



Structural Bioinformatics Approach in Bioactive Peptide Research: Tomato Vicilin Case Study

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Bioactive Peptides



- Bioactive peptides (BAP) are derived from food proteins and exert a positive effect in humans due to its health-promoting properties
- Both animal and plant proteins are known to contain potential bioactive sequences, most studies to date have involved milk proteins.
- BAPs have direct effects on human system & health

- gastrointestinal system
- the cardiovascular system
- the immune system
- the nervous system



BAP Production

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION (227.



Daliri, E. B.-M., Lee, B. H., & Oh, D. H. (2018). Critical Reviews in Food Science and Nutrition, 58(13), 2273–2284.

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BAP prediction: Protein Structure ?

 There are several bioinformatics tools used in identification of BAPS based on amino acid sequence of the protein and the digestion patterns of different proteases.



Tertiary Structure



Structural Biology approach

Plant BAPs: Tomato seed proteins

- Tomato seed proteins extracted
- in silico proteolyis
- >experimental proteolysis
- ➢ in vitro activity tests
- ➢ sequencing of bioactive peptides

4 major protein bands ; vicilin (45 kDa), legumin α (30 kDa), 14-3-3 (22 kDa) legumin β (18 kDa)

vicilin was selected for further analysis.



Analysis of tomato seed proteins on 12% SDS-PAGE. L:ladder, 1-2: different extraction protocols

Structural modeling

>vicilin precursor [Solanum lycopersicum]

MAIFTKPKLL FIFFLILSLV LVSQCYDQNP RGYQDPQEKL RECQQRCERQ QPGQQKQLCKQRCEQQYRKE QQQQHGGETG EDDLGNRGPD KSYKRLQECQ RRCQSEQQGQ RLOECOORCOOEYOREKGOH OGETNPOWEO OEKSNNPYLF ESQRFRSRFR ASHGDFRILE RFNQRSQLLKGIEKYRVAIL ELEPQSFVLP HHCDGEAIYV VVKGQGVINI AEQDNKNSFN LQKGDVIRLFAGSNVYLLNK DNNEKLFVYV LAKSVNAPGN LOEYFSAGGO NPESFYRAFS SDILESAFNNPRDKLERLFG OHKEGIIIKA SEEOIRAISE HASRSTOOTR GRTOGPFNLM KERPVFESRFGQFFEARPER YEQLRDLDAA VGFMNINQGG MVLPYYNTKS TKLVMVIEGN ARFEMACPHLGROSOSPWSR GQGREQEREQ EQEQEEGDVH YQKIRGNLNV GDVLVIPAGH PITFVATGNSNLRIVGFGVD AENNKKNFLA GKONIWRNID REAKELSFSM PGREVEEIFO RODOSYFVPEHROORERG EEGRRGODOY LSSILDFVF



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RaptorX: Protein Structure and Function Prediction Powered by Deep Learning

RaptorX is developed by Xu group, excelling at secondary, tertiary and contact prediction for protein sequences without close homologs in the Protein Data Bank (PDB). RaptorX predicts protein secondary and tertiary structures, contact and distance map, solvent accessibility, disordered regions, functional annotation and binding sites. RaptorX also assigns confidence scores to predicted structures. See details below and <u>HERE</u>.



Vicilin experimental structures:

Gray: Crystal structure of the vicilin from S. melongena 5vf5 Pink: Structure of vicilin from Capsicum annuum, 5yjs

Tomato seed vicilin structural model

• Region Pro147-Phe567 modeled



Model shown as cartoon colored from N terminus to C terminus (blue to red)



