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ORIGINAL ARTICLE

Novel angiotensin-converting enzyme inhibitory peptides from caseins and whey proteins of goat milk

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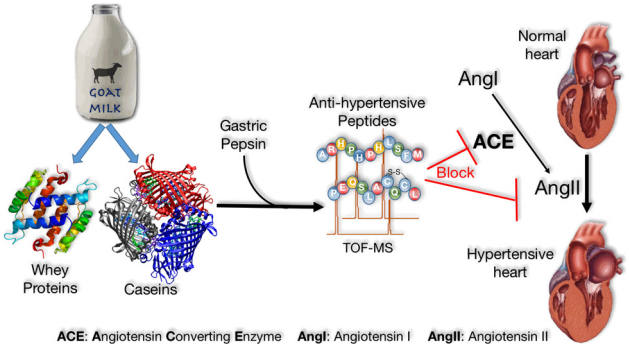
GRAPHICAL ABSTRACT



Abbreviations: GCP, goat casein proteins; GWP, goat whey proteins; P-GCP, pepsin digested-GCP; P-GWP, pepsin digested-GWP; ACE, angiotensin I-converting enzyme; HHL, hippuryl-histidyl-leucine; HA, hippuric acid; HL, histidyl-leucine; TNBS, 2,4,6-trinitrobenzene sulfonate; TNP-, 2,4,6-trinitrophenyl; MALDI-TOF/MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

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ABSTRACT

Angiotensin-converting enzyme (ACE) plays a central role in blood pressure regulation by producing the vasoconstrictor angiotensin II. The inhibition of ACE with natural inhibitors, as alternatives to avoid the side effect of synthetic drugs, is a major target in the prevention of hypertension. In this study, we examined the separated caseins and whey proteins of goat milk for the presence of ACE inhibitory peptides. Digestion of isolated whey proteins and caseins of goat milk by gastric pepsin generated soluble hydrolysates exhibiting significant inhibition of ACE compared to weak inhibition by undigested proteins. The hydrolysates were fractionated by size exclusion chromatography, Sephacryl S-100 column, into four fractions (F1–F4). The late-eluting fraction (F4) of either whey or caseins exhibited greater ACE inhibition. Peptides in both F4 fractions, isolated by RP-HPLC, exhibited variable ACE inhibitory activities with the hydrophobic peptide peaks being the most potent ACE inhibitors. MALDI-TOF MS/MS resulted in identification of three potent ACE inhibitory peptides: PEQSLACQCL from β-lactoglobulin (residues 113–122), QSLVYPFTGPI from β-casein (residues 56–66), and ARHPHPHLSFM from κ-casein (residues 96–106). The peptides from whey and caseins exert significant ACE inhibitory activities comparable to that of captopril, an antihypertensive drug, exhibiting IC50 values of 4.45 μM and 4.27 μM, respectively. The results introduce, for the first time, new potent ACE-inhibitory peptides that can be released by gastric pepsin of goat milk whey and caseins and thus may pave the way for their candidacy as anti-hypertensive bioactive peptides and prevention of associated disorders.

Introduction

Milk proteins are the major source of bioactive peptides released upon enzymatic hydrolysis during gastrointestinal transit or food processing. Such peptides are being identified in dairy protein hydrolysates and shown to possess opioid, immunemodulatory, antimicrobial, antithrombotic, growth stimulating or antihypertensive properties [1,2]. The milk protein-derived bioactive peptides have the potential to be formulated into foods to provide their health promoting effects in human. The major difference between drugs and milk protein-derived bioactive peptides is that synthetic drugs are normally not present within the human body unless they are intentionally administered. On the other hand, bioactive peptides may be present in human as they may arise from digestion of food [2]. These differences may bring additional challenges to the discovery of milk bioactive peptides and to the evaluation of their therapeutic efficacy.

Milk of bovine is the most commonly searched for dairy bioactive peptides. In the past few years, developments in molecular biology, genomics, and proteomics have highlighted the extreme complexity and variability of milk proteins across species [3]. However, in most dairy species, other than bovine, the repertoire of potential milk bioactive proteins or their derived peptides remains to be unraveled. It is one of the greatest challenges facing milk science to provide the basis for health-promoting properties of milk proteins and peptides of dairy species other than bovine. The importance of goat milk is intensifying because cow's milk is a common cause of food allergy in infants [4,5]. In addition, goat milk proteins are more digestible and medically is being recommended for newborn when human milk is lacking [6]. In newborns, milk feeding contributes to protect against oxidative stress and the associated diseases such as cardiovascular disorder [7,8].

Hypertension is recognized as a serious risk factor for cardiovascular disease [9]. Angiotensin I-converting enzyme (ACE) is a key enzyme in regulation of blood pressure through two different reactions in the renin-angiotensin-aldosterone system (RAAS) and the kinin nitric oxide system (KNOS). For this, many synthetic ACE inhibitors, such as captopril, enalapril, fosinopril, lisinopril, and ramipril were identified and used for the treatment of hypertension. However, these synthetic inhibitors have side effects including coughing, taste disturbance and skin rash [10,11]. Thus, one of the major challenges to today's world healthcare sectors is to identify ACE inhibitors from natural resources.

Milk bioactive peptides constitute alternatives for this, serving directly as ACE inhibitors, or providing a scaffold for the engineering of novel molecules with clinical potential. In earlier work we found that gastric pepsin digestion of goat milk proteins generated various bioactive peptides with potent antioxidant activities [12]. The current study aimed to explore the ACE inhibitory activities of hydrolysates and peptides of separated whey proteins and caseins of goat milk, liberated upon cleavage with gastric pepsin. The structures of goat milk peptides for inhibition of ACE enzyme and the potential of whey proteins, by-products of cheese industry, as a source of new therapeutic peptides against hypertension are discussed.

Material and methods

Materials

Goat milk was obtained from three goats, Egyptian Baladi breed, at the animal station of the South Valley University (Qena, Egypt). Angiotensin I-converting enzyme (ACE) from rabbit lung, its substrate hippuryl-L-histidyl-L-leucine (HHL), and pepsin were purchased from Sigma-Aldrich (Tokyo, Japan). Captopril and 2,4,6-trinitrobenzene sulfonate (TNBS) were products of Nacalai Tesque Inc. Sephacryl S-100 was a product of Amersham-Pharmacia Biotech (Tokyo, Japan). All other reagents were of analytical grade.