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Predictive modelling of angiotensin converting enzyme inhibitory dipeptides

Roseanne Norris [a], Fergal Casey [c,d], Richard J. FitzGerald [a,b,*], Denis Shields [c,d,*], Catherine Mooney [c,d]

[a] Department of Life Sciences, University of Limerick, Limerick, Ireland
[b] Food for Health Ireland, University of Limerick, Limerick, Ireland
[c] Complex and Adaptive Systems Laboratory, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland
[d] School of Medicine and Medical Science, University College Dublin, Dublin, Ireland

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ABSTRACT

The ability of docking to predict angiotensin converting enzyme (ACE) inhibitory dipeptide sequences was assessed using AutoDock Vina. All potential dipeptides and phospho-dipeptides were docked and scored. Peptide intestinal stability was assessed using a prediction amino acid clustering model. Selected dipeptides, having AutoDock Vina scores $\leqslant -8.1$ and predicted to be 'stable' intestinally, were characterised, using LIGPLOT and for ACE-inhibitory potency. Two newly identified ACE-inhibitory dipeptides, Asp-Trp and Trp-Pro, having Vina scores of −8.3 and −8.6 gave IC_{50} values of 258 ± 4.23 and 217 ± 15.7 μM, respectively. LIGPLOT analysis indicated no zinc interaction for these dipeptides. Phospho-dipeptides were predicted to have a good affinity for ACE. However, the experimentally determined IC_{50} results did not correlate since, for example, Trp-pThr and Pro-pTyr, having Vina scores of −8.5 and −8.1, respectively, displayed IC_{50} values of >500 μM. While docking allowed identification of new ACE inhibitory dipeptides, it may not be a fully reliable predictive tool in all cases.

1. Introduction

Angiotensin converting enzyme (ACE, EC 3.4.15.1) is a key target for the treatment of hypertension due to its involvement in a number of blood pressure-related systems, e.g., the renin–angiotensin system (RAS) and the kinin nitric oxide system (KNOS). In the RAS, ACE cleaves angiotensin I into the potent vasoconstrictor angiotensin II, and in the KNOS, ACE inactivates the hypotensive peptide, bradykinin (Eriksson, Danilczyk, & Penninger, 2002). Therefore, excessive action of ACE leads to increased vasoconstriction and hypertension. The discovery of the first ACE-inhibitory peptides in the venom of the snake, *Bothropa jararace*, in the 1970s, led to the development of synthetic ACE inhibitors, such as Captopril, Lisinopril and Enalapril (Meyer, Essenburg, Smith, & Kaplan, 1982). However, the use of these pharmacological ACE inhibitors is associated with a range of side-effects, including angiodema, cough, skin rashes, reduced renal function and fetal abnormalities (Libby, Bonow, Mann, & Zipes, 2008).

There has been an ongoing search for natural food-derived ACE inhibitory peptides. These peptides have been identified in a variety of food proteins and they become active when released from their parent proteins through enzymatic hydrolysis in food processing and/or digestion (Murray & FitzGerald, 2007). Although these peptides are less potent than are synthetic ACE inhibitors (IC_{50} values in μM range), they have potential as active components in the diet by integration into functional food products. At present, the search for ACE inhibitory peptides has focused primarily on the production and characterisation of peptides isolated from microbial fermentation or enzymatic digests of proteins with limited studies involving the relationship between structure and activity of peptide inhibitors (FitzGerald & Meisel, 2000). However, some structure–activity features of ACE inhibitory peptides have been identified. Potent ACE-inhibitory peptides are generally short sequences (2–12 amino acids) and contain aromatic or hydrophobic residues at their C-terminus, with many containing Pro, Trp and Lys residues (FitzGerald & Meisel, 2000; López-Fandino, Otte, & van Camp, 2006; Murray & FitzGerald, 2007; Pripp, Isaksson, & Stepaniak, 2004). ACE also seems to prefer positively charged functional groups at the N-terminus. Bulky and hydrophobic side chains seem to be features favoured by ACE inhibitory dipeptides (Wu, Aluko, & Nakai, 2006a). ACE, being a metallopeptidase, contains a zinc-binding motif, HEXXH (X = any amino acid residue), which is located on both the C- and N-domains of somatic ACE (Ehlers & Riordan, 1991). The active sites of the two-domain somatic ACE are located within the cleft of the two domains. The Zn^{2+} molecule binds to the two histidines of the motif, and is known to directly interact with inhibitors at the active site. The cleft is partially covered by an N-terminal 'lid' thus allowing for only small peptides to reach the active

