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Research progress of metal chelating peptides

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Wei-Tao Lu conceived and drafted the first draft of this review and translated it; Chun-Ming Dong supervised the writing of the manuscript and guided the revision, review and editing of the manuscript.
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Abbreviations
His, histidine; Cys, cysteine; Glu, glutamic acid; Asp, aspartic acid; SFE, supercritical fluid extraction; RSM, response surface methodology; DBP, deer bone peptide; DBPCC, deer bone peptides chelated calcium; HBSGPH, highland barley brewer's spent grain protein hydrolysates; MS, mass spectrometry; NMR, nuclear magnetic resonance; UV-vis, ultraviolet-visible; FTIR, Fourier transform infra-red; CD, circular dichroism; XRD, X-ray diffraction; CPs, small-molecular-weight collagen peptides; IMAC, immobilized metal ion affinity chromatography; HAC, hydroxyapatite chromatography; IEC, ion exchange chromatography; RP-HPLC, reversed-phase high performance liquid chromatography; TOF-MS, time-of-flight mass spectrometry; IDA, iron-deficiency anemia.
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Abstract
Some metal elements, especially trace elements, have obvious nutritional and physiological functions on the human body, which can promote the growth and development of the body, regulate the function of the body and maintain the metabolism of the human body. Compared with the elements in the form of inorganic salts, metal chelating peptides are more easily absorbed by the human body. Metal chelating peptides efficiently provide various trace elements necessary for life activities, have antioxidant, antibacterial and other biological activities, and have broad application prospects in food, medicine, cosmetics and other fields. In this paper, the methods of preparation, separation and purification of metal chelating peptides from animals and plants were reviewed, the structure-activity relationship and antioxidant activity of metal chelating peptides were analyzed, and the development prospect of metal chelating peptides was prospected.

Keywords: chelation; peptides; metal; structural characterization; separation and purification; bioavailability

Background

Some metal elements, especially trace elements, have obvious nutritional and physiological functions to the human body; and are an indispensable part of human life activities. They maintain a certain concentration in human body fluids and various organs, and are not only part of the human body, but also components of enzymes and vitamins; maintain the pH and electrolytic balance of blood; participate in endocrine, affect gonad development, fertility, sexual function and glucose metabolism; closely related to material transport and energy metabolism [1-4]. Their content in the body is too low or too high, it will cause disease, and be harmful to health [5]. However, at present, the absorption rate of mineral supplements in the market is not high, and there are side effects such as increasing kidney burden [6-8], so people turn their attention to metal chelating peptides using food-derived peptides as dietary metal carriers [9].

Metal chelating peptides are formed by the coordination of the N-terminal amino group, C-terminal carboxyl group, amino acid side chain and carbonyl and imide groups with metal ions [10]. Peptides containing histidine (His), cysteine (Cys), glutamic acid (Glu) and aspartic acid (Asp) usually have strong metal ion chelating activity and are easy to bind to metal ions such as Ca^{2+}, Fe^{2+} and Zn^{2+} through coordination covalent binding or adsorption binding [11]. The prepared chelates have the advantages of high biological titer, fast absorption, and strong nutrition, as well as antioxidant, antibacterial, immunomodulatory, hypolipidemic and hypoglycemic activities. Now in the United States, Western Europe and other developed countries, the metal chelating peptide nutritional supplement industry has become more mature; as a more efficient and safer new supplement, peptide metal ion chelate has a better development prospect. This paper reviews the preparation and absorption mechanism of metal chelating peptides from animals and plants at home and abroad, in order to provide a reference for the future application and further research of metal chelating peptides.

Preparation and optimization of metal chelating peptides

At present, the preparation of metal chelating peptides is focused on the preparation of peptides and the chelating process of peptides with metal elements. With the extensive and in-depth study on the comprehensive utilization of food resources, more and more animal and plant raw materials from different sources are being used in the preparation of metal chelating peptides, especially the by-products of food processing. Foong [12] and others use supercritical fluid extraction (SFE) to extract active peptides from rice bran (rice processing by-products) to prepare metal chelating peptides. Peptides extracted from processed fish skeletons (such as cod) have the ability to bind calcium and can be used to prepare peptide calcium chelates [13-15].