Trypsin, sequence based vicilin domain

Results of enzyme action

NPYLF ESQR - FR - SR - FR - ASHGDFR - ILE R - FNQR - SQLLK - GIEK - YR - VAIL ELEPQSFVLP HHCDGEAIYV VVK - GQGVINI AEQDNK - NSFN LQK - GDVIR - LFAGSNVYLLNK -DNNEK - LFVYV LAK - SVNAPGN LQEYFSAGGQ NPESFYR - AFS SDILESAFNNPR - DK - LER - LFG QHK - EGIIIK - A SEEQIR - AISE HASR - STQQTR - GR - TQGPFNLM K - ER - PVFESR - FGQFFEAR - PER - YEQLR - DLDAA VGFMNINQGG MVLPYYNTK - S TK - LVMVIEGN AR - FEMACPHLGR - QSQSPWSR - GQGR - EQER - EQ EQEQEEGDVH YQK - IR - GNLNV GDVLVIPAGH PITFVATGNSNLR - IVGFGVD AENNK - K - NFLA GK - QNIWR - NID R - EAK - ELSFSM PGR - EVEEIFQ R - QDQSYFVPEHR - QQR - ER - G EEGR - R - GQDQY LSSILDFVF

Location of released peptides

[1-10], [11-12], [13-14], [15-16], [17-24], [25-29], [30-33], [34-38], [39-42], [43-44], [45-74], [75-88], [89-96], [97-101], [102-113], [114-119], [120-128], [129-154], [155-170], [171-172], [173-175], [176-182], [183-188], [189-196], [197-205], [206-211], [212-214], [215-224], [225-226], [227-232], [233-240], [241-243], [244-249], [250-275], [276-279], [280-290], [291-300], [301-308], [309-313], [314-317], [318-334], [335-336], [337-366], [367-379], [380-380], [381-387], [388-392], [393-397], [398-400], [401-410], [411-419], [420-430], [431-433], [434-435], [436-441], [442-442], [443-457]

BIOPEP: Search for active fragments

No	Peptide ID	Sequence	Location	Name	Function	Activity	Monoisotopic mass	Chemica mass
1	3258	IR	[335- 336]	beta-lactokinin	Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)	ACE inhibitor	287.1850	287.3480
2	7592	FR	[11-12]	ACE inhibitor		ACE inhibitor	321.1690	321.3650
3	7592	FR	[15-16]	ACE inhibitor		ACE inhibitor	321.1690	321.3650
4	8130	EAK	[398- 400]	peptide derived from dried bonito		antioxidative	346.1730	346.3690
5	8215	IR	[335- 336]	Antioxidative peptide	Oxygen radical scavenging	antioxidative	287.1850	287.3480
6	8246	IR	[335- 336]	renin inhibitor	Inhibitor of Renin (EC 3.4.23.15) (MEROPS ID A01.007)	renin inhibitor	287.1850	287.3480
7	8247	IR	[335- 336]	CaMPDE inhibitor	Inhibitor of calmodulin-dependent phosphodiesterase 1 (abbrev. CaMPDE) (EC 3.1.4.17).	CaMPDE inhibitor	287.1850	287.3480
В	8780	FR	[11-12]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)	dipeptidyl peptidase IV inhibitor	321.1690	321.3650
9	8780	FR	[15-16]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)	dipeptidyl peptidase IV inhibitor	321.1690	321.3650
10	8806	IR	[335- 336]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)	dipeptidyl peptidase IV inhibitor	287.1850	287.3480
11	8944	YR	[43-44]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)	dipeptidyl peptidase IV inhibitor	337.1640	337.3580
12	9484	YR	[43-44]	DPP-III inhibitor	Inhibitor of Dipeptidyl peptidase-III (DPP-III) (EC 3.4.14.4) (MEROPS ID: M49.001)	dipeptidyl peptidase III inhibitor	337.1640	337.3580
13	9501	FR	[11-12]	DPP-III inhibitor	Inhibitor of Dipeptidyl peptidase-III (DPP-III) (EC 3.4.14.4) (MEROPS ID: M49.001)	dipeptidyl peptidase III inhibitor	321.1690	321.3650
14	9501	FR	[15-16]	DPP-III inhibitor	Inhibitor of Dipeptidyl peptidase-III (DPP-III) (EC 3.4.14.4)	dipeptidyl peptidase	321.1690	321.3650

Proteolysis prediction based on structure



Chymotrypsin structure



Trypsin only cleaves the peptide bonds after (on the C-terminal side of) the basic amino acids **lysine** and **arginine** Chymotrypsin prefers cleaving after large **hydrophobic** amino acids such as **phenylalanine**, **tyrosine** and **tryptophan**, and also **leucine** and **methionine**

