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Metal solubility enhancing peptides derived from barley protein

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ABSTRACT

Mineral supplements are required to be soluble as their bioavailability is highly correlated to their solubility in body fluids. In this study, metal binding capacity of barley protein hydrolysates and their purified fractions was investigated and expressed as increase in solubility of metal ions. Metal ions in the presence of hydrolysates exhibited a remarkable increase in solubility: 118, 32, 10, 29 and 35-fold for Fe^{2+}, Fe^{3+}, Ca^{2+}, Cu^{2+} and Zn^{2+}, respectively. A mixture of low molecular weight peptides possesses a synergistic combination of both charged and hydrophobic residues and achieves the best binding metal ions. Electrostatic interactions via charged side chains and coordination binding with His and Cys, initially attract the metal ions and, afterward, hydrophobic interactions and aromatic ring stacking stabilize the positioning of metal ions in the structure of the peptide. Barley hordein hydrolysates show potential as dietary supplements that enhance both mineral solubility and bioavailability.

1. Introduction

Metal ions are essential and play important roles in all classes of biological processes. Metal ions are closely related to the functional three-dimensional structure of up to one third of all proteins (Lyons & Eide, 2006). Mineral absorption is a complex process since absorption efficiency depends not only on a number of host-related factors such as physiological, biochemical, and hormonal condition, but also on both the dietary intake of the mineral and its solubility. Bioavailability, defined as the incorporated portion of the ingested mineral into the body is largely related to the solubility of the minerals in body fluids. Many studies have discussed the solubility of minerals as a proper predictor of the bioavailability (Kim, Lee, & Lee, 1995; Perales, Barbera, Lagarda, & Farre, 2005; Perales, Barbera, Lagarda, & Farre, 2006). The vast majority of mineral absorption occurs in the proximal part of small intestine, where the pH drops from 7 at fasting to around 5.5 after ingestion of food. The mild acidic condition of intestinal content favors the absorption of minerals in the proximal duodenum, while the gradual increase in pH (to 7.49 ± 0.46) by the terminal ileum decreases mineral solubility and therefore, the absorption (Karava & Mahoney, 2011). Research of "chelating supplements" used in food fortification to compensate for mineral deficiency is currently very intense (Huang, Ren, & Jiang, 2011). The metal binding activity of proteins and peptides is also associated with an antioxidant capacity, because metal ions coordinated by amino acids in the prosthetic groups are unable to generate reactive oxygen species and act as prooxidants (Saiga, Tanabe, & Nishimura, 2003).

In recent years bioactive peptides with chelating properties have attracted a growing interest in the food and pharmaceutical industry. Peptides are capable of binding to other substances and improving their stability, solubility and bioavailability due to their various residues with potential donor side chains and spatial structures (Kozlowski, Bal, Dyba, & Kowalik-Jankowska, 1999). They also facilitate the absorption of chelated micro-and macroelements in the digestive tract (Clemente, 2000).

Previous research reported that hydrolysates as a source of chelating peptides can be derived from milk whey (Kim et al., 2007), egg yolk phosvitin (Choi, Jung, Choi, Kim, & Ha, 2005) and shrimp processing by-products (Huang et al., 2011). The highest chelating capacity was reported for phosphopeptides obtained from milk protein hydrolysis exhibiting chelating properties toward zinc (Miquel & Farre, 2007), iron (Bouhallab et al., 2002) and calcium (Cross, Huq, & Reynolds, 2007). Most of the research focuses on peptides chelating one or two metal ions such as iron, zinc or calcium. Systematic research on binding ability of peptides toward different minerals is extremely limited. In particular, in the large body of published studies concerning the mineral-binding peptides, there is no evidence of metal-binding peptides derived from plant proteins. Emerging biofunctionalities of plant proteins have raised their market demand due to lower cost and energy consumption compared to animal protein sources.

Recently, growing attention has been focused on the potential functionalities of barley proteins as a valuable by-product of the brewing industry (Mohamed, Hojilla-Evangelista, Peterson, &

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Biresaw, 2007). Barley proteins, consisting mainly of hordein and glutelin, have a high amount of glutamine and hydrophobic and non-polar residues. The unique structural features of barley proteins, attributed to their amino acid sequence, offer the possibility of producing bioactive peptides upon proteolytic degradation (Bamdad, Wu, & Chen, 2011). In our previous work, enzymatic hydrolysis of barley protein resulted in peptides with a high content of charged residues, due to partial deamidation of Gln side chains. Metal binding via the exposure of such charged groups is the main mechanism responsible for their strong ferrous ion chelating capacity. Further purification of the hydrolysates by PR-HPLC improved their ferrous ion chelating activity. The sequences identified in purified fractions contained diverse amino acids such as Ser, Ala, Glu, Val, and Pro, which may enhance the ferrous ion chelation capacity in addition to exposing charged groups. Therefore, it is hypothesized that barley protein may bind well many other mineral ions after enzymatic hydrolysis. This research investigates the binding properties of barley protein hydrolysates toward different metal ions including Fe^{2+}, Fe^{3+}, Ca^{2+}, Cu^{2+} and Zn^{2+}. Barley hordein was hydrolyzed by different proteolytic enzymes and then fractionated based on molecular weight using ultra-filtration and hydrophobicity using RP-HPLC. The metal binding capacity of barley protein hydrolysates and their purified fractions were systematically investigated and expressed as an increase in metal ion solubility. The metal binding capacity is discussed in relation to peptide structure. Value-added opportunities exist for barley hordein as a new metal ion binding ingredient in dietary supplements to enhance mineral solubility and bioavailability, and to benefit barley producers and processors to enhance their market sustainability.

