Food Bioscience 22 (2018) 19–25

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Identification and mechanism of peptides with activity promoting osteoblast proliferation from bovine lactoferrin

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ARTICLE INFO

Keywords:
Bovine milk
Lactoferrin
Peptides
Osteoblasts
Molecular docking

ABSTRACT

Lactoferrin (LF) is a basic glycoprotein and a dominant functional component from the whey protein in bovine milk. As a novel bone growth factor, it can fulfill its biological functions of preventing osteoporosis by regulating the growth and metabolic processes of bone. However, it has not been clarified that LF plays a role of osteogenesis in a form of molecular fragments after enzymatic digestion. In this study, a novel peptide with osteoblast proliferation activity, ENLPEKADRDQYEL, was identified using UPLC-Q-TOF-MS/MS and Mascot analysis. The mechanism of promoting proliferation of osteoblast activity was also analyzed by molecular docking. Results demonstrated that ENLPEKADRDQYEL can significantly promote the proliferation of osteoblasts. The main interaction forces of ENLPEKADRDQYEL with epidermal growth factor receptor (EGFR) were the hydrophobic and hydrogen bonding. ENLPEKADRDQYEL had similar target domain (Lys13-Leu14-Thr15-Gln16-Leu98-Ser99-Ser418) with the key structure of EGFR compared with epidermal growth factor (EGF). This work established a theoretical foundation for the peptide from lactoferrin used as a functional component in functional dairy products.

1. Introduction

Osteoporosis is an age-related skeletal disorder leading to low bone mass and weakening bone microarchitecture (Lerner, 2006). It occurs due to unbalanced bone remodeling and altered functions of the osteoclasts and osteoblasts (Chim et al., 2013; McClung et al., 2013). To treat and prevent bone loss, there are several drugs available either suspend or prevent bone loss or bone reconstruction. Latest osteoporosis treatments are anti-resorptive medicines which inhibit osteoclastic bone resorption but not promote new bone formation, including risedronate, alendronic acid, bisphosphonate, zoledronic acid and alendronate, have been widely used in the clinical treatment to reduce the risk of fracture (Amso et al., 2016; Calabria et al., 2016; Eriksen, Diez-Perez, & Boonen, 2014; Park et al., 2014). However, these treatments may cause several side effects, including a risk of an inflamed esophagus, nausea and abdominal pain. Therefore, the discovery of novel bone-anabolic agents with less toxicity, greater safety and the ability to increase bone strength, bone mass and potentially reversing structural damage are attracting more attentions.

In recent years, a variety of osteogenic peptides with less side effects to establish an alternative strategy were reported as natural alternative bioactive peptides. A peptide named osteogenic growth peptide (OGP) was reported to serve as an important bone growth factor (Pigossi, Medeiros, Saska, Cirelli, & Scarel-Caminaga, 2016). OGP is a 14-amino acid motif (ALKRQGRTLYGFGG) with a primary structure identical from C-terminus of histone H4. It has been verified that OGP had promoting effect on bone regeneration, mainly in stimulating the differentiation, proliferation, and matrix mineralization of osteoblast via MAPK signal pathway (Maia et al., 2014). Atsuhiro et al. also reported a synthetic peptide (NSVNSKIPKACCVPTELSAI), relating to residues 68–87 of BMP-2, recruited osteocalcin-positive osteoblasts to induce ectopic calcification (Saito, Suzuki, Ogata, Ohtsuki, & Tanihara, 2005). Besides, the principal cytokines such as insulinlike growth factor-1 (IGF-1), transforming growth factors -α and -β (TGF-α and TGF-β), epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) (Bikle & Wang, 2011; Cinque et al., 2015; Crane & Cao, 2014; Plonka et al., 2017; Tian, Guo, Zhuang, Chu, & Zhang, 2014) showed a critical role in all aspects of skeletal development and bone remodeling (Soon et al., 2015). Although being the most potential therapy currently available, the high cost of isolation and labor-intensive of purification has caused cytokines hard to be a potent treatment.