Trypsin structure



Kaplan Türköz, Burcu

pdb:2ptc





Predicted Solvent Accessibility

		20	40	60	80	100	120	140
Sogue								
beque	ALCE PAIFINFI		MERGIQDEQERENCEQQRCER	QÕL QÕÕVÕTCVÕKCTÕÕ1	KKEQQQQIIGGE I GEDDLGMF	GFDK51KKLQLCQKKCQ5	PÖÖGÖVPÖPCÖÖVCÖÖPIÖVI	INGOIDGEINE OMEOU
Predi	ction 61223323	800000001100000001123	4443464146435544533554	446654445345634644	45464645646644554445	5444663643662464265	564553454444424544554	445444464534443
	Values r	ange from 0 (buried	residue) to 9 (highly	exposed residue)				
								1
)	160	180	200	220	240	260	280	300
QEKSNNPY	LFESQRFRSRFRASH	GDFRILERFNQRSQLLKGIEKYF	VAILELEPQSFVLPHHCDGEAIY	VVVKGQGVINIAEQDNKNS	FNLQKGDVIRLFAGSNVYLLNK	DNNEKLFVYVLAKSVNAPGN	LQEYFSAGGQNPESFYRAFSSDI	LESAFNNPRDKLERLI
134433200	102374243313263	01010023134323104102200	00101034300000110102000	0001130000002473433	2303311001010100000003	5732201000000133374	13401111344231001102450	0341150446104401
	320	340	360	380	400	420	440 40	50
ALFGQHKEG.	IIIKASEEQIRAISE	HASRSTQQTRGRTQGPFNLMKE	RPVFESRFGQFFEARPERYEQLRI	DLDAAVGFMNINQGGMVLP	(YNTKSTKLVMVIEGNARFEMA	CPHLGRQSQSPWSRGQGREQ	EREQEQEQEEGDVHYQKIRGNLI	NVGDVLVIPAGHPITFV
101553653	100302453344145	3254334444334422130343	424142620202101374041033	30100001020343101001	L010300000000313020000	0222423433444444444	4444444444444143041303	34100000011000002
480		500	520	540		560		
		500	520	540		500		
FVATGNS	NLRIVGFGVDA	ENNKKNFLAGKQNIWRN	IDREAKELSFSMPGREVE	EEIFQRQDQSYFVAG	PEHRQQRERGEEGRRGQ	DOVISSILDFVF		
0201563	2000001020	54332110002300043	034501320171437204	30163343220020	16444544446645543	3632023003223		

Chymotrypsin, sequence based vicilin domain

Results of enzyme action

N - PY - L - F - ESQRF - RSRF - R ASH - GDF - RIL - E RF - N - QRSQL - L - KGIEKY - RVAIL - EL - EPQSF - VL - P H - H - CDGEAIY - V VVKGQGVIN - I AEQDN - KN - SF - N - L - QKGDVIRL - F - AGSN - VY - L - L - N - K DN - N - EKL - F - VY - V L - AKSVN - APGN - L - QEY - F - SAGGQ N - PESF - Y - RAF - S SDIL - ESAF - N - N - PRDKL - ERL - F - G QH - KEGIIIKA SEEQIRAISE H - ASRSTQQTR GRTQGPF - N - L - M - KERPVF - ESRF - GQF - F - EARPER Y - EQL - RDL - DAA VGF - M - N - IN - QGG M - VL - PY - Y - N - TKS TKL - VM - VIEGN - ARF -EM - ACPH - L - GRQSQSPW - SR GQGREQEREQ EQEQEEGDVH - Y - QKIRGN - L - N - V GDVL - VIPAGH - PITF - VATGN - SN - L - RIVGF - GVD AEN - N - KKN - F - L - A GKQN - IW - RN - ID REAKEL - SF - SM - PGREVEEIF - Q RODOSY - E - VPEH - ROOPERG FEGRRGODOY - L - SSIL - DF - VF