Figure 1 depicts the flow chart for the synthesis of metal chelating peptides. Generally speaking, the first step in the preparation of metal chelating peptide is to extract the peptide (to obtain the carrier of metal chelating peptide). The main methods for preparing metal chelated peptide carriers are solvent extraction, enzymatic hydrolysis, chemical synthesis and so on. Because there are some safety problems such as high cost and chemical residue in solvent extraction and chemical synthesis, enzymatic hydrolysis is mainly used at present. Enzymatic hydrolysis has the advantages of mild conditions, high safety and easy to control the hydrolysis process. Still, Due to the differences in protease digestion sites, the chelating effect of the prepared peptides is often different. At present, the research on enzymatic hydrolysis is mainly focused on the screening of suitable enzymes, the optimization of enzymatic hydrolysis conditions and the improvement of the enzymatic hydrolysis process, in order to obtain peptides with high binding metal activity [16]. The condition of enzymatic hydrolysis is the key factor affecting the chelation rate [17]. The chelating conditions and the conditions of chemical modification of peptides also have significant effects on the chelation rate and chelate yield. Optimizing these conditions can achieve the purpose of fully using raw materials and maximizing economic benefits.

In the research at home and abroad, single factor experiment combined with orthogonal experimental design or response surface design (response surface methodology, RSM) is often used to optimize the enzymatic hydrolysis conditions and chelating conditions, in order to obtain a better metal ion chelation rate [18], in which the enzymatic hydrolysis conditions determine the degree of enzymatic hydrolysis. When the degree of enzymatic hydrolysis is in a certain range, the metal chelation rate is positively correlated with the degree of hydrolysis. Chelating reaction is affected by temperature, pH and other conditions. Polypeptide chelating metal reaction will release heat. If chelating temperature is too high, it will inhibit the reaction. when the temperature is low, the molecular movement speed is slow, resulting in low chelation rate [19]. Bi et al. [9] used pepsin to obtain calcium binding protein hydrolysate from deer bone. The optimum conditions for the preparation of peptide calcium chelate were determined by a single factor test. The optimum conditions were as follows: the concentration of deer bone peptide (DBP) was 1.535 mg/mL, the concentration of Ca^{2+} was 7.5 mmol/L, the concentration of pH was 7, the chelating time was 30 min and the chelating temperature was 37 ℃. Fang et al. [20] used alkaline protease to extract peptides from Manzhouli walnut, and then chelated with Ca^{2+} to obtain peptide calcium chelate. The optimum conditions for the preparation of peptide calcium chelate were determined by response surface methodology (RSM). The optimum conditions were as follows: peptide calcium mass ratio 3:1, pH 8, chelating time 40 min, chelating temperature 45 ℃, and the chelating rate was 69%. Wang [21] used trypsin to hydrolyze sesame protein to prepare metal (Fe^{2+}, Zn^{2+}) chelating peptides. The optimum enzymatic hydrolysis parameters were determined by a single factor test. The results showed that the optimum trypsin hydrolysis conditions were as follows: substrate concentration 5.0%, enzyme dosage 20 U/g, time 5 h. The peptides prepared under these conditions showed that the chelating ability of Fe^{2+} and Zn^{2+} were 90.9% and 93.5%, respectively. Hao et al. [22] extracted porcine blood protein to prepare peptide calcium chelate. Through orthogonal experiment, the optimum chelating conditions were determined as follows: peptide calcium mass ratio 3:1, pH 7.0, chelating time 45 min, chelating temperature 100 ℃. Under these conditions, the yield of calcium chelate is 63.8%. Thus, after the optimization experiment, the chelation rate of metal ion chelating peptides has been improved to a certain extent.

