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Towards the design of hypolipidaemic peptides: Deoxycholate binding affinity of hydrophobic peptide aggregates of casein plastein

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ABSTRACT

Plastein is a thixotropic product of protease-induced peptide aggregation. This study evaluated the effects of the modified peptide structure of plastein on its affinity for a secondary bile acid. Casein plastein formation resulted in decreased free amino nitrogen and increased mean particle size, surface hydrophobicity and hydrophobic-to-hydrophilic amino acid ratio of the peptides. The net negative surface charge of casein hydrolysate (CH) was decreased ($P < 0.05$) in crude plastein (CPc) and completely lost in the isolated plastein (CPi) resulting in the formation of insoluble aggregates. The surface and structural changes in CPi were associated with 38.5 ± 0.0% binding of physiological level of sodium deoxycholate, higher ($P < 0.05$) than the activities of CPc (23.1 ± 0.0%) and CH (12.8 ± 4.4%). Maximum specific ligand binding (B_{max}) was higher ($P < 0.05$) for CPc (0.391 ± 0.021 mM/mg protein) than CH (0.246 ± 0.043 mM/mg protein) and CPi (0.234 ± 0.005 mM/mg protein). However, CPi had the strongest affinity for sodium deoxycholate with the lowest dissociation constant (K_d) of 2.719 ± 0.146 mM, which was 2.5- and 3-folds lower ($P < 0.05$) than those of CH and CPc, respectively. Understanding deoxycholate-binding affinity of peptides can facilitate the design of potent food-based BA sequestrants for managing hypercholesterolaemia (dyslipidaemia) and associated health conditions.

1. Introduction

Abnormal endogenous lipid metabolism (dyslipidaemia) is an important risk determinant of metabolic syndrome (Cleeman, Grundy, Becker, & Clark, 2001) that can increase the risk of developing cardiovascular disease, a leading cause of global mortality (World Health Organization, 2012). Dyslipidaemia, particularly hypercholesterolaemia, can be clinically modulated by bile acid (BA) sequestration in the gastrointestinal tract (Chen, Ma, Liang, Peng, & Zuo, 2011; Insull, 2006; Staels, Handelsman, & Fonseca, 2010). Primary BAs are products of hepatic cholesterol catabolism with a rate-limiting step catalysed by cholesterol 7α-hydroxylase or cytochrome P450 7A1. Primary BAs are secreted in the colon where microbial activities convert them into secondary BAs; the BAs in the colon

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Abbreviations: BA, bile acid; B_{max}, maximum specific ligand binding; CH, casein hydrolysate; CPc, crude casein plastein; CPi, isolated casein plastein; DCA, deoxycholic acid; HHR, hydrophobic-to-hydrophilic amino acid ratio; H_o, calculated hydrophobicity; K_d, ligand dissociation constant; SDC, sodium deoxycholate; S_o, surface hydrophobicity

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are mostly recycled through enterohepatic circulation. Sequestering BA in the colon can lead to the formation of insoluble complex and this can disrupt BA reabsorption from the ileum leading to an increase in hepatic cholesterol metabolism and a decrease in endogenous cholesterol levels (Chen et al., 2011; Staels et al., 2010). Moreover, BA sequestration can also alter BA-facilitated absorption of dietary lipids. Deoxycholic acid (DCA) is a major secondary BA produced when intestinal bacteria dehydroxylate cholic acid (primary BA) at the C-7 position. Besides its role in dietary fat absorption, prolonged exposure of colon cells to DCA can lead to the development of colon cancer (Bernstein et al., 2011). Therefore, BA sequestration can have a variety of positive effects on these human health conditions.

Food-derived peptides and protein hydrolysates have been proposed to exhibit hypolipidaemic activities in the intestine, hepatocytes and adipocytes (Howard & Udenigwe, 2013; Udenigwe & Rouvinen-Watt, 2015). Particularly, peptides have been reported to exhibit BA-binding capacities *in vitro* (Barbana, Boucher, & Boye, 2011; Choi, Adachi, & Utsumi, 2002; Ma & Xiong, 2009; Udenigwe, Mohan, & Wu, 2015; Yoshie-Stark, Wada, & Wäsche, 2008) and in experimental rat model (Higaki et al., 2006). To date, there is scanty information on the structure–function relationship of food protein-derived BA-binding peptides. In some instances, the low digestibility and high amounts of hydrophobic amino acid residues of peptides are thought to be crucial for bile acid sequestration (Higaki et al., 2006; Matsuoka et al., 2014). However, protein hydrolysate samples of similar amino acid profiles were recently reported to possess varying BA-binding capacity (Udenigwe et al., 2015; Udenigwe & Rouvinen-Watt, 2015). Therefore, there is a need to provide more clarification on the structural requirements of peptides for bile acid sequestration.

