ASSESSMENT OF ACE INHIBITORY ACTIVITY OF THERMOLYTIC DIGEST OF CATFISH MUSCLE PROTEIN

Masomeh Ghassem[1], Fatemeh Mahmoodani[1], Abdul Salam Babji[1] and Keizo Arihara[2]

[1]School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia;

[2]Department of Animal Science, Kitasato University, 35-1 Higashi-23-Bancho, Towada-shi, Aomori 034-8628, Japan.

Abstract – Thermolytic digests of catfish muscle protein exhibited inhibitory activity towards Angiotensin Converting Enzyme (ACE) and were purified with the aid of ultrafiltration, gel filtration and RP-HPLC. The amino acid sequence of hydrolysate with the highest ACE-inhibitory activities was determined using ESI-TOFQ mass spectrometry. The sequence of GPPP (IC_{50} = 0.86 µM) corresponding to the fragments 986-989 of myosin-I heavy chain was identified for the muscle protein hydrolysate. The results demonstrate that hydrolysate of catfish muscle protein obtained by thermolysin may contain bioactive peptides.

Key Words – Tandem mass spectrometry; ACE-inhibitory activity; Thermolytic digest; Bioactive peptides.

I. INTRODUCTION

By the year 2020, it is estimated that hypertension will surpass infectious diseases as the most important preventable causes of cardiovascular morbidity and mortality [1]. The most therapeutic approach in treatment of high blood pressure is the inhibition of ACE. The Asian catfish, "*Clarias batrachus*", species of freshwater amphibious air-breathing fish found primarily in Southeast Asia. This fish has a great market demand due to its nutritive, invigorating and therapeutic value in Malaysia [2]. Therefore, the objective of this study was to evaluate the ACE-inhibitory activity of thermolytic digests of *C. batrachus* muscle protein. Hydrolysate was purified with the aim of ultrafiltration, gelfiltration and reversed phased high-performance liquid chromatography (RP-HPLC). The molecular weight, sequence and structural information of the most active potent fractions were identified and characterized using liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS).

II. MATERIALS AND METHODS

Muscle protein was digested by thermolysin for 0.5 h at 37ºC. The hydrolysate was then fractionated using 10 and 3 kDa UF membranes. The UF 3 hydrolysate was separated through gelfiltration and RP-HPLC. Peaks with highest ACE inhibitory activity were injected to LC-MS for structural information. Molecular mass, peak list generation and peptide sequence of all spectra of the purified catfish muscle protein fractions were determined through the Data Analysis 4.0 (Bruker Daltonics, Germany) and MASCOT (Matrix Science, v2.204) search engine software against Swiss Prot and NCBI database. ACE inhibitory assay was performed as described by Shalaby [3].

III. RESULTS AND DISCUSSION

Results show that ACE-inhibitory activity of muscle protein hydrolysate (DH= 80%) increased with decrease of molecular weight. Among all fractions, the 3 kDa permeates of muscle (IC_{50}=1.364 mg/mL) protein hydrolysate exhibited the highest ACE-inhibitory activity. Several reports have indicated that ultrafiltrated hydrolysates with very low molecular weights had higher ACE-inhibitory activity [4]. Four peaks were collected from gel filtration which fraction S4 indicated the highest inhibition activity of 72% at peptide concentration of 85 µg/mL. This fraction was again separated on RP-HPLC C_{18} column and consequently, five major peaks were observed at absorbance of 214 nm, which among those fractions, S4-3 exhibited the highest inhibition activity of 67% at peptide concentrations of 50 µg/mL. The amino acid sequence of fraction S4-3 was evaluated using LC-MS/MS instrument. From the mass spectrum profile in Fig. 1A it appeared that fractions S4-3

