Hypocholesterolemic peptides from soy protein: study on the absorption across Caco-2 cells and degradation by DPP-IV activity

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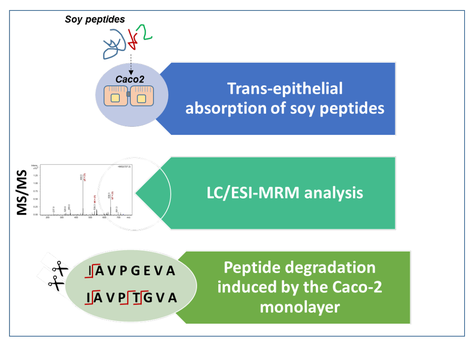
Introduction

IAVPGEVA, IAVPTGVA, and LPYP, three peptides deriving from soy protein hydrolysis, inhibit the activity of 3-hydroxy-3-methylglutaryl CoA reductase (HMGCoAR) [1-2] and modulate cholesterol metabolism in HepG2 cells. In order to assess whether these hypocholesterolemic peptides can be absorbed across the epithelium barrier, experiments were performed using human intestinal Caco-2 cell monolayers grown in two-compartments systems. Each peptide (500 μM) was incubated in the apical compartment for a time spanning from 15 to 120 min and quantified in the basolateral compartment using a highly sensitive LC-MRM method. The peptides were partially absorbed across the Caco-2 monolayers, but they were also hydrolyzed to shorter fragments by brush border peptidases. A main role in this metabolism is probably played by dipeptidyl peptidase IV (DPP-IV), since biochemical experiments showed that these peptides are substrates of this enzyme acting as competitive inhibitors. In silico docking simulations suggested that some metabolites may retain a hypocholesterolemic activity.

Aim and Workflow

The objectives of the present work were i) the evaluation of the transepithelial transport of IAVPGEVA, IAVPTGVA, and LPYP across Caco-2 monolayers, ii) the development of a sensitive LC-MS method for the quantitation of these peptides, iii) the investigation of their possible degradation induced by Caco-2 cells.



Results

1. Absorption experiments with Caco-2 cell monolayers

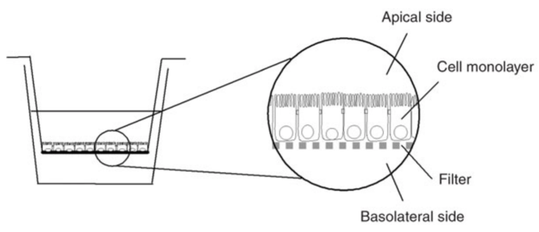
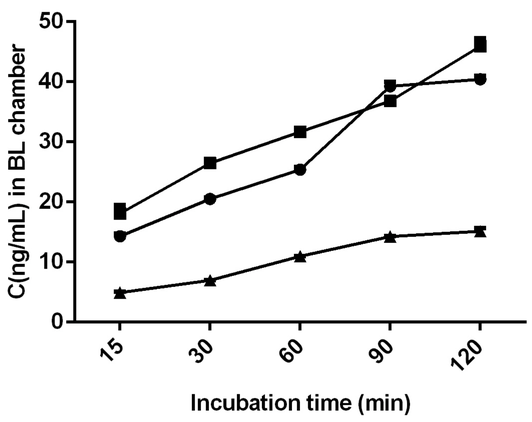


Figure 1: Diagram of a Caco-2 monolayer grown on a permeable filter support.



A)

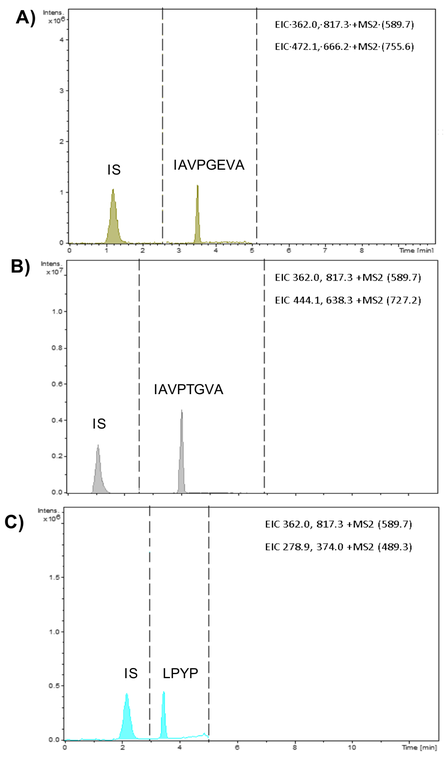
GRADIENT ELUTION	
Time (min)	% B
0	3
4	80
5	10
10 (post run)	3

B)

