Food Chemistry 141 (2013) 2011–2016

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Oxidative modification of a proline-rich gliadin peptide

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ARTICLE INFO

Article history:
Received 11 October 2012
Received in revised form 19 March 2013
Accepted 2 May 2013
Available online 24 May 2013

Keywords:
Protein oxidation
Degradation
Gliadin
Proline
Dityrosine
Fenton reaction
Coeliac disease
ELISA

ABSTRACT

Prolamins are proline-rich proteins occurring in cereal grains. Prolamins of wheat, barley and rye, or gluten protein, can cause coeliac disease in individuals not tolerating gluten. Degrading harmful prolamins can reduce their toxicity. A model peptide sequenced in α-gliadin, 33-mer (LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF), was chosen for our study. The metal-catalysed oxidation of 33-mer was studied, instead of enzymatic hydrolysis. Peptide 33-mer was treated in several oxidative systems. Iron-catalysed hydrogen peroxide-induced oxidation showed the greatest modification of 33-mer. Carbonyl groups and dityrosine cross-links were readily formed. At best, the immunological activity of 33-mer was reduced to 18% of its initial level after 24 h of oxidation. Oxidative treatment can be further applied for the modification of cereal prolamin proteins, since it appears to be a potential alternative for reduction of coeliac immunological activities in gluten proteins.

1. Introduction

Prolamins of wheat, barley and rye are characterised by a high proline (P) content. These proteins, the main constituents of gluten, contain toxic sequences that can trigger coeliac disease. Shan et al. (2002) suggested that a particular peptide, consisting of 33 amino acids, including 13 proline residues, is involved in the toxicity. This peptide, the 33-mer, is often used as a model in coeliac disease-related studies (Qiao et al., 2004; Shan et al., 2002).

By degrading the harmful prolamins of wheat, barley and rye, it is possible to decrease their toxicity and develop new gluten-free products or ingredients. When germination occurs, cereal grains develop proteolytic activity that effectively hydrolyses the cereal storage proteins, including prolamins (Hartmann, Koehler, & Wieser, 2006). Loponen et al. (2009) showed a 99.5% decrease in immunological activity after degradation of rye prolamins by endogenous rye proteases in a sourdough process. Proline-specific endopeptidases of microbial origin have also been applied for degrading prolamins in gluten-free food applications (Edens et al., 2005; Luoto et al., 2012; Stepniak et al., 2006). Such extensive gluten protein hydrolysis also eliminates the dough-forming properties of wheat proteins.

Oxidation is another mechanism for protein degradation. Free radical-mediated oxidation often occurs in food and biological systems. Proteins, lipids and carbohydrates are major targets of oxidation in food. Protein oxidation can cause fragmentation of polypeptide chains, generation of protein–protein cross-linkages, and oxidation of amino acid side chains (Stadtman & Levine, 2003). Reactive oxygen species (ROS) can be formed by various mechanisms such as electromagnetic radiation, ultrasound treatment and Fenton and Haber–Weiss-type reactions. Hydroxyl radical (·OH), a very strong ROS, can be formed through Fenton-type reactions (reaction 1), where hydrogen peroxide (H_2O_2) reacts with a reduced transition metal ion (e.g. Fe^{2+}, Cu^{+}) (Davies, 2005). The highly reactive free radicals stimulate oxidative modification of proteins, peptides and amino acids. Additionally, at low concentrations, ascorbic acid (AH_2) can reduce metals and act as a catalyst in the Fenton reaction. In the presence of molecular oxygen, pro-oxidation may be initiated through reactions 2 and 3. A hydroxyl radical is formed through the continuation of reaction 1 (Buettner & Jurkiewicz, 1996).