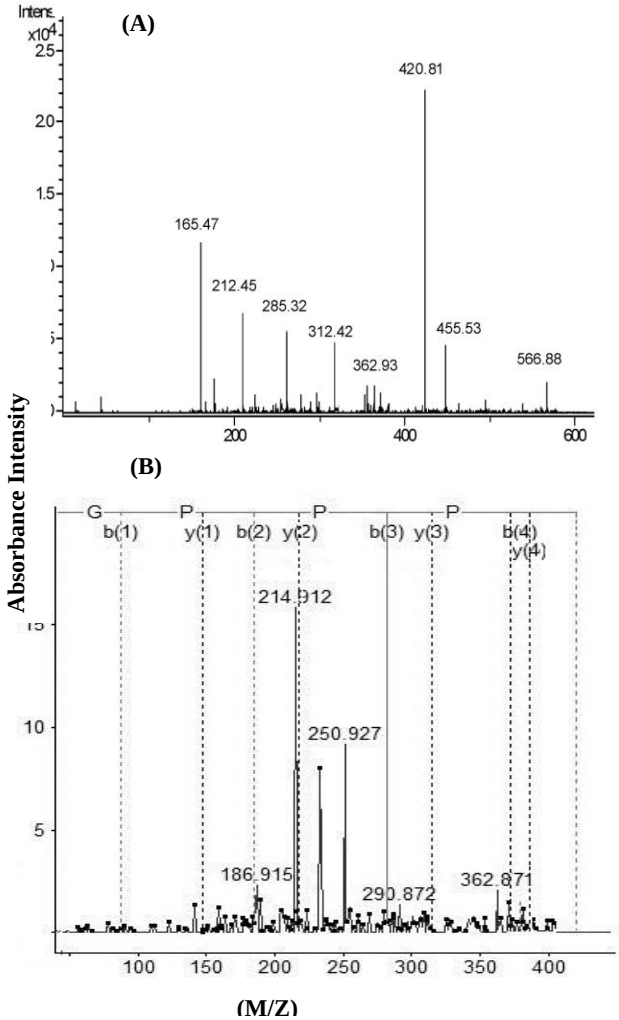

Figure 1. ESI-MS spectrum of fractions S4-3 (A) and ESI MS/MS spectrum of ions m/z 420.8 (B) corresponding to the f(986-989) of myosin-I heavy chain. The sequence of peptide is displayed with the fragment ions observed in the spectrum. Dotted lines represent identified y and b ions of the ACE inhibitor.

contained a peptide giving charge to mass ratio of 420.8 m/z. The database search for peptide identification was performed using Data Analysis 4.0 and MASCOT search engine software against Swiss Prot and NCBI database. According to the molecular mass and tandem MS, the sequences of GPPP corresponding to the fragments 986-989 of myosin-I heavy chain, was determined for the fraction S4-3. The fragmentation pattern of the peptide GPPP indicated a major ion at 214.9 m/z which was identified as a y_2 fragment ion resulting from the cleavage C-terminal to Pro (Fig. 1B). This amino acid is associated with very abundant *y* and *b* ions that are often easily identifiable because of their intensity. Incorporation of hydrophobic amino acids such as Trp, Tyr, Phe and Pro at the C-terminus may enhance the ACE inhibition activity of peptide by locking the carboxyl group at the C-terminus of peptide to the conformation more suitable for interaction with charged residues at three subsites of C-domain catalytic site of the somatic form of the ACE. The naturally occurring ACE-inhibitory peptides have been derived from a wide range of enzymatically hydrolysate proteins including thermolytic digest of porcine myofibrillar protein, chicken muscle and haruan myofibrillar protein hydrolysates. Moreover, various interesting ACE inhibitory peptides with significant antihypertensive effect on human have been derived from milk proteins such as two lactotripeptides IPP and VPP from fermented milk casein [5] and LL from trypsin digest of whey protein [4]. Also it has been reported that several potent ACE inhibitory peptides with –PEW (IC_{50} = 1-5 µM) and –IPP (IC_{50} = 4-5 µM) residues at their C-terminus were isolated from thermolytic digests of α-lactalbumin and β-casein, respectively [6], which are almost similar to the IC_{50} value of isolated peptide GPPP (0.86 µM) from thermolytic digest of catfish muscle protein in the present study.

IV. CONCLUSION

In the present study, biologically active peptide from enzymatic digestion of catfish muscle protein was identified by the use of HPLC system coupled to a tandem mass spectrometer after a series of purification steps. This technique allowed us to identify ACE-inhibitory peptides in 3000 Da permeates of catfish muscle protein hydrolysate with the sequence of GPPP (IC_{50} = 0.86 µM). The implementation of QTOF mass spectrometer combined with the database searching and interpretation of fragmentation patterns assisted in generation of accurate mass and structural information of the isolated peptides. The ACE-inhibitory activity of the extracted peptides from fish muscle proteins may lead to their applications as bioactive ingredients in the functional foods in

order to prevent hypertension. However, the nutraceutical quality of a protein depends on the physiological activity of its specific amino acids after digestion and absorption in the gastrointestinal tract. Therefore, further studies are required to determine the *in vivo* antihypertensive activity of the purified potent ACE-inhibitory peptides.

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