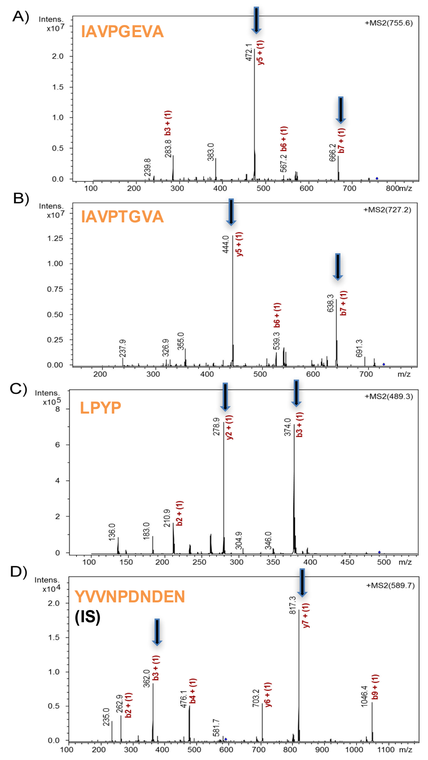
Time (min)	% B
0	3
6	60
8	5
13 (post run)	3

C)

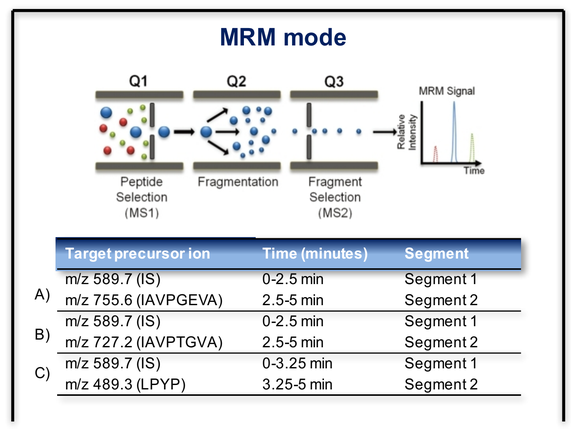
Time (min)	% B
0	5
5	80
6	5
11 (post run)	5



2. Optimization of LC-MRM assay

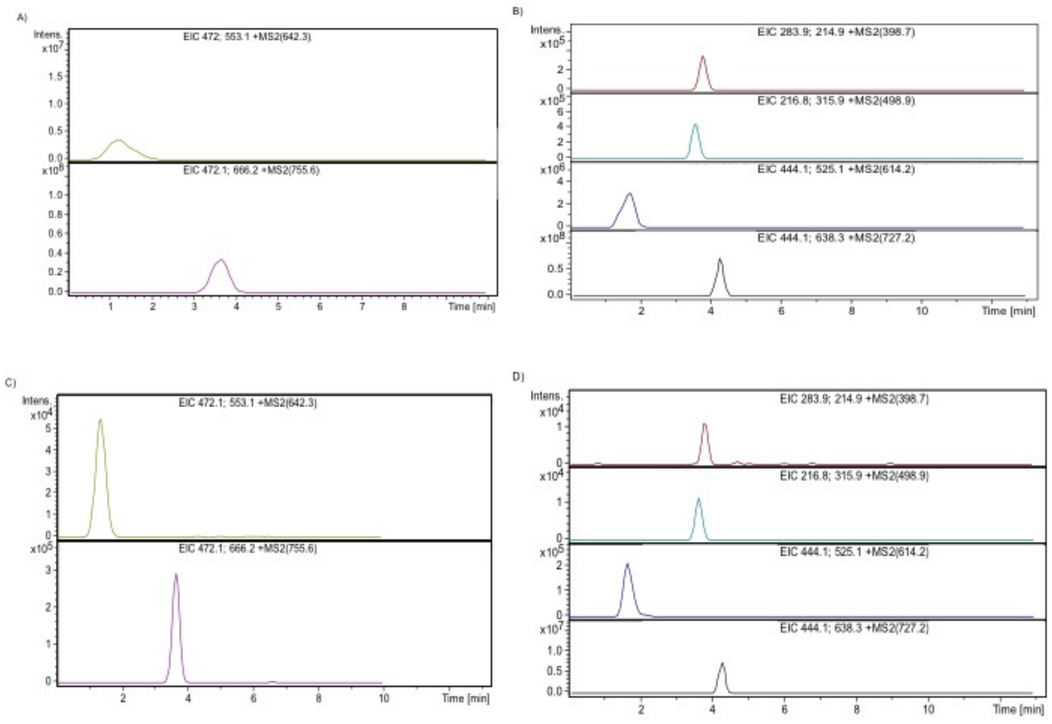


	Peptides	[M+H]+	Transitions	fragments
A)	IAVPTGVA	727.4	727.4→444.1 727.4→638.3	*y5* *b7*
B)	IAVPGEVA	755.6	755.6→472.1 755.6→666.3	*y5* *b7*
C)	LPYP	489.3	489.3 → 278.9 489.3→ 374.0	y2 b3
D)	YVVNPDNDEN (IS)	589.7	589.7→817.3 589.7→362.0	y7 b3



