities of Lactoferrin. It is worth noting that these peptides may be naturally produced in the human intestine after milk ingestion. Interestingly, peptides deriving from proteolytic cleavage of Ovotransferrin, a Lactoferrin homologous protein present in reptiles and birds, displays the same protective activities. This mini-review summarizes the most relevant protective activities of peptides deriving from Lactoferrin and Ovotransferrin.

Keywords: Lactoferrin; Ovotransferrin; Nutraceutic peptides

Introduction

Lactoferrin (Lf) (formerly known as lactotransferrin) is an iron-binding glycoprotein, belonging to the transferrin protein superfamily, together with serum transferrin, melanotransferrin and the inhibitor of carbonic anhydrase. Lf was firstly isolated from bovine milk by Sorensen and Sorensen in 1939 [1] and after twenty years it was recognized to be the main iron binding protein present in bovine milk [2]. In most mammalian species, including humans, Lactoferrin is produced and released by mucosal epithelial cells and also by polymorphonuclear leukocytes [3].

Lactoferrin displays a non-specific protection against infections and other pathologies linked to its antimicrobial, anti-inflammatory and anticancer activities, being a crucial component of innate immunity in mammals [4]. Ovotransferrin (Otrf), a glycoprotein of egg white albumen, is the Lf homologous in reptiles and birds and it is present in large quantity in hen's egg [5].

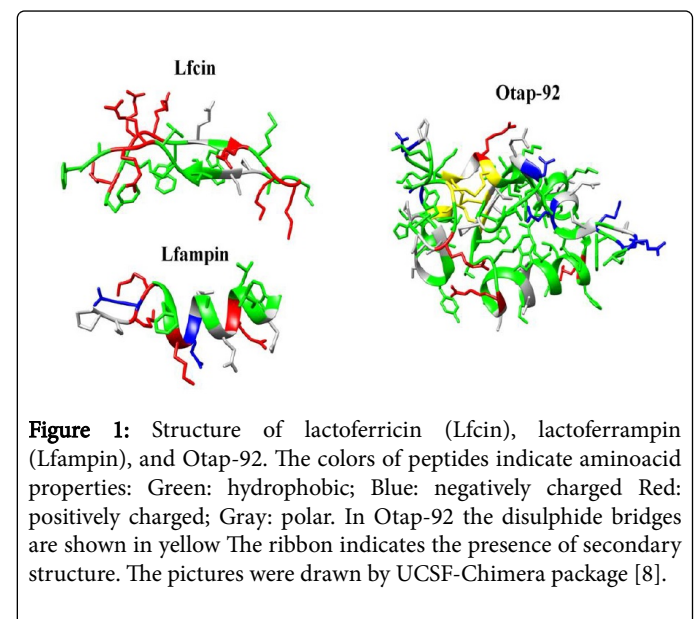
Both Lf and Otrf are glycosylated proteins of about 80 kDa consisting of about 700 amino acids and showing high homology among different species. Their three-dimensional structures consist of a single polypeptide chain folded into two symmetrical lobes (N and C lobes), each one subdivided in two domains (N1 and N2, C1 and C2, respectively), with high (33-41%) inter-lobe and interdomain homologies [6,7].

Lactoferrin peptides

Several functions have been ascribed to peptides deriving from the proteolytic hydrolysis of Lf, and their relevance is particularly important since it is possible that they could be naturally produced in the human intestine after the ingestion of human or bovine milk.

In particular, three peptides deriving from the N-lobe of Lf show significant antimicrobial activities. These peptides are amphiphilic and

their antimicrobial activity is due to their hydrophobicity, cationic charges, and secondary structure (Figure 1). These three peptides are called Lf (1-11), Lfcin and lactoferrampin (Lfampin), respectively.



