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Characterisation of Maillard reaction products derived from LEKFD – A pentapeptide found in β-lactoglobulin sequence, glycated with glucose – By tandem mass spectrometry, molecular orbital calculations and gel filtration chromatography coupled with continuous photodiode array

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ABSTRACT

Maillard reaction peptides (MRPs) contribute to taste, aroma, colour, texture and biological activity. However, peptide degradation or the cross-linking of MRPs in the Maillard reaction has not been investigated clearly. A peptide of LEKFD, a part of β-lactoglobulin, was heated at 110 °C for 24 h with glucose and the reaction products were analysed by HPLC with ODS, ESI-MS, ESI-MS/MS and HPLC with gel-filtration column and DAD detector. In the HPLC fractions, an imminium ion of LEK*FD, a pyrylium ion or a hydroxymethyl furylium ion of LEK*FD, and KFD and EK were detected by ESI-MS. Therefore, those products may be produced by the Maillard reaction. The molecular orbital of glycated LEKFD at the lysine epsilon-amino residue with Schiff base form was calculated by MOPAC. HPLC with gel-filtration column showed cross-linking and degradation of peptides.

1. Introduction

The Maillard reaction is one of the most important reactions involved in food processing, storage, and cooking, and it plays a role in baking bread and roasting coffee, soy sauce, soybean paste and meat (Ogasawara, Katsumata, & Egi, 2006a). The reaction between the amino group and the carbonyl group of the food components occurs non-enzymatically, and various changes are produced when heat is applied and the sugar concentration increases.

In the food industry, Maillard reaction products are widely used as functional substances that can be generated safely and efficiently and contribute to taste, aroma, colour, texture and biological activity (Chobert, Gaudin, Dalgalarrondo, & Haertlé, 2006; Jing, Melissa, Wong, & Kitts, 2011; Lertittikul, Benjakul, & Tanaka, 2007; Liu et al., 2012; Sun, Hayakawa, Puangmanee, & Izumori, 2006). In addition, various toxic substances are generated *via* the Maillard reaction, such as heterocyclic amines (HCAs), acrylamide and 4(5)-methylimidazole (Moon & Shibamoto, 2011). *In vivo*, advanced glycation end-products (AGEs) are linked to development of various diseases associated with hyperglycaemia, such as diabetes, cataracts, kidney disorders, arteriosclerosis, neurodegenerative amyloidal diseases such as Alzheimer's and cell aging processes (Baynes, 2001). However, dietary AGEs are not harmful to human health (Ames, 2007). This is partly because the efficiency of absorption is generally much less than 100%. Furthermore, most of the AGEs that are absorbed are rapidly excreted by the kidneys.

In recent years, it has become known that the role of peptides in Maillard reactions is important. Several researchers have reported the taste properties of MRPs. The MRPs that are located in the 1000–5000 Da fraction of hydrolysed soybean proteins enhanced the "kokumi" sensation, which contributes to mouthfulness and continuity in the umami solution and in consommé soup (Ogasawara, Katsumata, & Egi, 2006). Further, these MRPs (1000–5000 Da) derived from hydrolysed soybean proteins produced a biphasic effect on human salt taste perception, enhancing NaCl responses at low concentrations and suppressing it at high concentrations (Katsumata et al., 2008). With regard to the flavour formation from MRPs, mainly glutathione (Zhang, Chien, & Ho, 1988; Zhang & Ho, 1989, 1991a, 1991b) or glycine-derived peptides, such as diglycine, triglycine and tetraglycine (Lu, Hao, Payne, & Ho, 2005; Oh, Shu, & Ho, 1991), have been studied. Recently, flavour compounds, namely pyrazines, have been produced from Maillard reaction dipeptides containing lysine (Van Lancker, Adams, & De Kimpe, 2010). Several researchers reported that the

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MRPs from hydrolysed proteins had antioxidant activity. MRPs derived from the protein hydrolysates of chicken residues exhibited good antioxidant activity, as determined by their reducing power and ability to inhibit lipid oxidation in sausages during storage (Sun, Zhao, Cui, Zhao, & Yang, 2010). MRPs from soy protein hydrolysates showed a higher reducing power and Fe^{2+} chelating activity compared with the original hydrolysates (Liu et al., 2012).

Mass spectrometric techniques have been widely used for the investigation and characterisation of MRP structures (Cucu, De Meulenaer, Kerkaert, Vandenberghe, & Devreese, 2012; Lapolla et al., 2004; Meltretter, Seeber, Humeny, Becker, & Pischetsrieder, 2007; Mennella, Visciano, Napolitano, Del Castillo, & Fogliano, 2006). Matrix-assisted laser desorption ionisation (MALDI) and electrospray ionisation (ESI) have been used to determine the structure of MRPs (Frolov, Hoffmann, & Hoffmann, 2006; Lapolla et al., 2004; Moreno, Quintanilla-López, Lebrón-Aguilar, Olano, & Sanz, 2008). Recently, tandem mass spectrometry (MS/MS) was also applied to MRPs sequencing (Lapolla et al., 2004). These studies were performed with instruments that utilised collision-induced dissociation (CID). Thus, the weakest bonds on the modified side-chain in the peptide tend to dissociate preferentially, resulting in high abundance ions corresponding to various neutral losses (i.e., H_2O, $2H_2O$, $3H_2O$, $4H_2O$, $3H_2O + HCHO$ and $C_6H_{10}O_5$) (Frolov et al., 2006; Lapolla et al., 2004; Zhang, Frolov, et al., 2007). The neutral-losses may indicate the presence of MRPs, especially loss of 150 amu showing the presence of 1-amino-1-deoxy-2-ketose known as an Amadori product (Yaylayan & Sporns, 1989).

