The Major Peanut (*Arachis hypogaea*) Allergens Potentially Contain Antibacterial and Antiviral Peptides: An *In Silico* Study

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INTRODUCTION

Peanut (*Arachis hypogaea*) seeds are known source of proteins, carbohydrates, lipids, vitamins, and minerals (Settaluri et al., 2012). Its protein includes defensive seed storage proteins or allergens that can induce allergic reactions (Portnoy, 2015). Its major allergens are Ara h 1 (A1), a 7S trimeric vicilin, Ara h 2 (A2), a 2S albumin, and Ara h 3 (A3), an 11S hexameric legumin (Palladino et al., 2018). They can be hydrolyzed into peptides that may exhibit bioactive properties such as antibacterial (AB) and antiviral (AV).

Literatures are mostly focused on the molecular mechanisms of the immune responses triggered by these allergens. Moreover, this study aims to explore the potential bioactive peptides of these allergens through proteolytic hydrolysis, which can reduce its allergenicity and provide a basis for antibiotic alternative.

OBJECTIVES

- ☐ to generate peptides from the major allergens of peanut seeds through *in silico* proteolysis
- ☐ to determine the antibacterial and antiviral probability of the peptides, and correlate with its GRAVY values
- ☐ to analyze the residue-level interaction of selected AB and AV peptide with protein receptor using molecular docking

METHODOLOGY

Gather sequence in NCBI using accession no.:

A1	ADQ53858
A2	QGA89290
A3	ADQ53859

Hydrolyze protein in ExPASy Peptide Cutter using:

proteases

pepsin
trypsin
chymotrypsin

Screen generated peptides using:

AB	AV
iAMPpred MLAMP	Meta-AVP

Determine GRAVY of the peptides using GRAVY calculator

GRAVY (Kyte et al., 1982)

+	hydrophobic
-	hydrophilic

Correlate probability and GRAVY using Spearman's

Spearman's Correlation (Mukaka, 2012)

r_s Value	Strength
± 0.00 - 0.30	Negligible
± 0.30 - 0.50	Low
± 0.50 - 0.70	Moderate
± 0.70 - 0.90	High
± 0.90 - 1.0	Very high

Determine residue-level interaction of peptide and receptor (6RYP and 3G7T) using Chimera

IMGT Hydropathy Classification (Pommié et al., 2004)

AA	Hydropathy Index	IMGT Classification	AA	Hydropathy Index	IMGT Classification
I	4.5	Hydrophobic	G	-0.4	Neutral
V	4.2		T	-0.7	
L	3.8		S	-0.8	
F	2.8		Y	-1.3	
C	2.5		P	-1.6	
M	1.9		H	-3.2	
A	1.8		N	-3.5	Hydrophilic
W	-0.9		D	-3.5	
K	-3.9	Hydrophilic	Q	-3.5	
R	-4.5		E	-3.5	

RESULTS AND DISCUSSION

Table 1. Number of antibacterial and antiviral peptides generated per major allergen.

Parent Protein	Generated Peptides	Antibacterial Peptides		Antiviral Peptides	
		iAMPpred	MLAMP	iAMPpred	Meta-iAVP
A1	114	75	40	37	26
A2	31	22	5	18	5
A3	83	53	28	33	15

- ☐ The peptide is considered as antibacterial and antiviral if it has probability ≥ 0.5.
- ☐ The differences on the number of potential bioactive peptides are due to the differences in databases and algorithms of the tools.

Table 2. Correlation of GRAVY and bioactive probabilities per allergen-derived peptides.

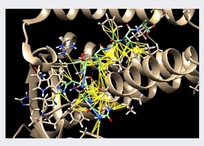
Property	Tool	Spearman's Coefficient (r_s)		
		Ara h 1	Ara h 2	Ara h 3
Antibacterial	iAMPpred	0.184	0.558	0.095
	MLAMP	0.816	0.400	0.739
Antiviral	iAMPpred	0.366	0.493	0.299
	Meta-iAVP	0.151	0.308	0.629

- ☐ There is a considerable positive monotonic relationship between GRAVY and bioactive probabilities (AB and AV), but not significantly linear.
- ☐ This signifies that GRAVY influenced the bioactive tendencies of the peptides.

Table 3. Residue level interaction of ^{393}NAHTIVVA^{400} (left) and ^{64}QCAGVA^{69} (right) peptide with bacterial and viral receptor summarized from 158 and 69 contacts, respectively.

Peptide	Receptor	Interacting Residue
Ala 2	Phe 129	O
Ile 5	Leu 139	O
	Val 143	O
Val 6	Ile 74	O
	Leu 99	O
	Leu 139	O
	Val 143	O
	Leu 78	O
His 3	Pro 130	N
	Thr 140	N
Val 7	Val 143	O
	Ile 146	O
	Leu 99	O
Ala 8	Ile 146	O
	Phe 81	O

I – hydrophilic | O – hydrophobic | N – neutral



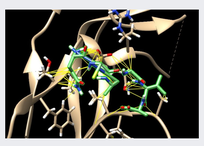
Antibacterial peptide: ^{393}NAHTIVVA^{400} derived from Ara h 3

Receptor: lipoprotein-processing enzyme (LspA) of MRSA

- ☐ Predominant interaction: hydrophobic (O)
- ☐ Antibacterial peptides mainly inflict damages on bacterial membrane (Pelegrini et al., 2011).

Peptide	Receptor	Interacting Residue
Gln 1	Asp 235	I
Ala 3	Val 91	O
	Cys 92	O
Val 5	Ala 88	O
	Phe 90	O
Ala 6	Cys 92	O
	Val 91	O

I – hydrophilic | O – hydrophobic | N – neutral



Antiviral peptide: ^{64}QCAGVA^{69} derived from Ara h 3

Receptor: dengue virus type I envelope protein

- ☐ Predominant interaction: hydrophobic (O)
- ☐ Antiviral peptides cause interferences in protein-protein interaction between host and viral proteins (Lou et al., 2014).
- ☐ The peptide interacts with residues near the binding site of the viral receptor.

CONCLUSIONS

- ☐ Several potential antibacterial and antiviral peptides were generated from the peanut major allergens.
- ☐ For antibacterial property, non-negligible correlations between GRAVY and probability were observed using MLAMP for A1 and A3 peptides. For antiviral, non-negligible correlations were observed using iAMPpred for A1 peptides and using Meta-iAVP for A3 peptides. For A2 peptides, non-negligible correlations were observed using all tools, but mostly low positive.
- ☐ The predominating interaction of the antibacterial and antiviral peptide with corresponding receptors are hydrophobic. This indicates that GRAVY influence the bioactivity of peptides through its mechanism of action.

RECOMMENDATION

- ☐ *In vitro* experiments to verify the bioactivity of the peptides as antibacterial and antiviral agents.
- ☐ Development of *in silico* tools which consider GRAVY as a physicochemical property in calculating the bioactive probabilities of the peptides.
- ☐ Exploration of other storage proteins for their potential bioactive peptide content.

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