$$Cu^{+}/Fe^{2+} + H_2O_2 \rightarrow \cdot OH + OH^{-} + Cu^{2+}/Fe^{3+} \quad \text{(reaction1)}$$

$$AH_2 + 2Cu^{2+}/Fe^{3+} \rightarrow A + 2H^{+} + 2Cu^{+}/Fe^{2+} \quad \text{(reaction2)}$$

$$AH_2 + O_2 \rightarrow A + H_2O_2 \quad \text{(reaction 3)}$$

Uchida, Kato, and Kawakishi (1990, 1992) and Kato, Uchida, and Kawakishi (1992) studied metal-catalysed oxidation (MCO) of proline-containing peptides and collagen. It was reported that oxidation of proline-containing peptides in a Cu^{2+}/H_2O_2 system not only caused oxidative modification of proline residues, but also triggered oxidative cleavages of prolyl peptide bonds, and the generation of 2-pyrrolidone compounds. Collagen has a triple helical

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http://dx.doi.org/10.1016/j.foodchem.2013.05.066

structure and a repeated sequence, Glycine-X-Y, where X and Y are often proline and hydroxyproline. The unique structure and amino acid sequence of collagen contribute to the breakdown by MCO. Wheat and barley prolamins also contain repeating sequences with proline residues, and this suggests that breakdown of repeating structures could occur in prolamins as in collagen.

A characteristic of prolamins is their relatively high resistance to breakdown by gastric and pancreatic enzymes. Shan et al. (2002) digested alpha-gliadin, using gastric and pancreatic enzymes, and analysed the resulting peptides with liquid chromatography and mass spectrometry. They found that a peptide of 33 amino acids (LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF; α_2-gliadin residues 56–88) remained intact while most other fragments were cleaved into small fragments. This peptide, the 33-mer, has three epitopes, PFPQPQLYP, PYPQPQLPY (two copies) and PQPQLPYPQ (three copies) that stimulate T-cell. In addition, the deamidated 33-mer is the strongest T-cell stimulator known among gluten peptides (Qiao et al., 2004). It is anticipated that the breakdown of 33-mer into small fragments can reduce its toxicity. A proline-endopeptidase was reported to be a potent form of peptidase therapy for coeliac disease because of its post-proline specificity (Shan et al., 2002).

The aim of our study was to examine the non-enzymatic oxidation of the model 33-mer under different metal-catalysed oxidation conditions. Our hypothesis was that the 33-mer can be degraded by non-enzymatic oxidation and, further, its coeliac active epitopes can be modified to reduce its activity in immunological detection.

2. Materials and methods

2.1. Synthetic peptides and chemicals

The synthetic 33-mer peptides (MW = 3911.5 g/mol, purity 95.1%) and the peptide of nine amino acids (9-mer, PFPQQPFPQ, MW = 1085.24 g/mol, purity 98.5%) were purchased from GenScript Corporation (Piscataway, New Jersey, USA). Tripeptide leucine-glutamine-proline (LQP, MW = 356.43 g/mol, purity 96.96%) was purchased from LifeTein LLC (New Jersey, USA). $CuSO_4{\cdot}5H_2O$, $FeSO_4{\cdot}7H_2O$, amino acid glutamine (Q, MW = 146.14 g/mol), ascorbic acid, hydrogen peroxide (30%), and HCl were obtained from Merck (Darmstadt, Germany). Reagent 2,4-dinitrophenylhydrazine (DNPH) was from Sigma–Aldrich (42210). All chemicals were of analytical grade.

2.2. Oxidation of 33-mer peptide

The reaction solution (100 μl) contained 2 mg/ml of 33-mer peptides, and 0.05 mM CuSO4 or $FeSO_4$ in pH 4.5 milli-Q water (adjusted by 2 M HCl). Cu^{2+}/H_2O_2 and Fe^{2+}/H_2O_2 reactions were initiated by dispensing 10 μl of 50 mM hydrogen peroxide (freshly made from 30% hydrogen peroxide) into 2 ml brown Eppendorf tubes to avoid decomposition of the hydrogen peroxide. The Cu^{2+}/AH_2 and Fe^{2+}/AH_2 reactions were initiated by dispensing 10 μl of freshly made 50 mM AH_2 into a 15 ml tube to provide enough oxygen. Incubation temperature was 37 °C and incubation time was 24 h. The reactions were stopped by adding 11 μl of 1 mM EDTA.