Entropy-driven aggregation of peptides, mediated by proteases, results in hydrophobic clusters of peptides known as plastein (Gong, Mohan, Gibson, & Udenigwe, 2015). Plastein was previously explored for incorporating amino acids into dietary proteins in order to enhance their nutritional quality (Yamashita, Arai, Tsai, & Fujimaki, 1971). Subsequently, plastein reaction was used in debittering protein hydrolysates (Synowiecki, Jagietka, & Shahidi, 1996); it was thought that the bitterness-contributing hydrophobic amino acid residues of protein hydrolysates are hidden in the peptide aggregate's core, thereby reducing their interaction with taste receptors (Gong et al., 2015). Recently, plastein was reported to possess enhanced bioactivity compared to its hydrolysate precursor including antioxidative (Udenigwe, Wu, Drummond, & Gong, 2014; Zhao, Wu, & Li, 2010) and angiotensin converting enzyme inhibitory activities (Sun & Zhao, 2012), although it is still not known how the aggregate structure contributed to bioactivity. Moreover, plastein reaction was also found to enhance the BA-binding capacity of chicken meat protein hydrolysates and this was associated with an increase in surface hydrophobicity of the peptide aggregates (Udenigwe et al., 2015; Udenigwe & Rouvinen-Watt, 2015). Plastein derived from dairy proteins have also demonstrated BA-binding capacity (unpublished data). In order to develop food-based physiologically potent BA-binding agents, it is imperative to ensure that the molecules possess high affinities for BAs, particularly high maximum ligand binding (B_{max}) and low dissociation constants (K_d). There is a dearth of literature information on the binding parameters for food peptide-based BA sequestrants. Therefore, the objective of this study was to evaluate the relationship of secondary BA (sodium deoxycholate, SDC)-binding affinity with the surface and molecular properties of casein hydrolysates and plastein.

2. Materials and methods

2.1. *Formation and isolation of casein plastein*

Casein was isolated from commercial bovine milk by isoelectric precipitation at pH 4.6 followed by centrifugation at 10,000 × *g* for 10 min and freeze drying of the resulting precipitate. The casein isolate (5%, w/v) was hydrolysed with papain (E.C. 3.4.22.2; Sigma Aldrich, St. Louis, MO, USA) at E/S ratio of 1:100 at 65 °C and pH 7.0 for 5 h using a pH-Stat system (Metrohm AG, Herisau, Switzerland). Hydrolysis was terminated by heating the mixture at 95 °C for 15 min; the cooled hydrolysate was centrifuged and the supernatant was freeze-dried to obtain the casein hydrolysate (CH). Aqueous suspension of CH (40%, w/v) was mixed with papain (E/S 1:100) and incubated near neutral pH a 37 °C for 24 h. A portion of the resulting gel-like product was freeze dried for 48 h to obtain the crude casein plastein (CPc). The other portion was mixed with equal volume of 10% trichloroacetic acid to precipitate the aggregates and placed on ice for 10 min followed by centrifugation at 2798 × *g*. The resulting residue was washed two times each with 2 mL acetone, centrifuged and then freeze dried to obtain the isolated casein plastein (CPi).

2.2. *Characterization of the casein hydrolysates and plastein*

2.2.1. *Free amino nitrogen assay*

The free amino nitrogen present in the hydrolysate (CH) and plastein (CPc and CPi) was analysed using *O*-phthalaldehyde reagent containing sodium dodecyl sulphate and dithiothreitol. The reagent (250 μL) was mixed with 33 μL of samples (1 mg/mL) and absorbance was measured at 340 nm after 2 min. Serine (0.1 mg/mL) was used as the standard and the results were calculated as milliequivalent of serine NH_2/g of protein (Nielsen, Petersen, & Dambmann, 2001).

2.2.2. *Surface hydrophobicity (S_o) analysis*

The surface hydrophobicity of CH, CPc and CPi was determined by the fluorescence method using a hydrophobic probe, 8-anilino-1-naphthalenesulphonic acid. The samples were analysed at concentrations of 0.0009–0.015% at excitation and emission wavelengths of 390 nm and 470 nm, respectively. Surface hydrophobicity was determined as the slope of the fluorescence vs. concentration plot (Horax, Hettiarachchy, Chen, & Jalaluddin, 2004).

2.2.3. *Determination of zeta (ζ)-potential*

Zeta (ζ)-potential of CH, CPc and CPi was determined using Zetasizer Nano Series Nano-ZS (Malvern Instruments Ltd., Malvern, UK). Samples were dispersed in deionized water and