The type of protease determines the amino acid sequence of the peptide, which in turn determines the metal ion chelation of the peptide. At present, multi-enzyme combination is often used for simultaneous enzymatic hydrolysis or step-by-step enzymatic hydrolysis, or ultrasonic, microwave-assisted, pulsed electric field and other auxiliary means are used to improve the chelation rate of metal ions. Cui [23] and others use double enzymatic hydrolysis of protein raw materials to prepare bioactive peptides. Compared with single enzymatic hydrolysis, the yield of protein peptides is significantly increased. Ikram [24] studied the effects of ultrasound and heat treatment on the enzymatic hydrolysis of highland barley brewer's spent grain protein hydrolysates (HBSGPH). The results showed that the metal chelating activity of the peptide was enhanced after treatment. Wang et al. [25] used papain, alkaline enzyme and trypsin as raw materials to improve the extraction efficiency of sesame protein by enzymatic reaction of papain (0.5-2 U/mg, pH 7.0, 50 ℃), alkaline enzyme (≥ 2.4 Au/g, pH 8.5, 60 ℃) and trypsin (250 U/mg, pH 8.0, 55 ℃), respectively. Generally speaking, the degree of protein hydrolysis is greatly affected by raw materials, proteases, enzymatic hydrolysis conditions and other factors, so it is necessary to determine the best protease hydrolysis process for different substrates in order to efficiently prepare bioactive products with the assistance of modern technology.

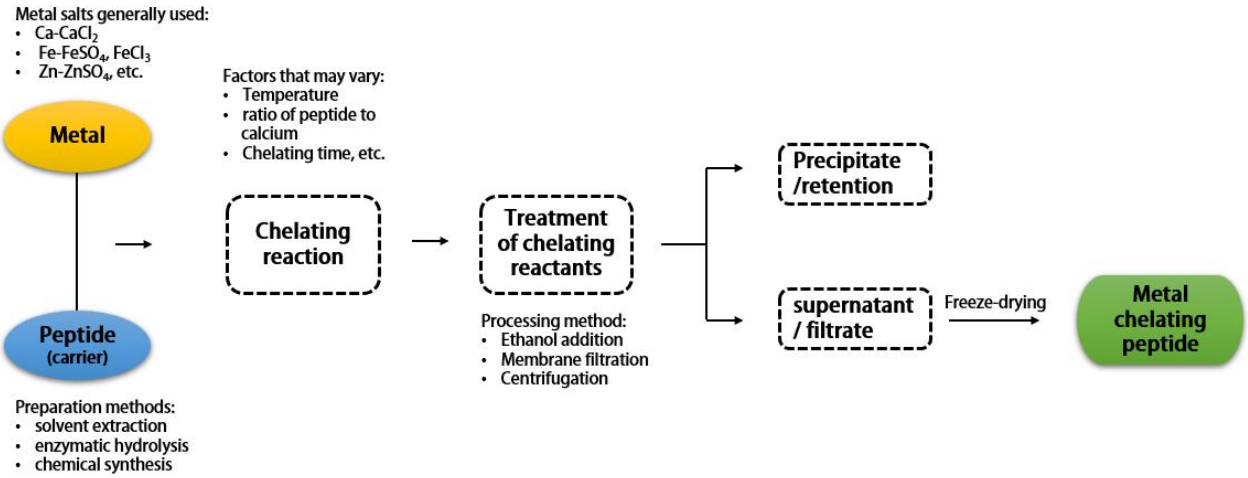

Figure 1 Flowchart of preparation of metal chelating peptides

Structural characterization and analysis of metal chelating peptide

The differences in the amino acid sequence and structure of the peptides and the types of metal ions determine the different chelation sites [11]. Peptides and metal chelates are two different substances. The common methods to identify chelating groups and analyze the structure and amino acid composition of metal chelating peptides are mass spectrometry (MS), nuclear magnetic resonance (NMR), ultraviolet-visible (UV-vis) absorption spectroscopy, Fourier transform infra-red (FTIR), circular dichroism (CD), X-ray diffraction (XRD) and so on [26]. The different structure of metal chelating peptides is bound to affect the function of chelates, so the study of the structure-activity relationship of chelates is of great significance to the development and utilization of metal chelating peptides.

