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Inhibitions of renin and angiotensin converting enzyme activities by enzymatic chicken skin protein hydrolysates

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ABSTRACT

Enzymatic hydrolysates from chicken skin protein were investigated for their in vitro inhibitions of angiotensin converting enzyme (ACE) and renin activities. Enzyme hydrolysis of the chicken skin protein from the thigh and breast muscles was done using alcalase or a combination of pepsin/pancreatin (PP) at enzyme concentrations of 1–4%. The chicken skin protein hydrolysates (CSPH) were then fractionated by membrane ultrafiltration into different molecular weight peptides (<1, 1–3, 3–5 and 5–10 kDa). Results showed that degree of hydrolysis (DH) of the hydrolysates increased significantly with protease concentration for all the samples (72.61–81.88%) and correlated positively with peptide yield. The alcalase hydrolysates generally had significantly higher ($p < 0.05$) ACE-inhibitory activity when compared to PP hydrolysates. ACE inhibition was inversely related to size of ultrafiltration membrane peptides. A moderate renin-inhibitory activity was observed (15–36%), which was dependent on the type of protease; the PP hydrolysates showed significantly higher ($p < 0.05$) inhibition than alcalase hydrolysates. These results suggest that CSPH can be considered a potential ingredient for the development of functional foods and nutraceuticals that can attenuate catalytic activities of ACE and renin.

1. Introduction

Hypertension is a chronic condition in which blood pressure in the arteries becomes elevated and usually is persistently at or above 140 mm Hg for the systolic blood pressure (SBP) or 90 mm Hg diastolic blood pressure (DBP) or both (Ahhmed & Muguruma, 2010). It is a leading cause of death, affecting about 24% of the adult population in the developed world especially the US and is a major problem in developing countries (Daien et al., 2012). Globally, hypertension is a leading risk factor for cardiovascular disease, stroke, end stage renal disease and premature death (Erdmann, Cheung, & Schroder, 2008; Girgih, Udenigwe, Li, Adebiyi, & Aluko, 2011; Lee, Qian, & Kim, 2010). Among the many approaches that are currently being used to address this problem is increased consumption of proteins, which has been reported to lower the risk of hypertension and heart diseases probably due to the presence of biologically active peptide sequences within the primary structure (Vercruysse, Van Camp, & Smagghe, 2005; Erdmann et al., 2008; Samanarayaka, Kitts, & Li-Chan, 2010). Antihypertensive peptides are the best known and researched among the different groups of bioactive peptides, notably, the inhibitors of angiotensin converting enzyme (ACE).

The renin–angiotensin system (RAS) has long been known to play a very crucial physiological role in maintaining blood pressure and associated cardiovascular diseases (CVD) such as congestive heart failure and hypertension (Erdmann et al., 2008; Fujita, Yokoyama, & Yoshikawa, 2000; Qian, Je, & Kim, 2007; Raghavan & Kristinsson, 2009). RAS mainly controlled by 2 enzymes, renin and ACE, plays vital roles in the maintenance of blood pressure homeostasis, fluid and electrolyte balance in the human body (Girgih et al., 2011; Lee et al., 2010). Renin, an aspartyl protease, is responsible for catalyzing the initial step, converting angiotensinogen to angiotensin-I for the subsequent conversion by ACE (Udenigwe, Lin, Hou, & Aluko, 2009). ACE (EC 3.4.15.1) belongs to the class of zinc proteases located in the vascular endothelial lining of the lungs and is present in several body tissues where it serves as an integral part in the moderation of blood pressure as well as the normal functioning of the heart (Je, Park, Kwon, & Kim, 2004; Ryan, Ross, Bolton, Fitzgerald, & Stanton, 2011). ACE is a decapeptidylcarboxylase that cleaves dipeptides from the C- terminus of oligopeptides, and in the process, catalyzing the hydrolysis (conversion) of the inactive decapeptide, angiotensin I into the potent vasoconstrictor, angiotensin II (Martinez-Maqueda, Miralles, Recio, & Hernandez-Ledesma, 2012). ACE also inactivates bradykinin (a vasodilator), which leads to blood pressure elevation (Vercruysse et al., 2005; Je et al., 2004; Raghavan & Kristinsson, 2009).

