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Structure-function relationships of hydroxyl radical scavenging and chromium-VI reducing cysteine-tripeptides derived from rye secalin

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ABSTRACT

The aim of the study was to determine the activity of four rye peptides and molecular descriptors responsible for the detected biological function. The activity was determined using hydroxyl radical scavenging and chromium-VI (Cr(VI) reducing assays while the density functional theory (DFT) was used for molecular descriptors (i.e. structure-activity relationships). It was found that at pH 7.4, peptide CQV had the highest Cr(VI) reducing activity (76%) followed by QCA (30.8%) while other peptides had less than 25% reduction. All tested peptides were less active at pH 3.0 and this was due to poor spatial proximity of thiol and amine on the glutamine side chain. In the hydroxyl radical scavenging assay, CQV had the highest activity with 28.9 ± 1.3% inhibition of the formation of HO˙ radicals compared to 19.0–13.6% for other peptides. Cysteine at the N-terminal was important for both the reduction of chromium (pH 7.4) and the HO˙ activity because S–H bond energies at that position were lower based on DFT calculations.

1. Introduction

The toxicity of hexavalent chromium (Cr(VI)) is due to the formation of Cr-DNA adducts and to the oxidation of biomolecules by excessive production of hydroxyl radicals (Ye et al., 1999). Small molecules, such as ascorbate and lipoic acid, can reduce the toxicity of Cr (VI) as a result of radical scavenging activity and rapid reduction of Cr (VI) (Sugiyama, Tsuzuki, & Ogura, 1991; Zhitkovich, 2011). Other reducers are cysteine and glutathione (Wiegand, Ottenwälder, & Bolt, 1984). In a previous work, hydrolyzed oat proteins were demonstrated to possess Cr(VI) reducing activity (Tsopmo, Gao, & Baakdah, 2014) but other hydrolysates or sulfur containing peptides have not been investigated. Cereal storage proteins (i.e. prolamins) are characterized by unusual amino acid compositions with high proline and amide nitrogen concentrations. Their common names are secalins (rye), gliadins (wheat), and hordeins (barley). The nutritional quality of this group of proteins is low because of their lesser amount of the essential amino acid lysine (Tatham & Shewry, 2012). Proteases have been used to produce hydrolysates or peptides with activities, such as antioxidant and anti-hypertensive, from wheat and barley (Bamdad, Wu, & Chen, 2011). Although, amino acids, such as Tyr, Trp, His and Cys, can contribute to the antioxidant activity of peptides, there is little information on structure-activity relationships of peptides as most works have focussed on polyphenols (Borgohain, Guha, Pratihar, & Handique, 2015; Cai, Chen, Xie, Zhang, & Hou, 2014). The discovery of novel bioactive peptides can then be accelerated by investigating their structure-activity relationships using computational methods. Previous works have shown that parameters, such as bond dissociation energy, ionization potential, steric hindrance, hydrogen bonding energy, proton dissociation enthalpy and proton affinity, can be related to functionalities of molecules (Xue, Zheng, An, Dou, & Liu, 2014; Yehye et al., 2015). A computational analysis based on the density functional theory (DFT) was used to calculate and rationalize the antioxidant activity of thiosemicarbazide derivatives (Nazarbahjat et al., 2014). DFT was also used to show that the reaction of aromatic amines with alkoxyl radicals in aprotic solvents depended on the N–H bond dissociation energy and the stability of the aminyl radical (Lucarini et al., 1999). Thousands of small peptides (2–6 amino acids) were generated with *in silico* methods from proteins of fifteen food commodities, and many were predicted to possess potent angiotensin converting enzyme (ACE)-inhibiting properties (i.e. IC_{50} < 10 μM) (Gu, Majumder, & Wu, 2011). Secalins are major storage proteins present in rye but there is no computational study regarding the activity of its peptides. This might be because rye is of lesser economic importance compared to other cereals. The aim of this study was to investigate the ability of peptides derived from a rye secalin protein to reduce chromium VI and scavenge hydroxyl radicals

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that generally accompany this reduction. It also aimed to determine molecular descriptors responsible for the activity.

2. Materials and methods

2.1. Chemicals and materials

Potassium phosphate monobasic, glutathione (Glut), sodium phosphate monobasic dihydrate, citric acid monobasic, potassium dichromate, ferrous sulfate hepahydrate ($FeSO_4{\cdot}7H_2O$), 1,10-phenanthronine and hydrogen peroxide were purchased from Sigma-Aldrich Co (Oakville, ON, Canada). Microplate spectrophotometer model Epoch™ controlled by Gen5™ data analysis software (Fisher Scientific, Nepean, ON) was used to measure the absorbance of samples. Rye secalin (UniProtKB/TrEMBL i.d. Q9FR41) was digested using proteinase K through a simulated digestion using UniProt PeptideCutter available at http://web.expasy.org/cgi-bin/peptide_cutter/peptidecutter.pl? Q9FR41. Two tripeptides containing cysteine were identified, CQV and QCA. These were then synthesized at 95% purity by GenScript Inc. (Piscataway, NJ, USA). The cysteine positions were also changed to obtain, QCV and QVC.

