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Analytical Methods

A HPLC-UV method for the determination of angiotensin I-converting enzyme (ACE) inhibitory activity

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ABSTRACT

To determine the angiotensin-converting enzyme (ACE) inhibitory activity of a fish hydrolysate, different methods were tested. Finally, a sensitive, extraction-free HPLC method using N-(3-[2-furylacryloyl]-Phe-Gly-Gly (FAPGG) as substrate was preferred. This method relies on the UV-titration of the peptide 2-furylacryloyl-L-Phe (FAP) resulting from the hydrolysis of the FAPGG after a chromatographic separation on a reverse phase column. The experimental conditions (enzyme/substrate ratio, incubation time, NaCl concentration) were optimised for linearity, sensitivity and precision. The assay was adequate for the study of ACE inhibition by Captopril, used as reference, and several peptides. Captopril and the fish hydrolysate had IC₅₀ values, respectively of 0.19 ng and 43 µg with standard deviations of 0.09 ng and 5 µg. Afterwards, the determination of the Hill coefficient sustained the hypothesis that active peptides present in the fish hydrolysate were low-molecular weight molecules. This result was confirmed by the activity measurement of the fish hydrolysate fractions obtained by gel filtration.

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1. Introduction

The angiotensin I-converting enzyme (ACE, EC 3.4.15.1) plays an important role in regulating blood pressure in the renin–angiotensin system because it catalyses the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, and inactivates bradykinin, a potent vasodilator (Skeggs, Kahn, & Shumway, 1957). So, the result of ACE action is an elevation of blood pressure. Apart from this, angiotensin I-converting enzyme is also involved in other physiological processes, and so abnormal levels of ACE activity in serum have been related with the apparition of some diseases (Beneateau-Burnat & Baudin, 1991; Lieberman, 1975). Thereby synthetic ACE inhibitors, such as Captopril and Enalapril, are currently used in the treatment of hypertension. However, synthetic ACE inhibitors can have side effects including cough, taste disturbances and skin rashes. Therefore, interest for natural inhibitor peptides has increased (Ferreira, Eça et al., 2007; Ferreira, Pinho et al., 2007; Fujita, Yokoyama, & Yoshikawa, 2000). In the food area, studies have been mainly focused on the identification of food components,

principally peptides, able to inhibit ACE activity with the aim to control hypertension and then to prevent cardiovascular diseases through diet. In fact nutrition has been reported as one of the main factors influencing blood pressure.

Numerous antihypertensive peptides are originating from milk proteins. Consequently, a variety of naturally formed bioactive peptides have been found in fermented milk, and there are already a few commercial dairy products supplemented with milk protein derived bioactive peptides as described recently by Ferreira, Eça et al. (2007) and Ferreira, Pinho et al. (2007). Many other ACE inhibitory peptides isolated from a wide variety of foods such as casein (Maruyama, Nakagomi, Tomizura, & Suzuki, 1985; Otte, Shalaby, Zakora, & Nielsen, 2007; Tauzin, Miclo, & Gaillard, 2002), soy (Wu & Ding, 2002), egg (Miguel, Alvarez, Lopez-Fandino, Alonso, & Salaices, 2007; Yoshii et al., 2001), or fish (Fujita & Yoshikawa, 1999; Jung et al., 2006; Kawasaki et al., 2000; Kohama et al., 1988) have been identified and reported. For example, LKPNM derived from fish protein showed an IC₅₀ value of 2.4 µM, FFVAP from casein exhibited an IC₅₀ value of 6.0 µM and DLP from soy protein showed an IC₅₀ value of 4.8 µM.

In order to facilitate the identification and isolation of ACE inhibitors peptides, establishment of a simple, sensitive and reliable in vitro inhibition assay is desirable. Numerous methods for the measurement of ACE activity have been reported, including spectrophotometric, fluorometric, radiochemical, high-performance liquid chromatography and capillary electrophoresis methods. Different substrates are suitable to measure ACE activity.

Abbreviations: ACE, angiotensin-converting enzyme; FAPGG, N-(3-[2-furylacryloyl]-Phe-Gly-Gly); FAP, 2-furylacryloyl-L-Phe; HHL, hippuryl-L-histidyl-L-leucine; HA, hippuric acid; TNBS, 2,4,6-trinitrobenzene sulphonate; HPLC, high-performance liquid chromatography.

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