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The mechanism by which ACE inhibitors lower blood pressure involves maintenance of a balance between the vasoconstrictive plus salt retentive abilities of angiotensin II and the vasodilatory property of bradykinin. This is achieved by decreasing the production of angiotensin II while at the same time reducing the hydrolytic degradation of bradykinin (Ryan et al., 2011). Inhibition of these processes has long been the basis for the use of synthetic ACE inhibitors such as captopril, enalapril, lisinopril, and fosinopril in the treatment of hypertension, congestive heart failure and myocardial infarction (Erdmann et al., 2008; Fujita et al., 2000). In this respect, naturally occurring bioactive peptides are becoming attractive as potential antihypertensive agents because they are not known to exhibit negative side effects such as cough, skin rash and pain that are associated with synthetic drugs (Je et al., 2004).

ACE-inhibitory peptides with demonstrated activities have been isolated and extensively studied from several foods such as milk, whey, eggs, meat, soybean, fish and animal by-products (Aluko & Monu, 2003; Erdmann et al., 2008; Martinez-Maqueda et al., 2012; Samanarayaka et al., 2010; Vercruysse et al., 2005). They have also been isolated from chicken bone protein extracts (Cheng, Liu, Wan, Lin, & Sakata, 2008; Cheng, Wan, et al., 2008; Nakade et al., 2008), chicken breast muscle extracts (Fujita et al., 2000; Saiga et al., 2003, 2006) as well as chicken collagen (Saiga et al., 2008). Renin inhibitory activities has equally been studied in hemp protein hydrolysates (Girgih et al., 2011) and flaxseed protein hydrolysates (Udenigwe et al., 2009).

Chicken skin is a by-product derived from chicken meat processing, is highly underutilised and constitutes a huge waste disposal burden and danger to the environment (Feddern et al., 2010). Several attempts have previously been made at developing novel products such as meat balls (Bhat, Kumar, & Kumar, 2011), collagen (Bonifer & Froning, 1996; Cliché, Amiot, & Gariepy, 2003), sausages (Biswas, Chakraborty, Sarkar, Barpuzari, & Barpuzari, 2007), and chicken meat frankfurter (Babji, Chin, Chempaka, & Alina, 1998) in order to diversify chicken skin utilization and reduce environmental waste burden. However, an area of research that is yet to be explored is the development of chicken skin based products with functional and health promoting values. The high protein content (dry weight basis) of chicken skins could, in addition to contributing to nutrition, also serve as a very active source of value-added products, including bioactive peptide-containing hydrolysates. According to Badr (2005), differences in the proximate composition of chicken skins may be due to differences in age and diet. However the chicken breast skin was shown to have a much lower fat content and higher protein content than the leg muscle skin. In this study, we produced enzymatic hydrolysates from chicken thigh and breast muscle skin proteins using alcalase and a combination of pepsin and pancreatin at concentrations of 1–4%. After fractionation using ultrafiltration membranes of 1, 3, 5 and 10 kDa molecular weight cut-offs (MWCO). Therefore, the objective of this study is to determine the potential antihypertensive effect of chicken skin protein hydrolysates by measuring their in vitro inhibitory activities against renin and ACE.

2. Materials and methods

2.1. Materials

Chicken skins from the thigh and breast muscles used for this study were supplied by Granny's Poultry (Winnipeg, MB, Canada). Pepsin (from porcine gastric mucosa, EC 3.4.23.1), pancreatin (from porcine pancreas), alcalase (from fermentation of *Bacillus licheniformis*, 3.4.21.62), ACE (from rabbit lung, EC 3.4.15.1), trinitrobenzene sulfonic acid (TNBS), N-(3-[2-furyl] acryloyl)-phenylalanylglycylglycine (FAPGG) and sodium dodecyl sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human recombinant Renin Inhibitor Screening Assay Kit was purchased from Cayman Chemicals (Ann Arbor, MI, USA). All other analytical grade reagents and ultrafiltration membranes (1, 3, 5 and 10 kDa molecular weight cut-offs) were purchased from Fisher Scientific (Oakville, ON, Canada).