Molecular size

It is found that the molecular weight of peptides has a very important effect on the chelation of peptides with metal ions, and some peptides with low molecular weight (< 500 Da) have good metal chelating activity [27]. The molecular weight first affects its chelating activity, and to a certain extent, the chelating ability is negatively correlated with the molecular weight of peptides. Guo et al. [28] identified the iron chelating peptide from Alaskan cod skin with a molecular weight of only 345 Da; Li et al. [29] found that low molecular weight peptides have higher iron chelating activity than high molecular weight peptides when they study the iron chelating peptides from broad bean protein. Guo et al. [30] extracted small-molecular-weight collagen peptides (CPs) from puffer fish skin, which has higher zinc chelating ability. Xia et al. [31] found that the iron chelating activity of small peptides from barley glutenin whose molecular weight is lower than 1 KDa is stronger.

Of course, it does not rule out that some polymer peptides also have good metal chelating activity. Miao et al. [32] isolated and identified four iron chelating peptides from casein hydrolysates, named casein hydrolytic peptides CHP-1, CHP-2, CHP-3 and CHP-4 with molecular weights of 830.6120 Da, 1012.5280 Da, 873.4440 Da and 829.4570 Da, respectively. When Seth and Mahoney [33] studied the binding of iron to chicken protein peptides, they found that most of the iron is bound to macromolecular peptides (> 10 kDa), while only about 10% is bound to small peptides and amino acids.

There are some inconsistencies in these results, partly because of the different sources and structures of peptides, and different binding properties of metal ions, and also because the methods used in different studies are different. Therefore, it is necessary to further explore the effects of different molecular weights on chelating activity.

Amino acid composition and sequence

The amino acid composition and sequence of peptides are also important factors affecting their metal chelating activity. Peptides usually containing histidine (His), cysteine (Cys), glutamic acid (Glu) and aspartic acid (Asp) have strong metal ion chelating activity and are easy to bind to metal ions such as Ca^{2+}, Fe^{2+}, Zn^{2+} through coordination covalent binding or adsorption. Some small peptides or special amino acid sequences such as "Asn-Cys-Ser" are considered to have high chelating activity [11]. Huang et al. [34] purified from shrimp enzymatic hydrolysate by ion exchange chromatography and Gmur25 dextran gel chromatography, obtained the peptide with the strongest chelating ability to Fe^{2+}, and found that the binding site of Fe^{2+} was glutamic acid (Glu) residue by infrared spectrum analysis. Three novel iron chelating peptides were isolated and purified from Pacific cod skin gelatin by Wu [35]. The amino acid sequences provide additional iron binding sites for GPAGPHGPPGKDGR, AGPHGPPGKDGR and AGPAGPAGAR, Lys, His and Asp. Torres-Fuentes et al. [36] found that small peptides containing 20%~30% histidine (His) from chickpea protein had higher copper chelating activity than other components with low histidine. Thus it can be seen that the chelating activity is easier to carry out when the peptide contains cysteine, glycine and histidine.

Special groups and chelating sites

By using infrared spectroscopy and nuclear magnetic resonance techniques, it was found that there were some amino acid groups and residues in the chelation process and binding sites between peptides and metal ions, such as carboxyl, carbonyl, sulfhydryl, negative charge and ionic bond can promote metal chelation [37]. For example, Wu [34] has isolated and purified three new iron chelating peptide groups from Pacific cod skin gelatin, in which the amino and carboxyl terminal groups and peptide bonds of the peptide skeleton, as well as the amino and imine groups of the arginine side chain are involved in the chelation. The coordination of metal ions with peptides occurs on amino, imino or carboxyl groups and is bonded in the form of monodentate covalent bonds, and it is found that the binding of small peptides to calcium is closely related to the carbonyl groups of peptides [38]. The chelating ability of metal chelating peptides containing residues Asp, Glu and His is mainly related to the carboxyl groups on Asp and Glu and imidazolyl groups on His [39].