However, the details of the MRPs are not explained completely by mass spectrometry (Frolov et al., 2006; Lapolla et al., 2004; Mennella et al., 2006; Zhang, Frolov, et al., 2007). Furthermore, the implementation of electron capture dissociation (ECD) on FTICR mass spectrometers has achieved an important advance in tandem mass spectrometry (Zubarev, Kelleher, & McLafferty, 1998; Zubarev et al., 2000). ECD provides more extensive sequence fragmentation of peptides, and allows modifications to remain intact, because bond dissociation occurs before energy redistribution. Very recently, electron transfer dissociation (ETD) fragmentation using a modified linear ion trap was developed (Syka, Coon, Schroeder, Shabanowitz, & Hunt, 2004). This technique was demonstrated in sequencing experiments of phosphopeptides. In addition, abundant peptide backbone c- and z-type ions were detected, resulting in almost complete sequence coverage in the ETD fragmentation spectra.

However, none of these studies addressed the thermal degradation or cross-linking of MRPs. The effect of temperature on the MRP-derived xylose–soybean peptide has been investigated (Lan et al., 2010), and it was found that both peptide degradation and peptide cross-linking occur in the Maillard reaction. To the best of our knowledge, any detailed information on the effects of temperature on MRPs is lacking. The initial stage of the Maillard reaction involves the condensation of the carbonyl group with the free amino group (Hodge, 1953). This reaction results in the formation of a Schiff base that rapidly rearranges to form the more stable Amadori products. Amadori products are degraded *via* various pathways, leading to the formation of furfurals, reductones and fragmentation products. Later stages in the Maillard reaction lead to reactions between those intermediates with amino compounds that lead to melanoidins. From the above, peptides are also considered to yield various Maillard products, but their structures and chemical propertied are still not well understood.

The Maillard reaction of milk proteins is known to occur during the processing and storage of milk and milk powder (Gaucher, Mollé, Gagnaire, & Gaucheron, 2008). Maillard products with improved functional properties, such as antioxidant, antimicrobial and antihypertensive properties, have been synthesised from milk proteins (De Block et al., 2003; Guyomarc'h, Warin, Muir, & Leaver, 2000; Sithole, McDaniel, & Goddik, 2005). Recent studies have reported that certain functional properties of β-lactoglobulin (β-LG), such as its thermal stability, emulsifying properties and foaming properties, are improved by the Maillard reaction (Chevalier, Chobert, Popineau, Nicolas, & Haertlé, 2001; Medrano, Abirached, Panizzolo, Moyna, & Añón, 2009; Wooster & Augustin, 2006). Some reports have focused on the antioxidant activity of Maillard reaction products from β-LG (Dong et al., 2012; Jiang & Brodkorb, 2012). For example, Maillard reaction products from β-LG-ribose exhibited increased antioxidant activity (Jiang & Brodkorb, 2012). In addition, several articles describing the mass spectrometric characterisation of glycated β-LG proteins or peptides have been published in the last decade (Corzo-Martínez, Lebrón-Aguilar, Villamiel, Quintanilla-López, & Moreno, 2009; Fenaille, Morgan, Parisod, Tabet, & Guy, 2003, 2004; Fogliano et al., 1998; Leonil et al., 1997; Morgan et al., 1998). However, research on peptide degradation and cross-linking of glycated β-LG peptides has not been previously reported.

In this work, we used *m*-aminophenylboronic acid affinity chromatography to specifically enrich glycated peptides of LEKFD, a pentapeptide found in the β-LG sequence. The resulting eluates were further separated by HPLC and analysed by ESI-MS and ESI-MS/MS. Several researchers showed that these glycated peptides can be quantitatively enriched from protein digests by

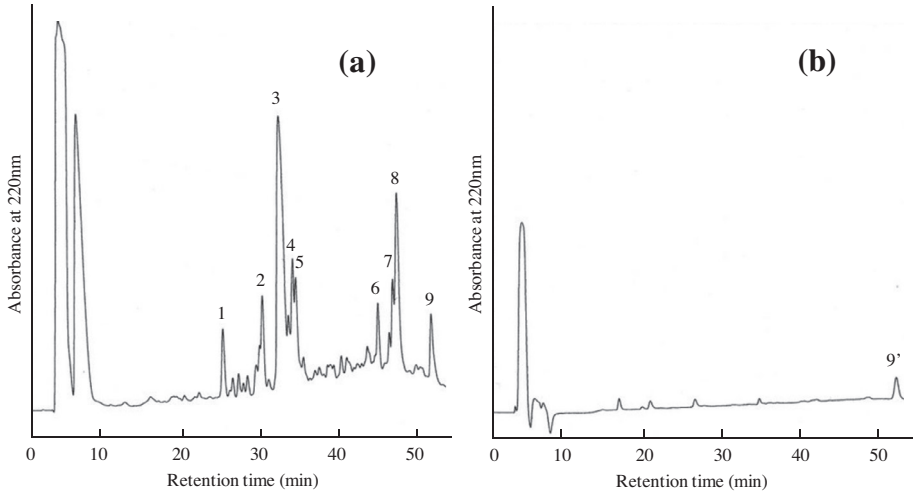

Fig. 1. HPLC chromatogram of (a) MRPs derived from the glycated peptide LEKFD not bound to the *m*-aminophenylboronic acid column, and (b) MRPs derived from the glycated peptide LEKFD bound to the *m*-aminophenylboronic acid column. Fractions of peaks 1–9, 9′ were analysed by ESI-MS, as described in Fig. 2.