2. Materials and methods

2.1. Chemicals and reagents

Barley grains (cv. Falcon) were kindly supplied by Dr. James Helm, Alberta Agricultural and Rural Development, Lacombe, AB, Canada). Alcalase 3.0T was obtained from Novo Nordisk (3 AU/g solid, Novo Nordisk, Bagsvaerd, Denmark). Flavourzyme ($\geqslant$500 U/g), pepsin (800–2500 U/mg protein) and trypsin (1500 U/mg) were obtained from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). All analytical grade chemicals and reagents were purchased from commercial sources. Certified metal standard solutions were obtained from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada).

2.2. Enzymatic hydrolysis of barley hordein

Barley hordein (91.6% protein content) was extracted using ethanol solution according to Wang et al. (2010). Hydrolysis was performed by different proteolytic enzymes (alcalase, flavourzyme, pepsin and trypsin) under continually monitored optimum conditions for each enzyme (pH 8, 50 °C; pH 7, 50 °C; pH 2, 37 °C and pH 7, 37 °C, respectively). At the end of the hydrolysis time, each enzyme was thermally inactivated and the hydrolysates were freeze dried and kept frozen until analyzed further.

2.3. Determination of mineral salt solubility at various pHs

In order to obtain the optimum pH condition for the chelation assay, mineral salts (including $FeCl_2$, $FeCl_3$, $CaCl_2$, $CuSO_4$ and $Zn(O_2CCH_3)_2$ were dissolved at three different concentrations (0.1, 0.5 and 1 mM) in buffer at different pHs (3.0, 4.0, 5.0, 6.0, 7.0, and 8.0). Various pHs were obtained using appropriate buffers at 0.1 M (KCl–HCl, phosphate buffer and HEPES buffer. After vigorous shaking at room temperature for 2 h, samples were centrifuged at 2500*g* for 20 min at 23 °C (Beckman Coulter Avanti J-E Centrifuge System, CA, USA) to remove the insoluble minerals. The amount of soluble minerals in the supernatant was measured by atomic absorption spectrophotometer (Fast Sequential Atomic Absorption Spectrometer, Varian AA240FS, Australia). The solubility was measured as the concentration (μg/ml) of metal ions in the supernatant, and percent solubility was calculated based on the dry weight of each mineral salt.

2.4. Determination of metal-binding capacity of barley hydrolysates

Based on the mineral solubility results, pH 7 was selected for Fe^{2+}, Fe^{3+} and Cu^{2+}, while pH 8 was selected for Ca^{2+} and Zn^{2+} binding assay. Barley protein hydrolysates were dissolved in HEPES buffer at pH 7 or 8 to a final protein concentration of 1% (w/v). Metal ion solutions were prepared in three concentration levels (0.1, 0.5 and 1 mM) and then added to hordein hydrolysate solutions, followed by shaking at room temperature (approximately 20 °C) for 2 h to form metal-bound peptides. The solubilized minerals when bound to peptides were recovered by centrifugation at 2500*g*, 10 min, 25 °C, and the resulting supernatants were subjected to atomic absorption analysis. A standard curve was generated in the appropriate concentration range provided by the instrument instruction manual. Metal-binding capacity was calculated as the increase in concentration (μg/ml) of metal ions in the supernatant in the presence of peptides compared to the control.

$$\text{Solubility of metal ions } (\%) = (C_{pep.}/C_{tot.}) \times 100$$

where $C_{pep.}$ represents the concentration (μg/ml) of metal ion in supernatant in the presence of peptides or positive control and $C_{tot.}$ represents the total concentration (μg/ml) of metal, corresponding to 0.1, 0.5 and 1 mM salt before addition of hydrolysates or peptide fractions. The control included the metal salt solution without the hydrolysates or peptide fractions. Glutathione (GSH), a well known metal binding short peptide, was used as positive control at 0.5 mM concentration.

2.5. Fractionation of metal-binding hydrolysates by ultra-filtration

The lyophilized barley hydrolysates were dissolved in deionized water and passed through an ultra/diafiltration system equipped with Centramate Cassettes filtration system (T-series Omega, PALL Life Science, Ann Arbor, MI, USA) using membranes with molecular weight cut off values of 10, 5 and 1 kDa. The fractions with molecular weight (M_w) distribution of >10 kDa, 5–10 kDa, 1–5 kDa and <1 kDa were collected, lyophilized and stored at 4 °C. The effect of peptide fractions in improving the solubility of Fe^{2+}, Fe^{3+}, Cu^{2+}, Ca^{2+} and Zn^{2+} were evaluated using the same method as described in Section 2.4. Mineral salt concentrations were 1 mM in these tests and the concentration of the peptide fractions was kept constant (1%, w/v) during all assays.

2.6. Fractionation of metal-binding hydrolysates by RP-HPLC

The most potent membrane fraction was further fractionated using Gilson PLC2020 system equipped with a semi-preparative reversed-phase column (Zorbax 300SB-C8, 4.6 × 250 mm). A linear gradient mixture of solvent A (0.1% trifluoroacetic acid (TFA) in water) and solvent B (0.1% TFA in acetonitrile (ACN)) was performed as follows: 5–40% B over 30 min, 40–90% B over 10 min and then 5 min at 90% B. The peaks corresponding to peptides were collected in five fractions and freeze-dried. The solubility of metals in the presence of these fractions was evaluated using the same method as described in Section 2.4.