When the chelating site was determined, the change of the characteristic absorption peak could reflect the binding of metal ions to the organic coordination groups on the peptide [40]. For example, Cui [41] extracted an octapeptide from sea cucumber egg pancreas and spontaneously combined with Ca^{2+} at a stoichiometric ratio of 1:1 to form a peptide calcium chelate. Infrared and Raman spectra show

that carboxyl oxygen atoms and amino nitrogen atoms located in Glu and Asp are potential calcium binding sites of sea cucumber polypeptides.

Phosphorylation is a common chemical modification method, the phosphorylation of peptides will affect the chelation with metal ions, and the chelation rate decreases with the decrease of peptide dephosphorylation level. Phosphorylation treatment can significantly increase the chelation rate when applied to metal chelating peptides [42]. Sun [43] and other enzymes hydrolyzed the protein in herring eggs and then phosphorylated with cyclic trisodium phosphate to obtain herring methionine phosphate peptide. Compared with the unphosphorylated peptide, the phosphopeptide had stronger calcium binding ability. It is speculated that the phosphopeptide has more carboxyl and serine phosphate residues that interact with Ca^{2+}. In summary, the chelating sites of peptide calcium chelation reaction are mainly amino group and carboxyl group, and the chelating activity of polypeptide with calcium ion is enhanced after chemical modification.

At present, the structural characterization of metal chelates at home and abroad can only determine the chelation between peptides and metal ions, which is limited to the analysis of chelating groups and special amino acids.

Isolation and purification of metal chelating peptides

Due to the continuity of the enzymatic hydrolysis process and the non-specificity of protease, the composition of enzymatic hydrolysis products is complex, and the target metal chelating peptides are mixed in the mixture of amino acids and short peptides, which need to be separated and purified. In order to further explore the structure, chelation sites and mechanism of the chelates, Table 1 shows preparation conditions and physiological activity characteristics of metal chelating peptides. Usually ultrafiltration membrane with specific molecular weight interception, immobilized metal ion affinity chromatography (IMAC) and hydroxyapatite chromatography (HAC) were used as the first step to roughly separate the enzymatic hydrolysates, and then further separated by dextran gel chromatography or ion exchange chromatography (IEC). Finally, the components with high purity and activity were obtained by reversed-phase high performance liquid chromatography (RP-HPLC), and their amino acid sequences were identified by mass spectrometry.

Table 1 Preparation conditions and physiological activity characteristics of metal chelating peptides