* Corresponding authors at: Department of Life Sciences, University of Limerick, Limerick, Ireland. Tel.: +353 0 61 202598; fax: +353 0 61 331490 (R.J. FitzGerald), Complex and Adaptive Systems Laboratory, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland. Tel.: +353 0 1 7165344 (D. Shields).
E-mail addresses: dick.fitzgerald@ul.ie (R.J. FitzGerald), denis.shields@ucd.ie (D. Shields).

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sites (Natesh, Schwager, Evans, Sturrock, & Acharya, 2003). Much of what is known about the structure–activity relationship of these peptides has been derived from qualitative analysis with chemically synthesized peptides or with analogues that have similar structures to known inhibitors, work which is both time-consuming and costly.

Computational methods, such as quantitative structure–activity relationship modelling (QSAR), artificial neural networks (ANN) and virtual substrate docking, are tools increasingly being applied in medicinal and pharmaceutical drug discovery. They allow for a statistical prediction of the potential of a small molecule (ligand) to bind to a macromolecule (receptor), while allowing for a better understanding of the molecular mechanisms involved (Pripp, Isakasson, Stepaniak, Sorhaug, & Ardo, 2005). There is significant scope for predictive approaches to be applied as an alternative method for identifying bioactive sequences in food proteins (Pripp et al., 2005). A limited number of QSAR and ANN studies have been carried out to date, focusing on food protein-derived ACE-inhibitory peptides (Majumder & Wu, 2010; Meisel, Walsh, Murray, & FitzGerald, 2006; Wu, Aluko, & Nakai, 2006a, 2006b). Docking studies have potential in the area of ACE-inhibitory peptides as they allow virtual screening of peptides for their theoretical inhibitory activity (Pripp, 2007). All possible docking or binding conformations can be assessed for their binding affinity to a receptor, and then 'scored' on their potential complimentarities. Although experimental verification is still required, if a correlation between theoretical and actual ACE-inhibitory potential is found, target peptides of interest may be identified without the need for a time-consuming conventional peptide discovery strategy (Majumder & Wu, 2010). Furthermore, quantitatively relating peptide sequence to inhibitory activity allows for physicochemical interpretation and for further molecularly-designed optimisation experiments (Pripp, 2007).

AutoDock Vina is a molecular docking programme which can be used for virtual ligand–protein screening. In the case of ACE, this programme allows the docking of peptide sequences with the active site, e.g., against PDB structure 1UZF (complex of captopril and human testicular angiotensin converting enzyme, Natesh, Schwager, Evans, Sturrock, & Acharya, 2004). Additional *in silico* approaches to characterise ligand–protein interactions are also available. The LIGPLOT programme allows the generation of schematic diagrams of protein–ligand interactions (Wallace, Laskowski, & Thornton, 1995) and has the potential to provide useful information on key interactive events during binding. The intestinal stability, and thus the bioavailability of peptides, is central to their ability to mediate a physiological response (Vermeirssen, van Camp, & Verstraete, 2004). The intestinal stability of peptides may be assessed using an amino acid clustering model (Foltz, van Buren, Klaffke, & Duchateau, 2009). This model predicts how stable a peptide may be under simulated small intestinal digestion conditions.

The objective of this study was first to assess the potential of using AutoDock Vina and LIGPLOT analysis, along with small intestinal stability modelling, for the *in silico* identification of potent ACE-inhibitory dipeptides. The second objective was to quantify the *in vitro* ACE-inhibitory potency of the identified dipeptides in order to validate the potential of the predictive computational methods in the discovery of potent ACE-inhibitory peptides.