In detail, Lf (1-11) is a short oligopeptide that includes the first eleven aminoacidic residues of the N-terminus of Lf. It has been demonstrated that Lf (1-11) is able to interact with the membranes of several bacteria and that it is active *in vitro* and *in vivo* against various pathogens (bacteria and yeast) with no clear synergistic effects with antibiotics. Lf (1-11) contains both hydrophobic and hydrophilic residues and the first two arginines at the N-terminus confer to Lf (1-11) a highly cationic nature that seems to be responsible of its antimicrobial effect [9].

Lfcin is an amphipathic, cationic peptide and it is the most studied anti-microbial peptide derived from milk proteins. It can be generated by the acidic pepsin-mediated digestion of Lf (aminoacid residues 17-41). The peptide has a significant percentage of basic residues, including Lysine and Arginine, as well as hydrophobic amino acids, like Tryptophan and Phenylalanine. Lfcin displays several biological activities including antiviral, antibacterial, antifungal and anti-inflammatory [10].

Lfampin comprises residues 268-284 in the N1 domain of Lf, and it is in close spatial proximity to Lfcin. Lfampin exhibits several antimicrobial actions against several Gram-positive and Gram-negative bacteria, yeast and parasites [11].

Lf- derived peptides demonstrate a considerably stronger antimicrobial activity compared to the native protein, displaying also a broader antibacterial spectrum at lower concentrations. Their activities are, however, not due exclusively to antibacterial properties and several other protective actions against microorganisms' infections have been found in Lf-derived peptides and are summarized in Table 1.

Activity		Peptide
Antibacterial	Gram positive	Lf(1-11), Lfcin, Lfampin
	Gram negative	Lf(1-11), Lfcin, Lfampin
Antiviral		Lf(1-11), Lfcin, Lfampin
Antifungal		Lf(1-11), Lfcin, Lfampin
Antiparasitic		Lfcin, Lfampin

Table 1: Anti-Infective activities of lactoferrin peptides. For further details and a complete list of references, [9,12].

Furthermore, in addition to their antimicrobial activity, Lf derived peptides possess other biological activities. Among these, the most important is the anticancer activity of Lfcin shown in Table 2.

Cancer type	Mechanism of anticancer action
Breast	LFcinB-CLICK and the chimeras composed of hLF11 and LFcinB1 in breast cancer cell line (MDA-MB-231)
Colon	Lfcin causes arrest in the at S phase through downregulation of cyclin E1 in CaCO ₂ cells
Gastric	Lfcin induces apoptosis human gastric cancer cell line AGS
Head, neck, and oral	Pepsin-digested-lactoferrin peptides induce apoptosis via JNK/SAPK activation in squamous cell carcinoma cell line SAS
Leukemia	LfcinB6 (RRWQWR) induces cytotoxicity via caspase-mediated and cathepsin B-mediated mechanism in T-leukemia cells
	Lfcin kills T-leukemia cells by triggering the mitochondrial pathway of apoptosis and through the generation of reactive oxygen species.
	LF11-322 (PFWRIRIR-NH ₂), peptide fragment derived from human lactoferricin, induces necrosis in leukemia cells (MEL and HL-60 leukemia cells)
	LFcinB-CLICK (FKCRRWQWRMKKLGAPSTCVRRAF) and the chimeras composed of hLF11 and LFcinB1 (GRRRRSVQWCA-P-RRWQWR-NH ₂) in Leukaemia cells (Jurkat)
Lung	Lfcin inhibits VEGF expression and induces apoptosis on non-small cell lung cancer H460.
NCS	Lfcin inhibits tumor growth and induces apoptosis through activation of caspases in neuroblastoma cells and <i>in vivo</i>

Table 2: Anticancer activities of Lfcin derivative peptides. For further details and a complete list of references [12,13].

Despite the fact that the mechanism(s) responsible for the anticancer activity of Lfcin has not yet been characterized, bLFcin6 (a very short hexapeptide derived from bovine lactoferricin) is able: i) to be rapidly internalized into HeLa cells, ii) to form stable electrostatic complexes with siRNA and iii) to deliver siRNA into cells, showing a significant knockout activity against both mRNA and protein, similarly to that mediated by TAT [14].