2.3. Size-exclusion chromatography (SEC)

SEC columns Superdex Peptide 10/300 GL and Superdex 200 10/300 GL (GE Healthcare Biosciences AB, Uppsala, Sweden) were combined in a model 1200HPLC system, to enhance the separation of the peptide mixture. The reaction solution was mixed with 100 μl of SEC running buffer, containing 0.1% trifluoroacetic acid in 30% acetonitrile, and centrifuged (18,500g, 10 min). Supernatants were collected for SEC analysis. The injection volume was 100 μl, flow rate 0.5 ml/min and stopping time 120 min. The samples were detected at wavelengths 210 nm and 280 nm. Peptide 9-mer, LQP, and amino acid glutamine were used as molecular markers.

2.4. Enzyme-linked immunosorbent assay (ELISA)

A competitive ELISA kit was used for the 33-mer immunological activity test (Ridascreen Gliadin Competitive R7021, R-Biopharm, Darmstadt, Germany). This method was based on antibody against pentapeptide QQPFP (R5). Fe^{2+}/H_2O_2 oxidation of 33-mer was incubated for 0.5, 4 and 24 h. The reaction was performed twice at each time point, and four measurements were taken for each sample in appropriate dilution. The control, containing 33-mer, had 100% immunological activity.

2.5. Characterization of dityrosine

Dityrosine cross-links were characterised by a Luminescence spectrometer (LS55, Perkin Elmer). Fe^{2+}/H_2O_2 oxidised 33-mer (0.5, 4, and 24 h) and intact 33-mer control were diluted to appropriate solution strength with 50 mM acetic acid, pH 3.0.

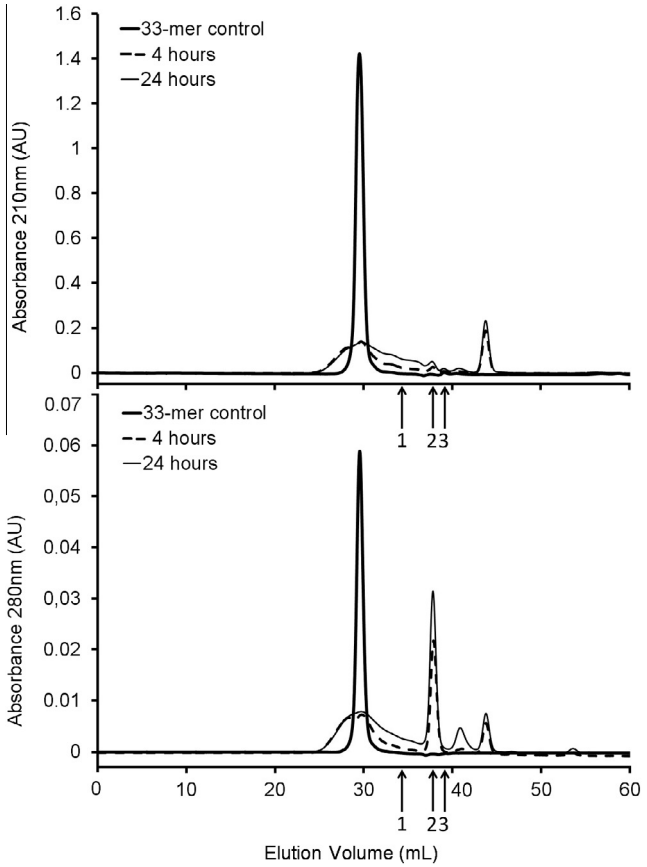

Fig. 1. Size-exclusion chromatography of 33-mer oxidation. Two milligrammes per millilitre of 100 μl of 33-mer peptide were incubated in the presence of 0.05 mM $FeSO_4$ pH 4.5, and 10 μl of 50 mM hydrogen peroxide were added. Incubation temperature was 37 °C. The reaction was stopped by adding 11 μl of 1 mM EDTA after 4 and 24 h of oxidation. (a) Absorbance 210 nm; (b) absorbance 280 nm. Arrows 1, 2 and 3 indicate the elution volume of molecular markers 1085.24, 356.43 and 146.16 g/mol.