Metal	Peptide source	Metal precursor	Optimum synthesis condition	Identified/proposed peptide sequence or proposed amino acids or side groups involved	Isolation and purification	Physiological activity/ structural characterization	Reference
Ca	Pacific cod bone	$CaCl_2$	11 g $CaCl_2$: 30 g Pacific cod bone hydrolysate, 50 ℃ , pH 7.0 , 60 min	KGDPGLSPGK	HAC RP-HPLC	Compared with inorganic calcium supplements, it is better for human intestinal absorption.	[44, 45]
	Deer Bone	$CaCl_2$	1.535 mg/mL Deer bone peptide, 7.5 mmol/L Ca^{2+}, pH =7, 37 ℃, 30 min	Aromatic amino acids (Phe, Tyr and Trp)	*	The size distribution of the DBPCC become larger than the DBP. The molecular weight distribution of the DBPCC was mainly ranging from 2,000 Da to 3,000 Da, larger than that of the DBP (< 1000 Da).	[9]
	Sea cucumber ovum	$CaCl_2$	Peptide:metal = 1:6 (molar ratio) , 50 ℃ , pH 8.0, 20 min	NDEELNK	UPLC-QTOF-MS/MS	Compared with $CaCl_2$, it is better for human intestinal absorption	[46]
Fe	Duck egg	$FeSO_4$	Peptide:metal = 2:1 (w/w) 0.2 g ascorbic acid/g peptide, 40 ℃, pH 5.5, 40 min	Pro-Val-Glu-Glu/ Arg-Ser-Ser	HPLC-ESI-MS /MS	Compared with $FeSO_4$, the complexes of duck white egg peptides-Fe is more beneficial for IDA (iron deficiency anemia) treatment	[47]
	Barley protein	$FeSO_4$	Peptide:metal = 2:1 (molar ratio) 25 ℃, pH 7.0, 120 min	SVNVPLY	IMAC MS/MS	Ferritin synthesis: Complex Fe-SVNPLY > $FeSO_4$	[48]
	β-lactoglobulin	$FeCl_3$	Peptide:metal = 40:1 (w/w) 25 ℃, pH 7.0, 30 min	Asp, Glu, and Pro	IMAC	Effectively improve hematological parameters and iron bioavailability	[49, 50]
Zn	Sesame	Zn(II)-IDA-chitosan (crosslinking reaction)	Not specified	Six new peptides: Asn-Cys-Ser, Arg-Gln-Arg, Arg-Lys-Arg, Ile-Ala-Asn, Leu-Ala-Asn, Ser-Met	IMAC HPLC–MS LC-MS/MS	The purified peptides have strong metal binding abilities. When these peptides are synthesized, they also show significant chelating activity.	[51]
	Bovine whey protein	$ZnSO_4$	10 mg/mL peptide and 50 uM Zn, pH 7.0, 60 min	Carboxylate ion, and sidechain carbonoxygen of Asp/Glu and Ser/Thr	*	Complexes of Zn-whey peptides improved Zn dializability	[52]

*, Nor identified or proposed; DBPCC, deer bone peptides chelated calcium; DBP, deer bone peptide

In the first stage of purification of metal chelating peptides, the high selectivity of IMAC makes it a good technology for the purification of proteins and peptides. It can separate peptides with different metal ion chelating abilities and is widely used in peptide separation [53]. Wang et al. [51] purified 6 zinc chelating peptides from sesame enzymatic hydrolysate by IMAC and RP-HPLC. Guo et al. [28] successfully isolated and identified iron chelated peptides from the hydrolysate of cod skin by IMAC.

RP-HPLC is usually used in the final purification stage of metal chelating peptides. The components isolated and purified by RP-HPLC can be used to determine the amino acid composition and peptide structure directly. RP-HPLC is widely used in the separation and purification of metal chelating peptides because of its high separation efficiency, good selectivity and low price of mobile phase [34]. Zhang et al. [40] used HAC and RP-HPLC to separate the hydrolysate of cod bone in Taiping Ocean, and obtained Peptide-K, which was identified as KGDPGLSPGK and showed high calcium binding activity. Cai et al. [54] used gel filtration chromatography and RP-HPLC to isolate and purify a new peptide (Phe-Tyr) with specific calcium binding ability from the genus Schizomonas.

After the enzymatic hydrolysis product was separated and purified to get a single component, the amino acid sequence of the peptide was identified by mass spectrometry. Zhao et al. [55] obtained a calcium binding peptide with molecular weight of 237.99 Da from whey protein hydrolysate by anion exchange chromatography, Sephadex G-25 gel filtration and RP-HPLC. Its amino acid sequence was identified as Gly-Tyr by time-of-flight mass spectrometry (TOF-MS), which was 122% higher than that of the whey protein hydrolysate complex. Jung et al. [56] separated and purified the polypeptide calcium chelate by hydroxyapatite affinity chromatography, and determined its amino acid sequence by electrospray ionization tandem mass spectrometry (ESI-QTOF). It was found that its amino acid sequence was similar to actin. There are many options for the separation and purification of peptides, but there are problems such as too long time, low efficiency, etc. There are many choices for the separation and purification of peptides, but there are some problems such as too long time and low efficiency. From an industrial point of view, it is necessary to develop more efficient technologies and develop and prepare value-added products on a large scale.