Location of released peptides

[1-1],[2-3],[4-4],[5-5],[6-11],[12-15],[16-20] 94],[95-102],[103-103],[104-107],[108-109 148],[149-152],[153-153],[154-156],[157-1 236],[237-237],[238-245],[246-248],[249-2 297],[298-298],[299-306],[307-330],[331-3 383],[384-389],[390-391],[392-393],[394-4

No	Peptide ID	Sequence	Location	Name
1	3384	VF	[456- 457]	ACE inhibitor
2	3492	VY	[108-	ACE inhibitor from sake
3	3492	VY	[122- 123]	ACE inhibitor from sake
4	7544	IW	[390- 391]	ACE inhibitor
5	7685	SF	[90-91]	ACE inhibitor from garlic
6	7685	SF	[403- 404]	ACE inhibitor from garlic
7	8224	VY	[108- 109]	antioxidative peptide
8	8224	VY	[122- 123]	antioxidative peptide
9	8320	VL	[57-58]	Glucose uptake stimulating peptide
10	8320	VL	[268-269]	Glucose uptake stimulating peptide
11	8804	IN	[261-262]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)
12	8807	IW	[390- 391]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)
13	8866	PY	[2-3]	dipeptidvl peptidase IV inhibitor
	Usefi	ıl links		st of publications concerning B

 i8],[59-61],[62-62],[63-69],[70-80],[

 ,[124-126],[127-131],[132-135],[13

)2],[203-219],[220-220],[221-221],

 72],[273-273],[274-280],[281-282],]

 34],[365-365],[366-370],[371-377],]

 49],[450-453],[454-455],[456-457]

6666 PY [2-3] diperitidy peritidase IV (DPP IV inhibitor) 8666 PY [270 diperitidy peritidase IV (DPP IV inhibitor) 8888 RN [332 diperitidy peritidase IV (DPP IV inhibitor) 6 8891 SF [90-9] diperitidy peritidase IV in (DPP IV inhibitor) 7 881 SF [403] diperitidy peritidase IV in (DPP IV inhibitor) 8 8917 VF [456 diperitidy peritidase IV in (DPP IV inhibitor) 9 8922 VL [75-8] diperitidy peritidase IV in (DPP IV inhibitor) 9 8922 VL [2586 diperitidy peritidase IV in (DPP IV inhibitor) 1 8923 VM [281 diperitidy peritidase IV in (DPP IV inhibitor) 2 822 VI [168 diperitidy peritidase IV in (DPP IV inhibitor) 3 8229 VY [128 diperitidy peritidase IV in (DPP IV inhibitor) 4 9074 DF [4544 ACE Inhibitor 5 9422 SF [4033 Renin inhibitor	1								
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7 9507 SM [405- 406] DPP-III inhibitor Useful links List of publications concerning BLOPEP-IWM database About BLOPEP-IWM Critics BLOPEP-IWM Contact	6	9432	SF	[403- 404]	Renin inhibitor				
Useful links List of publications concerning BIOPEP-INVM database About BIOPEP-IVVM Citing BIOPEP-IVVM Contact	7	9507	SM	[405- 406]	DPP-III inhibitor	(MEROPS ID: M49.001)		III inhibitor	
		Usef	ful links		ist of publications concerning l	BIOPEP-UWM database	About BIOPEP-UWM	Citing BIOPEP-UWM	Contact

Conclusions

- BAPs are very useful considering their therapeutic potential.
- Sequence based prediction does not take into account the buried residues.
- Structure based approaches will shed light on time consuming experimental studies in order to produce targeted peptides.



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Active Projects and Collaborators

- Identification and characterization of probiotic TIR domain proteins
- Characterization and Determination of Functional Properties of Bioactive peptides from plant food by-products ; Prof. Dr. Semih Ötleş
- Structural characterization of levansucrase and its use in functional food production; Prof. Dr. Taner Baysal, Prof. Dr. Yekta Göksungur
- Purification and characterization of collagen and related molecules from food industry by-products; Prof. Dr. Şebnem Tavman

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