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In silico methods to identify meat-derived prolyl endopeptidase inhibitors

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ABSTRACT

According to the World Health Organization (WHO), approximately 450 million people suffer from mental or neurological disorders and five of the ten leading causes of disability and premature death worldwide are psychiatric conditions. Social, biological and neurological sciences provided extensive understanding into the role of risk and protective factors in the development of mental disorders and poor mental health. Altered activity of a number of enzymes, such as prolyl endopeptidase (PEP, EC 3.4.21.26), has been linked to the prevention and treatment of a number of mental disorders, including anxiety, depression and Alzheimer's disease. The inhibition of PEP has potential for use in the prevention and in the treatment of mental disorders.

The objective of this work was to identify PEP-inhibitory peptides from meat proteins using *in silico* methods. In this paper, five proteins commonly found in meat by-products were evaluated as a substrate for use in the generation of PEP inhibitory peptides. These include serum albumin, collagen and myosin. These proteins were cleaved *in silico* using BIOPEP and ExPASy PeptideCutter and the generated peptides were compared to known PEP-inhibiting peptides in the database of BIOPEP. A number of novel PEP inhibitory peptide sequences were identified in this study, including PPL, APPH, IPP and PPG with corresponding IC_{50} values of 2.86, 3.95, 4.02 and 2.70 mM, respectively. This work demonstrates the usefulness of *in silico* analysis for predicting the release of PEP-inhibiting peptides from meat proteins.

1. Introduction

Health is defined by the World Health Organization (WHO) as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity (WHO, 2013). Mental health is a key component of health and 450 million people currently suffer from mental and behavioural disorders worldwide (WHO, 2004). Moreover, one in four people are expected to develop one or more mental health disorders during their lifetime, including unipolar depressive disorder, epilepsy, schizophrenia, Alzheimer's and other dementias (WHO, 2004). A number of studies point to the involvement of enzymes in the development of neurodegenerative disorders (Rosenblum & Kozarich, 2003). The use of bioactive peptides as enzyme inhibitors shows potential in the realm of functional food research as a strategy for potential prevention of mental health disorders through diet. Indeed, PEP inhibitory peptides were isolated previously from extracts of plant, red wine, fish, dairy products and bovine brain (Sørensen, Kildal, Stepaniak, Pripp, & Sørhaug, 2004; Wilson, Hayes, & Carney, 2011). Inhibition of enzymes like prolyl endopeptidase (PEP, EC 3.4.21.26) is thought to be important in the prevention of a number of mental health disorders (Rosenblum & Kozarich, 2003; Wilson et al., 2011).

PEP is a highly conserved serine protease which digests a variety of proline-containing small peptides including hormones, neuroactive peptides and various cellular factors, such as those shown in Fig. 1 (García-Horsman, Männistö, & Venäläinen, 2007). Researchers have demonstrated the involvement of PEP in several aspects and functions of the central nervous system (CNS), including learning, memory, mood, hypertension and eating behaviour, and

Abbreviations: PEP, prolyl endopeptidase; WHO, World Health Organization; CNS, central nervous system; UK, United Kingdom; MW, molecular weight; MW-SPPS, microwave-assisted solid phase peptide synthesis; RP-HPLC, reversed-phase-high-performance liquid chromatography; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; AF, average fluorescence; IEP, isoelectric point; SVM, support vector machine; GI, gastrointestinal; DPP-IV, dipeptidyl peptidase-IV; ACE-I, angiotensin-I-converting enzyme; GFAP, glial fibrillary acidic protein; SP, substance P; GnRH, gonadotrophin releasing hormone; TRH, thyrotropin releasing hormone; β-E, β-endorphin.

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some neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases (Lawandi, Gerber-Lemaire, Juillerat-Jeanneret, & Moitessier, 2010). Inhibition of PEP is thought to improve memory and/or learning disorders by blocking or lowering the metabolism of endogenous neuropeptides (Lawandi et al., 2010). Several studies were carried out previously in animal models which demonstrated the anti-amnestic, memory and cognition-enhancing properties of PEP inhibitors (Lawandi et al., 2010; Morain et al., 2002). However, only a few compounds have reached clinical trials in humans. These compounds include the PEP inhibitor S 17092, which is used as an anti-amnesic drug which prevents chemically induced amnesia and spontaneous memory deficits in humans (Morain et al., 2002). Although most of the published PEP inhibitors are chemically synthesised compounds based on the *N*-acyl-L-prolyl-pyrrolidine structure, a small number of naturally occurring peptidic PEP inhibitors have been reported to date (Wilson et al., 2011). For example, the peptides IHPFAQTQ and VYPFPGPI, corresponding to the amino acid sequences f(49–56) and f(60–67) of bovine and ovine β-casein and the peptide VYPFPGPIA, corresponding to the amino acid sequence f(60–68) of ovine β-casein were generated from milk proteins previously (Asano, Nio, & Ariyoshi, 1992). Moreover, the peptide MPPPLPARVDFSLAGALN, corresponding to amino acid sequence f(38–55) of glial fibrillary acidic protein (GFAP), was previously generated from bovine brain and was reported to inhibit PEP (Ohmori, Nakagami, Tanaka, & Maruyama, 1994). The use of *in silico* methods has been shown to be efficient in predicting the release of bioactive peptides from known protein sequences and in the selection of enzymes and proteins for the generation of bioactive peptides (Lafarga & Hayes, 2014). *In silico* tools were also used to identify bioactive peptides with angiotensin-I-converting enzyme (ACE-I; EC 3.4.15.1) and dipeptidyl peptidase-IV (DPP-IV; EC 3.4.23.15) from natural sources including meat by-products and milk proteins (Lafarga, O'Connor, & Hayes, 2014; Nongonierma, Mooney, Shields, & Fitzgerald, 2014). The use of *in silico* techniques for the prediction and identification of bioactive peptides from natural sources is becoming more efficient as new software is developed and novel peptides are identified and characterised. Due to their safe profile and their health promoting properties, PEP inhibitors generated from natural sources have the potential for use as ingredients in functional products for the prevention of mental health disorders. The presence of PEP inhibiting sequences in bovine brain proteins and the similarities between fish and meat proteins suggests that meat muscle and by-product proteins are potential precursors for PEP inhibiting peptides.