2. Materials and methods

2.1. Materials

Captopril, enalapril maleate, lisinopril, rabbit lung acetone powder, and sodium tetraborate decahydrate (borax) were purchased from Sigma Chemical Co. (Poole, Dorset, UK). *o*-Aminobenzoylglycl-*ρ*-nitro-L-phenylalanyl-L-proline (Abz-Gly-Phe-(NO_2)-Pro) and o-aminobenzoylglycine (Abz-Gly-OH) were from Bachem Feinchemikalien (Bubendorf, Switzerland). Synthetic peptides, Pro-Tyr, Trp-Thr, Tyr-Thr, Pro-pTyr, pTyr-pSer, Trp-Pro, Trp-pThr, Tyr-Ser, pTyr-pThr and Asp-Trp (where p represents a phosphorylated residue), were obtained from GenScript Corporation (Piscataway, NJ, USA).

2.2. Computational analysis

AutoDock Vina was used to dock all 529 possible dipeptides (from the twenty standard amino acids and three phosphorylated amino acids, Tyr, Ser and Thr) and six synthetic drug inhibitors (captopril, enalaprilat, lisinopril, RXPA380, kAF and kAW) against chain A of the PDB structure 1UZF (Natesh et al., 2004). The structures of the six drug inhibitors were taken from their respective PDB files (see Table 1) and the initial poses of the PDB-formatted structures of dipeptides were generated using the Open Babel Package, version 2.1.1 (Guha et al., 2006). AutoDockTools (ADT) was used to prepare the ligands and the 1UZF receptor and to determine the 'search space'. Dipeptides and inhibitors were then docked with the PDB structure, 1UZF, giving a Vina score i.e., the predicted affinity of the molecule to bind to the PDB structure, calculated in kcal/mol. A more negative score indicates that a molecule (ligand) is more likely to dock with the structure (receptor) and achieve more favourable interactions. The Vina scoring system was tuned using PDBbind and is described by Trott and Olsen (2010).

LIGPLOTs were generated for the six drug inhibitors (using their PDB files as input) and the highest ranked dipeptides, i.e., having Vina scores $\leqslant -8.1$ (using the Vina docking poses) in complex with ACE according to the protocol described by Wallace et al. (1995).

Dipeptides were assessed for their intestinal stability, using an amino acid clustering model adopted from Foltz et al. (2009). This model rates dipeptides as 'stable,' 'neutral' or 'unstable' with regard to small intestinal stability, using correlations between the N- and C-terminal amino acids of dipeptides and the stability of the dipeptide in the intestine.

Histograms and scatter plots were generated using the R package (R Development Core Team, 2004).

2.3. ACE activity assay

ACE inhibitory activity was determined using a fluorometric microtitre assay, as described by Sentandreu and Toldrá (2006) with some modifications. ACE was extracted from rabbit lung acetone powder as described by Murray, Walsh, and FitzGerald (2004). The reaction was carried out as follows: 50 μl of enzyme extract were added to 100 μl of assay buffer (100 mM sodium borate buffer, 300 mM NaCl, pH 8.3) and preincubated for 5 min at 37 °C. The reaction was initiated with the addition of the synthetic substrate, Abz-Gly-Phe-(NO_2)-Pro (0.45 mM), dissolved in assay buffer. The assay was run continuously for 30 min and fluorescence from the release of product (Abz-Gly) was quantified at time zero and time 30 min, using a BioTek Synergy HT plate reader (BioTek Instruments Inc., VT, USA). Excitation and emission wavelengths were 360 and 400 nm, respectively. One unit of ACE activity (U/ml) was defined as the amount of enzyme hydrolysing 1 μmol of Abz-Gly-Phe-(NO_2)-Pro per min at 37 °C.

For inhibition studies, an ACE activity of 10 mU/ml was used for each determination by diluting the enzyme solution appropriately with assay buffer. Dipeptides were analysed at final concentrations of 500, 250, 100, 50, 10, 1, 0.5, 0.1, 0.05, and 0.01 μM. For the synthetic ACE inhibitors, determinations were carried out at concentrations of 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001 μM. The reaction was carried out as above except that 50 μl of inhibitor and 50 μl of buffer were added in place of 100 μl of buffer. The %