Milk proteins are regarded to be the most important source of bioactive peptides, which attract more growing interests in the field of

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https://doi.org/10.1016/j.fbio.2017.12.011
Received 15 September 2017; Received in revised form 21 December 2017; Accepted 24 December 2017
Available online 27 December 2017

Table 1
Peptides profile of lactoferrin hydrolysate identified by UPLC-Q-TOF-MS/MS.

No.	*m/z* meas.	Mr. calc.	Scores	Length	Amino Acid Sequence	Range	Protein
1	747.4008	746.3963	50.01	7	APVDAFK	256-262	lactoferrin
2	805.3898	804.3865	35.43	7	ETAEEVK	352-358	lactoferrin
3	439.7389	877.4658	56.41	8	DSALGFLR	321-328	lactoferrin
4	516.7676	1031.5247	64.65	9	ETAEEVKAR	352-360	lactoferrin
5	355.1799	1062.5206	47.18	8	NLNREDFR	582-589	lactoferrin
6	540.786	1079.5611	25.19	10	VDSALYLGSR	333-342	lactoferrin
7	549.2539	1096.4978	57.48	9	YYGYTGAFR	542-550	lactoferrin
8	374.5603	1120.6604	30.11	9	YLTTLKNLR	343-351	lactoferrin
9	594.8117	1187.6146	27.59	10	NLRETAEEVK	349-358	lactoferrin
10	616.8148	1231.6197	45.61	11	ANEGLTWNSLK	461-471	lactoferrin
11	633.2929	1264.5764	59.09	11	EPYFGYSGAFK	206-216	lactoferrin
12	645.3371	1288.6663	77.95	11	SVDGKEDLIWK	278-288	lactoferrin
13	439.9214	1316.7452	53.53	12	LRPVAAEIYGTK	93-104	lactoferrin
14	660.8357	1319.6622	56.01	12	SFQLFGSPPGQR	304-315	lactoferrin
15	677.8224	1353.6353	68.59	11	EKYYGYTGAFR	540-550	lactoferrin
16	454.2441	1359.7146	32.91	12	KANEGLTWNSLK	460-471	lactoferrin
17	681.8386	1361.6688	84.43	12	GSNFQLDQLQGR	120-131	lactoferrin
18	461.2685	1380.7878	106.77	12	QVLLHQQALFGK	628-639	lactoferrin
19	472.5897	1414.7528	49.94	12	NLRETAEEVKAR	349-360	lactoferrin
20	492.5861	1474.7416	99.15	13	ANEGLTWNSLKDK	461-473	lactoferrin
21	745.8855	1489.7637	112.14	13	KGSNFQLDQLQGR	119-131	lactoferrin
22	498.9468	1493.8242	112.83	13	DLLFKDSALGFLR	316-328	lactoferrin
23	860.409	1718.8034	46.29	14	ENLPEKADRDQYEL	235-248	lactoferrin
24	502.614	1504.8249	62.87	14	IPSKVDSALYLGSR	329-342	lactoferrin
25	521.9375	1562.7954	42.47	14	SRSFQLFGSPPGQR	302-315	lactoferrin
26	531.2738	1590.8042	75.79	14	ESPQTHYYAVAVVK	105-118	lactoferrin
27	535.2843	1602.8365	58.38	14	ANEGLTWNSLKDKK	461-474	lactoferrin
28	540.2914	1617.8587	54.95	14	KGSNFQLDQLQGRK	119-132	lactoferrin
29	914.4367	1826.8686	18.51	18	GEADALNLDGGYIYTAGK	406-423	lactoferrin
30	646.3452	1936.0207	17.61	17	SFQLFGSPPGQRDLLFK	304-320	lactoferrin
31	747.3697	2239.0943	71.36	21	KADAVTLDGGMVFEAGRDPYK	407-423	lactoferrin
32	592.0757	2364.2801	38.65	22	DSALGFLRIPSKVDSALYLGSR	321-342	lactoferrin
33	672.3552	2685.4013	116.68	24	LGGRPTYEEYLGTEYVTAIANLKK	670-693	lactoferrin
34	699.8738	2795.4759	24.41	25	SFQLFGSPPGQRDLLFKDSALGFLR	304-328	lactoferrin
35	1095.2505	3282.7475	104.6	30	SAGWIIPMGILRPYLSWTESLEPLQGAVAK	141-170	lactoferrin