2.2. Preparation of chicken skin protein hydrolysates (CSPH)

Fresh thigh or breast chicken skins (approximately 250 g) were packed in freeze drying plates, frozen at −20 °C for 24 h and transferred to −80 °C for 6 h prior to freeze drying. The freeze dried samples were thereafter manually shredded and defatted repeatedly by mixing ~1 g with 10 mL of food grade acetone. The defatted skin samples were then air dried overnight in the fume hood chamber at room temperature and subsequently milled with a Waring blender to produce a fine powder that was stored at −20 °C. Proximate composition of the defatted samples were determined using standard methods of analysis (AOAC, 1990). For the initial screening test to optimise and select the best enzyme concentration, dried chicken skin powder from the thigh or breast muscles were mixed with water to give 5% (w/w protein basis) slurries. For the alcalase hydrolysis, the slurry was heated to 55 °C, adjusted to pH 8.0 using 2 M NaOH and hydrolysis initiated by addition of enzyme (1–4% w/v, skin protein basis); each mixture was stirred continuously for 4 h. For the pepsin + pancreatin (PP) hydrolysis, the slurry was heated to 37 °C, adjusted to pH 2.0 using 2 M HCl and the reaction initiated with the addition of pepsin enzyme (1–4% w/v, skin protein basis); the mixture was then stirred continuously for 2 h. After peptic hydrolysis, the reaction mixture was adjusted to pH 7.5 with 2 M NaOH, pancreatin was added (1–4% w/v, skin protein basis) and incubated at 37 °C for 4 h with continuous stirring. At the end of the incubation period, the enzyme reactions were terminated by heating the slurries to 95 °C for 15 min. The mixtures were thereafter centrifuged (7000 ×*g* at 4 °C) for 1 h and the resulting supernatant lyophilised and stored at −20 °C until needed for further analysis. The most active hydrolysate from each enzyme treatment was subsequently fractionated by sequentially passing the supernatant through ultrafiltration membranes with molecular weight cut-offs (MWCO) of 1, 3, 5 and 10 kDa in an amicon stirred ultrafiltration cell. Starting with 1 kDa MWCO, the retentate from each membrane was passed through the next higher MWCO membrane while the permeate from each membrane (1, 3, 5, and 10 kDa MWCO) was collected, lyophilised and stored at−20 °C as <1, 1–3, 3–5, and 5–10 kDa fractions, respectively. Protein content of the lyophilised CSPHs was determined by the modified Lowry method (Markwell, Haas, Biebar, & Tolbert, 1978). The above digestion and fractionation protocols were performed in triplicates and the lyophilised samples combined, analysed for protein content and used for the renin and ACE inhibition assays.

2.3. Determination of degree of hydrolysis

The percent degree of hydrolysis (DH) of CSPHs was determined according to the trinitrobenzene sulfonic acid (TNBS) method described by Adler-Nissen (1979). Defatted skin samples were digested under vacuum with 6 M HCl for 24 h and the digest used to determine total amino groups as L-leucine equivalent. The DH was calculated as percentage ratio of the leucine equivalent of CSPHs to that of defatted skin.

2.4. Peptide yield of CSPH and membrane fractions

The peptide yields (%) of CSPH and membrane fractions were calculated as the ratio of protein content of lyophilised CSPH to the protein content of unhydrolysed dried chicken skin samples. Also, the peptide yields (%) of the ultrafiltration membrane fractions were calculated as the ratio of the protein content of the lyophilised permeate to the protein content of the original chicken skin hydrolysates as described by Girgih et al. (2011).