2.2. Chromium reduction assays

Chromium (VI) reduction assays were performed based on a reported procedure at neutral and acidic conditions (Tsopmo et al., 2014). At pH 7.4, 0.1 M phosphate buffer (pH 7.4) was used to make 0.2 mM of a Cr(VI) solution from potassium dichromate $K_2Cr_2O_7$, 5 mM of peptides (CQV, QCV, QVC, QCA) and control (Glut). At pH 3.0, citric acid phosphate buffer (0.12 M, pH 3.0) was used to make 1 mM of $K_2Cr_2O_7$, and 1 mM solution of peptides and control. For analysis, 100 µl of each peptide was transferred in triplicate into a 96-well clear microplate followed by addition of 100 µl of Cr(VI). The control contained 100 µl of buffer instead of sample. The plate was sealed incubated at 37 °C for 1 h. Readings were recorded after at 30 min at 370 nm. A kinetic assay was performed to determine the reaction rate of each peptide. Concentrations were 0.2, 0.4, 0.6, 0.8 and 1.0 mM at pH 3.0 while they were 1.0, 2.0, 3.0, 4.0 and 5.0 mM at pH 7.4. Data were recorded at 15 s intervals for 60 min at 370 nm. Pseudo-first-order rate constants were derived from plots of $\ln(A_{obs} - A_{\infty})$ versus time.

2.3. Hydroxyl radical scavenging assay

The assay was performed based on the generation of HO· radicals from a Fenton reaction between ferrous ions and hydrogen peroxide (Vanvi & Tsopmo, 2016). Peptides and glutathione were prepared at 1.6 and 3.2 mM in phosphate buffer (0.1 M, pH 7.4). In a 96-well plate, the following were added in quadruplicate: peptide (50 µl), 1,10-Phenanthroline (50 µl, 3 mM) and iron(II) sulfate (50 µl, 3 mM). To initiate the reaction 50 µl of 0.03% hydrogen peroxide (H_2O_2) was added and the plate was incubated at 37 °C for one hour before absorbance reading at 536 nm. The percentage inhibition was calculated as reported (Alrahmany & Tsopmo, 2012).

2.4. Structure-activity study using density functional theory

DFT calculations were performed using GAMESS-US (Schmidt et al., 1993), with the 6-311G(d,p) basis set and the M06-2X functional (Zhao & Truhlar, 2008). Antioxidant molecules can function either through the homolytic bond dissociation that provides a hydrogen to saturate the oxidizing species or by giving an electron to saturate the oxidant. To determine which of the two mechanisms was dominant, thiol (–SH) bond dissociation energies (BDE) and ionization potentials (IPs) for the five molecules (CQV, QCV, QVC, QCA, and Glut) were calculated, using Eqs. (1) and (2), respectively.

$$E_{RS\cdot} + E_{H} - E_{RSH} = BDE \quad (1)$$

$$E_{RSH^{+}} - E_{RSH} = IP \quad (2)$$

Structural optimizations were carried out for all ionic and neutral, radical and closed-shell species in the two equations. The reported bond dissociation energies and ionization potentials include the contributions of zero-point energies.

2.5. Statistical analysis

Results for biological activities are presented as means ± standard deviation from replicates (n = 4). One way ANOVA was used, differences between means were calculated using Tukey's test. Statistical significance was set to $p < 0.05$. Analyses were completed with IBM SPSS software version 22 (Armonk, NY, USA). Pearson correlation coefficients were used to determine relationships between data.

3. Results and discussion

3.1. General

Computational or *in silico* methods are useful tools in structure-activity relationship analysis of peptides derived from food proteins. Secalins are major storage proteins present in rye but possess low nutritional value. To enhance the value of this group of proteins, *in silico* digestion was performed using Proteinase K, a protease with broad specificity that cleaves predominantly at bonds adjacent to the carboxyl group of aliphatic and aromatic amino acids with blocked alpha amino groups (Ebeling et al., 1974). Two tripeptides, CQV and QCA, were selected, as previous studies have shown that redox properties of thiol groups are important for metal reducing and antioxidant properties (Ramdon, Dixon, & Dasgupta, 2002; Wiegand et al., 1984). The position of cysteine was varied for structure-activity relationship investigations.

3.2. Chromium VI reducing activity

The Cr(VI) reducing properties of the four peptides and glutathione (Glut) were determined at both neutral and acidic environment to reflect what might happen *in vivo*. The concentration used was 1.0 mM Cr (VI) in acidic condition compared to 0.2 mM for neutral environment and this is because of difference in stabilities of dichromate at both conditions (Ramdon et al., 2002; Tsopmo et al., 2014). Data showed that at pH 7.4 (Fig. 1A), CQV was the most potent and reduced Cr(VI) by 73 ± 2.6%. It was followed by QCA and QCV with a reduction of 30.8 ± 2.8% and 25.5 ± 2.0%, respectively. The least active QVC, 11.7 ± 2.8 had similar activity to Glut. The Cr(VI) reducing activity found for Glut was not different to that of the same peptide from a previous study (Tsopmo et al., 2014). Cysteine at the N-terminal of CQV enhanced the activity by almost 3-fold compared to the one with cysteine at the C-terminal. It appeared that the proximity of the thiol group and the amide on the side chain of glutamine might have facilitated the formation of thiolate-Cr(VI) complex and subsequent reduction according to the general scheme proposed for some thiols (O'Brien, Wang, & Wyatt, 1992).

In acidic solution (Fig. 1A), QVC, QCA and Glut had similar reducing power 33.6–37.0% ($P > 0.05$) however, it was higher ($P < 0.05$) compared to the activity of CQV (24.5 ± 2.6) and QCV (24.7 ± 2.8). Peptide CQV had the highest activity at neutral pH but not at the acidic pH. This is in accordance with other studies where, for example, digested oat proteins in the presence of Cr(VI) behaved differently as well as depending on the pH of solutions (Tsopmo et al., 2014). Literature data showed that the reduction of chromate by thiol-containing molecules initially proceeded via formation of thiolate-Cr(VI) complex which may then at low pH decompose by acid-catalyzed pathway. However, in neutral solution, a second mole of thiol compound is useful for the reduction (Lay & Levina, 1996). Overall, tripeptides have