Several studies suggest that exogenous treatment with Lf and its derivatives can efficiently inhibit the growth of tumors and reduces susceptibility to cancer, alone or in combination with other therapeutic agents. It is interesting to note that in some studies Lf and its derivatives have already been used to increase the pharmacological effects of anticancer drugs. The relatively low cytotoxicity is likely to encourage the clinical use of Lf and its peptides in cancer treatment, despite lack of data about the mechanisms of action [15].

Ovotransferrin peptides

Otrf is the Lf homologous counterpart in reptiles and avian species and it can reach a very high concentration in hen egg whites. Figure 1 (panel B) shows the antibacterial peptides deriving from proteolytic digestion of Otrf.

OTAP-92 is the most important Otrf peptide with a mass of 9.9 kDa. It is made of 92 aminoacidic residues (Leu109-Asp200) and shows high sequence similarities with insect defensins. The antibacterial mode of action of OTAP-92 may be due to its relatively high isoelectric point and to the cysteine exposition [16].

Two other Otrf fragments: DQKDEYELL (hOtrf219-27) and KDLLFK (hOtrf269-301 and hOtrf633-638) show antiviral activities like Lf peptides. Indeed, treating chicken embryo fibroblasts (CEF) infected with Marek's disease virus (MDV) with these two fragments, it can be observed that they are able to inhibit antigen synthesis and counteract the infection. The highest blocking efficiency is shown by

the intact protein even though the peptides activity is also significant. Interestingly, these two Otrf peptides share sequence homology with two protein fragments derived from hLf and bLf [17,18] active against

Herpes Simplex Virus (HSV-1) [17]. Furthermore, other peptides derived from Otrf display biological activities, reported in Table 3.

Otrf Peptides	Activities	Mechanisms
Otrf peptide OTAP-92	Antimicrobial	Bacterial Membrane damage
Otrf peptides: 219–227; 269–301; 633–638	Antiviral	Viral adsorption inhibition
Reduced autocleaved Otrf (rac-Otrf)	Anticancer/ antiproliferation	Apoptosis induction
	Antioxidant	Autocleaved Otrf as preservative to prevent b-carotene discoloration
Otrf Peptides (IRW or IQW)	Antiinflammatory	Attenuate TNF- α -induced inflammatory responses
Otrf peptide (KVREGT)	Antihypertensive	Inhibition of Angiotensin I-Converting Enzyme.
Otrf Peptides (DLLFKDSAIMLK) (FFSASCVPGATIE)	Antioxidant	Catechin conjugation
Otrf peptides (mix obtained using: protamex, or alkalase, or trypsin, or α -chymotrypsin)	Antioxidant	Synergistic antioxidant effects with vitamin C, epigallocatechin gallate (EGCG), and caffeic acid.

Table 3: Physiological and pharmacological activities of Ovotransferrin's peptides. For further details and a complete list of references [19-22].

Conclusions

Lf proves to play an important role in many physiological functions. For its numerous activities, such as antioxidant, antiinflammatory and antimicrobial, Lf is one of the most studied nutraceutical proteins. Several studies also demonstrate its relevant potentiality in preventing the different stages of cancer including initiation, promotion, and progression [12,13] and references therein]. Peptides deriving from the proteolytic digestion of Lf show most of these protective activities.

Furthermore it is remarkable that Lf can have a systemic effect. In fact, Lf deriving from bovine milk, that undergoes proteolytic digestion, generates Lf peptides that may pass the intestinal barrier thanks to their low molecular weight. Interestingly, we can suggest that the same may occur also for Otrf and Otrf's peptides deriving from hen egg whites. Taking together all this evidence, we can conclude that Lf and Otrf, as well as their fragments, could improve human health and that the ingestion of bovine milk and of egg whites (preferably raw or cooked at low temperature) could be of greatest importance in normal and pathological human conditions.

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