Physiological activity of metal chelating peptides

Study on bioavailability

Peptide calcium chelates have good solubility, absorption and stability, and can effectively transport calcium ions through intestinal epithelial cells in a weakly alkaline environment with a pH of 7.2 in the gastrointestinal tract, and are absorbed and utilized by the body. This provides a good basis for application in food production [57]. However, when it is put into production as a human mineral supplement, *in vitro* digestion experiments, cell experiments and mouse experiments are needed to determine its safety and bioavailability.

Taking peptide-calcium chelates as an example, the main methods to evaluate the bioavailability of peptide-calcium chelates are cell models *in vitro* and animal experiments *in vivo*. Cell experiments can well determine the effect of metal chelates on promoting ion absorption. *In vitro* cell models include Caco-2 cell model and HT-29 cell model. Caco-2 cells come from human colon cancer cell line, and automatically differentiate into structural characteristics similar to human intestinal epithelial cells *in vitro*, and have a similar transport system and functional expression of marker enzymes to small intestinal epithelial cells. HT-29 cell line is often used to study the model of calcium absorption *in vitro* because of its ability to differentiate into different cell modes. Zhu et al. [58] found that zinc chelate with peptides from wheat germ protein has higher bioavailability of zinc in Caco-2 cells than $ZnSO_4$. Malison A et al. [59] identified a high content of calcium and isolated peptides with strong metal binding ability in the by-products of chicken claw soup. The toxicity and bioavailability of peptide calcium chelates were determined by cell experiments. These results show that peptide calcium chelates play an important role in promoting calcium absorption and transport.

Animal models are widely used to study bioavailability *in vivo*. Calcium chelated peptides can promote calcium absorption, increase bone mineral density and strength, and reduce the risk of osteoporosis [60]. Zhang [40] verified that the calcium binding peptide prepared from Pacific cod bone has anti-osteoporotic activity by establishing the model of osteoporosis in ovariectomized rats. The results show that the chelating peptide can increase the bioavailability of calcium and serum calcium, reduce the bone turnover rate, and improve osteoporosis.

IDA (iron-deficiency anemia) mouse model was used to study the mechanism of promoting iron absorption in peptide-ferrous ion chelates. Bo et al. [47] prepared peptide-ferrous ion chelates from desalted duck egg albumin peptide. After the application of IDA mouse model, compared with the absorption of ferrous inorganic acid, the chelate significantly increased the body weight of IDA mice and effectively improved the binding ability of transferrin such as hemoglobin (Table 1).

At present, there has been a more in-depth exploration of the mechanism of metal chelating peptides promoting absorption. It has been found that metal ions must be transformed into organic state with the help of coenzymes after being ingested by the human body. That is, chelates are formed with amino acids or peptides for absorption, transport, storage and utilization [61]. Because the body's demand for protein is not a single absorption of free amino acids, small peptides are also a form of absorption and have an independent absorption mechanism with free amino acids in the body [62]. After forming organic states with peptides, metal ions have some advantages that inorganic states do not have, such as the stable structure of chelates to avoid the precipitation or adsorption of mineral elements by other nutrients during intestinal absorption. In the chelated state, metal ions are absorbed through the absorption channels of amino acids and peptides rather than metal ions, so as to avoid antagonistic competition with other metal ions absorbed by the same channel and improve the absorption efficiency. Peptide-metal ion chelate is not only the main form of absorbing and transporting metal ions, but also the intermediate in the process of protein synthesis, which can reduce many biochemical processes and save energy consumption [63].

Generally speaking, metal chelates have higher digestive stability. Compared with inorganic metal element supplements, metal chelates have higher bioavailability and better effect of promoting iron absorption, so they have good application prospects.

