Gastrointestinal Endogenous Proteins as a Source of Bioactive Peptides

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Abstract

Gastrointestinal endogenous proteins (GEP) were investigated as a source of bioactive peptides. *In silico* and *in vitro* methods were used singly or in combination to study GEP-derived peptides after simulated digestion. The presence of bioactive peptides after *in vivo* digestion was determined using a porcine model. Bioactivity of the peptides was assessed using selected *in vitro* bioactivity assays, and peptides were characterised using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and mass spectrometry.

In the *in silico* study, twenty six different GEP and seven dietary proteins were subjected to simulated *in silico* gastrointestinal (SIGIT) digestion. The predicted resultant peptides possessing amino acid sequences identical to those of known bioactive peptides were identified by screening them against an online database of bioactive peptides (BIOPEP). The predicted number of bioactive peptides released after the SIGIT digestion of GEP ranged from 1 (secretin) to 39 (mucin-5AC), while those for dietary proteins ranged from 1 (gliadin) to 55 (myosin). Angiotensin-I-converting enzyme (ACE-I) inhibitory peptide sequences were found in abundance in both GEP and dietary proteins. The GEP mucin-5AC and the dietary protein myosin were predicted to release the highest number of ACE-I inhibitory peptides (38 and 49 peptides respectively), and were found to be comparable in their potential to release ACE-I inhibitory peptides.

Following SIGIT digestion of eleven representative GEP, nineteen novel GEP-derived peptide sequences were selected by applying quantitative structure-activity relationship rules, and were chemically synthesised. Two novel peptides with the

amino acid sequences RPCF and MIM, showing dipeptidyl peptidase IV (DPP-IV) inhibitory activity and five novel antioxidant (2,2-diphenyl-1-picrylhydrazyl (DPPH)- inhibitory and, or ferric reducing antioxidant power (FRAP) activity) peptides with amino acid sequences CCK, RPCF, CRPK, QQCP and DCR were identified. These results indicate that GEP may contain novel bioactive peptide sequences.

The potential release of bioactive peptides, from four GEP (trypsin, lysozyme, mucin, and serum albumin) and a dietary protein (chicken albumin), in the gastrointestinal tract (GIT) was investigated using an *in vitro* digestion model. The *in vitro* digests were screened for ACE-I-, renin-, platelet-activating factor acetylhydrolase (PAF-AH)-, and DPP-IV-inhibition, and antioxidant activity. All four *in vitro* GEP digests showed ACE-I inhibition comparable to that of the positive control captopril. In comparison to the unfractionated digests, the enriched fractions (<3 and <10 kDa) of lysozyme and serum albumin showed greater renin-, PAF-AH-, and DPP-IV-inhibition, and antioxidant potential. Over 190 peptide sequences were identified from these fractions using mass spectrometry.

Stomach chyme (SC) and jejunal digesta (JD) were collected from growing pigs that were fed a protein-free diet for a period of 3 days. The peptides extracted from SC and JD samples were characterized by SDS-PAGE, and their ACE-I-, DPPH-, and microsomal lipid peroxidation (MLP)- inhibition, FRAP activity determined. Potential bioactive peptides responsible for bioactivity were identified using mass spectrometry. SDS-PAGE analysis showed that all of the samples contained a heterogeneous mixture of peptides. Porcine JD samples inhibited ACE-I and DPPH, while SC samples inhibited MLP. Characterization studies identified over 180

peptide sequences from the enriched fractions of SC and JD samples that showed the highest activity. Further, a porcine serum albumin peptide sequence (FAKTCVADESAENCDKS) was found to be a sub-sequence of a larger sequence identified in the *in vitro* digest of human serum albumin. There was considerable inter-animal variation for the bioactivities. This may be attributed to sampling effects and, or natural variations in the gut contents, thus underlining the complexity involved in *in vivo* release of bioactive peptides.

Together, the results indicate: 1) GEP contain abundant encrypted bioactive peptide sequences; 2) GEP-derived bioactive peptides display a range of bioactivities; 3) GEP-derived bioactive peptides are released during gastrointestinal digestion in pigs; 4) GEP may contain numerous novel bioactive peptide sequences encoded within their primary sequence.

In conclusion, the evidence reported here suggests that, like the dietary proteins, GEP are also a potentially rich source of exogenously-derived bioactive peptides in the gastrointestinal tract. Beyond their primary functions, GEP may act as an important cryptomic source of bioactive peptides, given that the amount of GEP secreted into the gut is equal to or greater than the dietary protein ingested per day, and that up to 80% of GEP are known to be digested.

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Contents

Abbreviations

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

ACE-I: Angiotensin-I-converting enzyme

CaMPDE: Cyclic nucleotide phosphodiesterase 1

DPPH 2,2-Diphenyl-1-picrylhydrazyl

DPP-IV: Dipeptidyl peptidase IV

ENL: Endogenous nitrogen losses

ENS: Enteric nervous system

ESI-TOF LC-MS/MS: Electrospray ionization time-of-flight liquid chromatography tandem mass spectrometry

ESI-TOF MS: Electrospray ionization time-of-flight mass spectrometry

ExBP: Exogenous bioactive peptides

FAO (UN FAO): Food and agriculture organisation of the United Nations

FRAP Ferric reducing antioxidant power

GALT: Gut-associated lymphoid tissue

GEP: Gut endogenous proteins

GIT: Gastrointestinal tract

GLP-2: Glucagon-like peptide-2

MAFP: Methyl arachidonyl fluorophosphonate

MLP inhibition: Microsomal lipid peroxidation inhibition

MWCO: Molecular weight cut-off

NOD: Nucleotide-binding oligomerization domain-like receptors

PAF-AH: Platelet-activating factor acetylhydrolase

QS: Quorum sensing

QSAR: Quantitative structure-activity relationship

RAAS: Renin angiotensin aldosterone system

RFU: Relative fluorescence units

SDS-PAGE: Sodium dodecyl sulphate polyacrylamide gel electrophoresis

SIGIT digestion: Simulated *in silico* gastrointestinal digestion

TEnBP: Truly endogenous bioactive peptides

WPI: Whey protein isolate

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List of Publications

Note: All of my publications have Lakshmi A. Dave as my author name

- **Dave, L. A.,** Hayes, M., Mora, L., Montoya, C. A., Moughan, P. J., & Rutherfurd, S M. (2016). Gastrointestinal endogenous protein-derived bioactive peptides: An *in vitro* study of their gut modulatory potential. International Journal of Molecular Sciences, 17(4), 482. doi:10.3390/ijms17040482.

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