	Target precursor ion	Time (minutes)	Segment
A)	m/z 589.7 (IS)	0-2.5 min	Segment 1
	m/z 755.6 (IAVPGEVA)	2.5-5 min	Segment 2
B)	m/z 589.7 (IS)	0-2.5 min	Segment 1
	m/z 727.2 (IAVPTGVA)	2.5-5 min	Segment 2
C)	m/z 589.7 (IS)	0-3.25 min	Segment 1
	m/z 489.3 (LPYP)	3.25-5 min	Segment 2

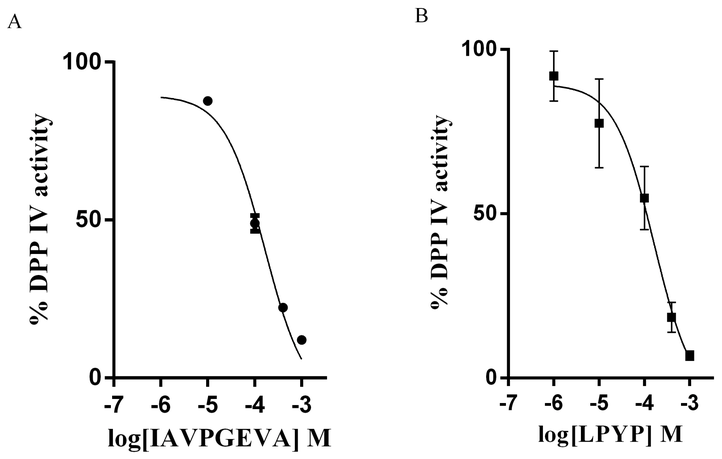
3. Analysis of the metabolites produced by Caco-2 cells

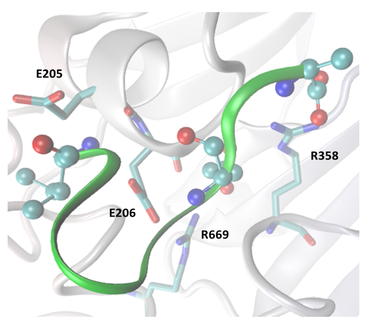


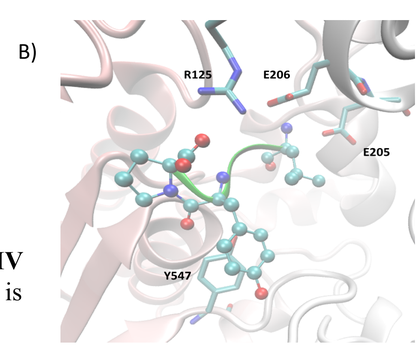
Extracted ion chromatogram (EIC) of precursor peptides and hydrolytic fragments after 120 min. A) EIC of AVPGEVA (*m/z* 642.3) and IAVPGEVA (*m/z* 755.6) at the AP side. B) EIC of IAVP (*m/z* 398.7), IAVPT (*m/z* 498.9), AVPTGVA (*m/z* 614.2) and IAVPTGVA (*m/z* 727.2) at AP side. C) EIC of AVPGEVA (*m/z* 642.3) and IAVPGEVA (*m/z* 755.6) at the BL side. D) EIC of IAVP (*m/z* 398.7), IAVPT (*m/z* 498.9), AVPTGVA (*m/z* 614.2) and IAVPTGVA (*m/z* 727.2) at BL side.

4. Inhibition of the catalytic activity of DPP-IV

Dose–response curves of the inhibitory action of IAVPGEVA (A) and LPYP (B) on DPP-IV. The estimated IC_{50} values were equal to 94.6 ± 0.04 μM and 164.3 ± 0.13 μM, respectively.

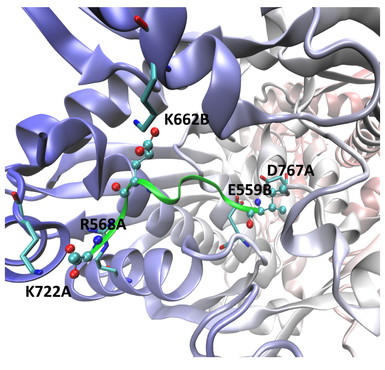






Main interactions stabilizing the putative complexes between DPP-IV and IAVPGEVA (A) and LPYP (B). The backbone of bound peptides is represented in green cartoon.

5. In silico simulation of the docking of the metabolites with HMGCoAR



Main interactions stabilizing the putative complexes between HMGCoA reductase and IAVPGEVA

shorter metabolites (IAVPT and IAVP) show seemingly less stable complexes in which they are able to maintain the ion-pairs elicited by their carboxyl terminus but lose the key contacts involving their amino group.

Conclusion

This findings provide the first evidence of a transepithelial absorption across Caco-2 cell monolayers of hypocholesterolemic peptides deriving from the hydrolysis of soy glycinin. The MS-based approach allowed detecting soy peptides as intact compound as well as metabolic fragments.

References

Lammi, C., Zanoni, C., & Arnoldi, A. *Journal of Functional Foods, 14*, 469-478.
Lammi, C., Zanoni, C., & Arnoldi, A. *Int J Mol Sci, 16*(11), 27362-27370