Antioxidant activity

Metal ions such as copper and iron ions *in vivo* contain unpaired electrons. As the main catalyst of Fenton reaction, metal chelating peptides which can bind copper and iron ions can block the production of free radicals and have certain antioxidant activity. By chelating with metal ions, peptides can change the physical position of transition metals and hinder the interaction between metals and lipids and peroxides, thus realizing antioxidation [64]. Therefore, metal chelating peptides are also considered to have certain antioxidant activity. As early as 1986, it was found that the copper chelated peptide obtained from chickpea protease hydrolysate had good antioxidant activity and could effectively prevent the oxidation of β-carotene [65]. Yuan et al. [66] hydrolyzed Grifola frondosa protein with alkaline protease to obtain hydrolysate, which was chelated with ferrous ions to obtain metal chelate. *In vitro* studies showed that peptide iron chelate had good immune enhancement activity on splenocyte proliferation and cytokine secretion, and the iron chelating peptide of Grifola frondosa remained this activity after digestion *in vitro*. Recently, it has been found that the antioxidant activity of metal chelating peptides is not only related to its ability to chelate metal ions to prevent the formation of free radicals, but also to some of its own active groups. Small peptides that can chelate metals usually contain indolyl, imidazole and sulfhydryl groups derived from

tyrosine, histidine and cysteine, which also play an important role in the antioxidation of peptides [67]. In a word, the further study on the antioxidant activity of metal chelating peptides will have an important impact on the further development and utilization of metal chelating peptides.

Antibacterial activity

Some peptides and metal ions have antibacterial activity themselves. after chelating with metal ions, the antioxidant or antibacterial activity of peptides is generally higher than that of the original peptides, and some peptide calcium chelates and peptide iron chelates have been proved to have antibacterial activity. Studies have confirmed that the antioxidant activity of peptide calcium chelates not precipitated by ethanol is equivalent to 94.43% of tocopherol, while chelates precipitated by 80% ethanol have certain antibacterial activity, such as inhibiting the growth of *Bacillus subtilis* and *Staphylococcus aureus* [68]. In addition, Ding [69] carried out antimicrobial circle experiments on polypeptide-calcium chelates and found that polypeptide-calcium chelates had certain inhibitory effects on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus Niger* and so on.

The antibacterial activity of some metal chelating peptides is close to or even better than some common antimicrobial agents which have been widely used at present, but as one of the raw materials of chelates, peptides come from natural animal and plant proteins, so there is almost no safety problem. Therefore, chelates as antimicrobial agents in food, cosmetics and other industries have great advantages and prospects.

Discussion and prospect

For a long time, the bioavailability of inorganic mineral supplements is low, and metal chelating peptides have higher bioavailability and stability than ordinary mineral supplements. At the same time, it has the advantages of fast absorption, and strong nutrition, as well as antioxidant, antibacterial, immunomodulatory, lipid-lowering and hypoglycemic activities. We always think of "the more bioavailable, the more reactive", but metal chelating peptides "protect" metal ions chemically or physically, thus achieving the effect of antioxidation and improving bioavailability; and metal chelating peptides can hinder the interaction between metal ions and dietary components and reduce side effects [70].

However, from the perspective of food industry standards, in order to consider the impact of the source of metal chelating peptides, it is necessary to further evaluate the bioavailability. Metal chelates obtained from protein hydrolysates can also be used as food ingredients, but the binding force, structure, mineral release and absorption of these chelates, especially food matrix factor, must be studied. At present, metal chelating peptides are not enough to be effectively applied to the food industry. These chelates can only be used as potential applications of food ingredients, and can only be applied to some special populations (For example, peptide iron chelates applied to anaemia population). At present, it is impossible to replace ordinary mineral supplements in the large-scale market of population consumption.

Generally speaking, metal chelating peptides still have the following problems: (1) In the process of production, people can not study it qualitatively or quantitatively, which makes it difficult to control the quality of metal chelating peptides; (2) There are few studies on its safety evaluation; (3) The efficient separation and purification of chelates need to be improved by 4.5%. In terms of industrial production, at present, the production cost of metal chelating peptides is high and the amount of synthesis is low. So people still tend to buy inorganic salt elements. Therefore, it is necessary to explore the optimal technological conditions of the chelating process, deeply study the chelating mechanism, develop low-cost, high-performance proteins and metal chelating peptides, and solve the problems in practical applications.

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