The aim of this study was to evaluate the PEP-inhibitory activity of a number of proline-containing peptides which were predicted to be released from proteins present in meat by-products of bovine and porcine origin and hydrolysed with a number of different commercially available enzymes using *in silico* analysis. A number of the resultant proline-rich peptides were chosen for chemical synthesis and PEP inhibitory activity *in vitro* was confirmed. Active PEP-inhibiting peptides were assessed further using an *in silico* digestion methodology in order to predict their stability following exposure to enzymes active during gastrointestinal (GI) digestion. A number of PEP-inhibiting peptide sequences were identified and found to be resistant to GI digestive enzymes *in silico*. However, the ability of these peptides to cross the blood brain barrier (BBB) was not confirmed.

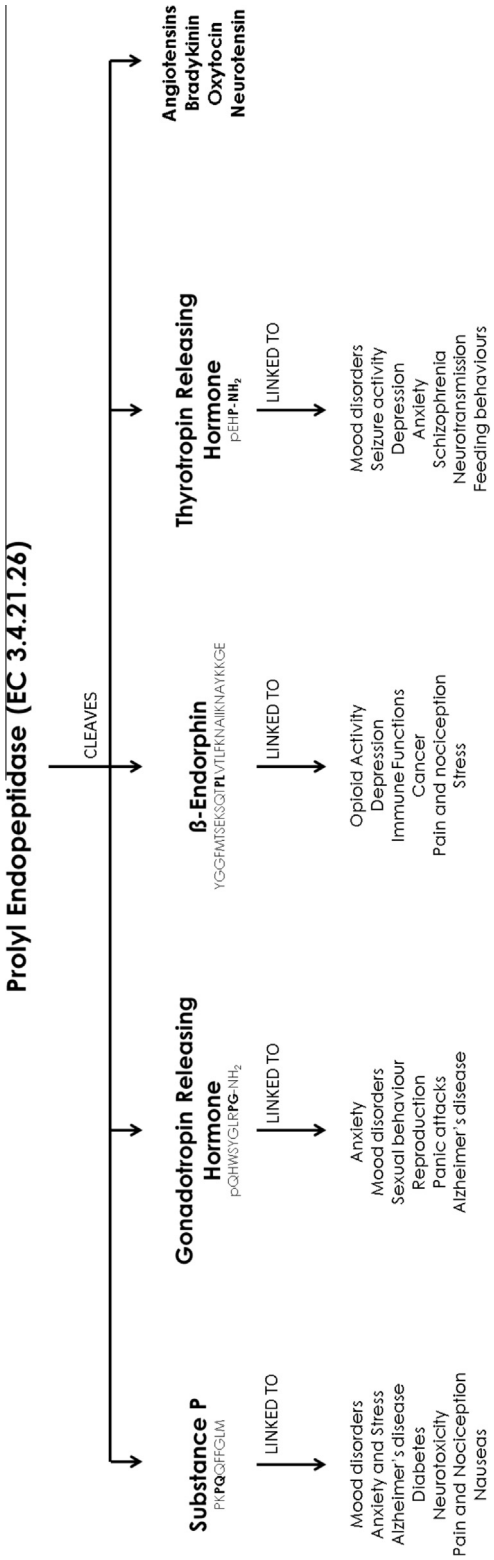

Fig. 1. Small proline-containing peptides susceptible to digestion by PEP and some of their associated diseases. Substance P (SP), gonadotrophin releasing hormone (GnRH), thyrotropin releasing hormone (TRH), β-endorphin (β-E), angiotensins, bradykinin or oxytocin are cleaved by PEP. Cleavage bonds are shown in bold. SP has been associated, for example, with the regulation of mood disorders, anxiety and stress, diabetes, pain and nociception or nauseas and nemesis (Diemunsch & Grelot, 2000; Zubrzycka & Janecka, 2000). GnRH is thought to be related to anxiety, mood disorders, reproduction, sexual behaviour and the development of Alzheimer's disease (Meethal, Smith, Bowen, & Atwood, 2005).

2. Materials and methods

2.1. Materials and reagents

Valproic acid, which was used as a positive control, was supplied by Sigma Aldrich (Steinheim, Germany). The fluorogenic