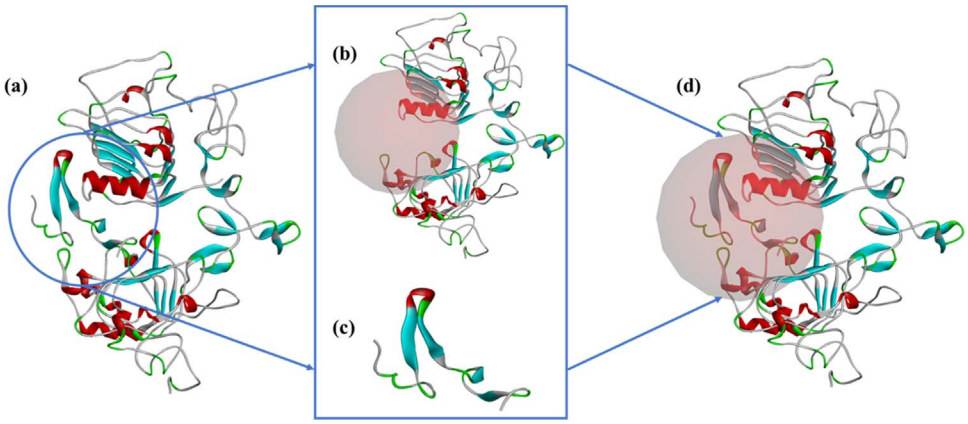

Fig. 1. Docking for the interaction of epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) (PDB:1IVO) used for validation. (a) 3D structure of EGF and EGFR complex as a surface image, generated by the surface menu of Discovery Studio 2017 software based on the PDB database. (b) Active site of EGFR and the selective site used for molecule docking. (c) Structure of EGF. (d) EGF and EGFR compound after docking.

health-beneficial functional foods (Mohanty, Mohapatra, Misra, & Sahu, 2016). Lactoferrin (LF), a dominant functional component from the whey protein in bovine milk, is a concerned glycoprotein for its regulatory effect on bone cells that results cured in some pathological conditions, such as osteoporosis (Gao et al., 2016; Li, Zhu, & Hu, 2015). In the previous study, lactoferrin was reported that it had impact on osteogenesis showing protective effects on bone resorption of ovariectomized rats, and had a significantly promoting effect on JNK1/2, ERK1/2 and p38 mRNA expression (Du et al., 2011). Therefore, lactoferrin as an excellent origin to produce bioactive peptides for osteoporosis has aroused considerable attention.

Molecular docking works based on the “lock-key principle” which represented the function of ligand and receptor, simulating interaction between the receptor and small molecular biological ligand (Rawendra, Chang, Chen, Huang, & Hsu, 2013). Molecular docking predicts the ligand best model which suitable to the binding site of a macromolecular target. It serves a quick and high-throughput screening method to produce bioactive peptide from protein according the protein-ligand bindings principle (Thomsen & Christensen, 2006).

In the present study, the molecular docking method was used to screen and reveal the mechanism of osteogenic peptides from lactoferrin hydrolysates. Trypsin was utilized to hydrolyze lactoferrin to obtain a peptide mixture and the sequence of peptides in hydrolysates were identified with UPLC-Q-TOF-MS/MS and Mascot analysis. The interaction of these peptides and epidermal growth factor receptor (EGFR) was analyzed by Molecular docking software, and the peptides with better affinity to EGFR were screened. The potential peptides with osteoblast proliferation promotion activity were synthesized based on