#720 Mapping Epitopic Regions of Cysteine Protease Allergen from *Phaseolus vulgaris.*

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Rationale: *Phaseolus vulgaris* triggers allergic reactions in Indian population. In the present study, B & T-cell epitopes of cysteine protease allergen were identified and validated using *in-silico* methods. **Methods:** Homology-model was generated by modeller9.19v software and epitopes were predicted by a combination of *in-silico* tools. Linear B-cell epitopes were predicted by ABCpred, BCpred, conformational epitopes by DISCOTOPE2.0 & Ellipro and T-cell epitopes by MHCpred, Propred & IEDB. Final prediction involves consensus sequence from both linear & conformational epitopes. Further, epitopes were assessed for secondary-structure, surface-accessibility, epitope-conservancy and Property-distance (PD) values using SDAP allergen database and population coverage analysis on IEDB server. Epitopes were validated using *in-silico* tools. **Results:** *Ricinus communis* protease taken as template has 63% homology with query sequence. 500 3D-model was generated and the best model with 96% overall-quality and 92% residues in most favourable region of Ramachandran plot was selected for prediction. Four B-cell B1(45-59 amino acid), B2(130-156), B3(173-183) and B4(210-225) and four T-cell T1(11-25), T2(190-199), T3(251-265) and T4(342-356) epitopes were predicted and were in coil-helical region except T3. PepCalc server showed 4 B-cell, T2 & T3 epitopes were in hydrophilic region while T1,T4 in hydrophobic region. Epitopes showed 52-95% conservation in B-cell and 4-69% in T-cell epitopes. Further, PD values(<10) showed similarity with allergens from *Ananas comosus*, *Actinidia delicosa*, *Carica papaya* and *Dermatophagoides farinae*. MHC-class II analysis showed about 80%-coverage in Indian population. **Conclusion:** B & T-cell epitopes were predicted by bioinformatic tools and validated by *in-silco* methods. They have cross-reactivity with known food allergens and have potential for diagnostics.

Background of Study

- Red kidney bean- major allergic sensitizer of Indian population (Arora N. et al., 2011).
- Cysteine proteases are well known potent allergens in both inhalant and food allergen sources. Reported as major allergens: HDMs (Der p 1, Der f 1), Ragweed pollen (Amb a 11), Kiwifruit (Act d 1, Act c 1), Insects (Pso o 1, Eur m 1).
- Kidney bean cysteine protease has shown to be a major allergen previously.
- No treatment for food allergy is available other than avoidance, epitopes may act as potential targets for immunotherapy.

Objective

To identify and perform allergenicity assessment of immunodominant epitopes of cysteine protease allergen and validation using *In-silico* approach.

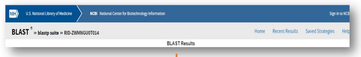
Methods

Homology modelling of protein
- **Modeller software 9.19v**
- **Best model was selected as per the criteria**

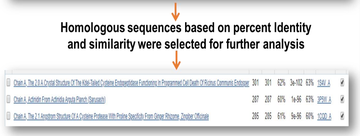
B and T-cell epitope prediction
- **Linear epitopes were predicted using protein sequence**
- **Conformational epitopes using 3D model**

***In silico* validation of predicted epitopes**
- ***In silico* allergenicity validation**
- **Cross-reactivity analysis**
- **Epitope conservancy analysis**

Query sequence was searched on NCBI BLASTp search engine against PDB database with BLAST algorithm



Homologous sequences based on percent Identity and similarity were selected for further analysis

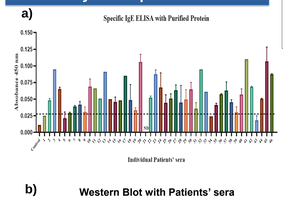


Prediction tools for B-cell epitopes*		Prediction tools for T-cell epitopes*
Linear B-cell epitopes	Conformational B-cell epitopes	
ABCpred	DiscoTope-2.0 server	IEDB server
BCpred	Ellipro server	MHCpred
Bcepred	CBTOPE web server	Propred
Bepipred-2.0 server		SYPPEITHI server
IEDB analysis tool		
SVMTrip		
DNASTAR Lasergene version 7.2		

(Tools having AUC > 0.7 are considered)*

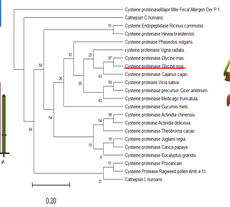
Results

Allergen Characterization of Cysteine protease

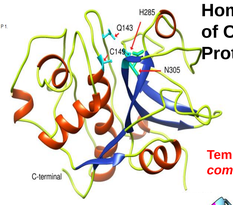


➢ Cysteine Protease of *Phaseolus vulgaris* has shown 89% sera reactivity

Phylogenetic relationship with other homologs

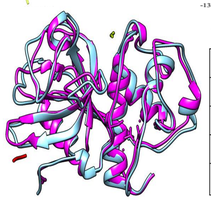


3D Model of Cysteine Protease



Homology Model of Cysteine Protease

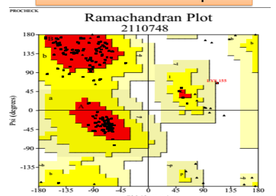
Template- *Ricinus communis* protease



Superimposition between template and modelled structure. (RMSD 1.3 Å)

Quality Assessment of Model on SAVES Server

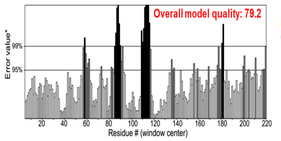
Ramachandran plot



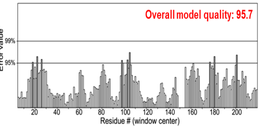
Residues in most favoured regions [A,B,L]	171	91.9%
Residues in additional allowed regions [a,b,l,p]	14	7.5%
Residues in generously allowed regions	0	0.0%
Residues in disallowed regions	1	0.5%
Number of non-glycine and non-proline residues	186	100.0%

ERRAT plot

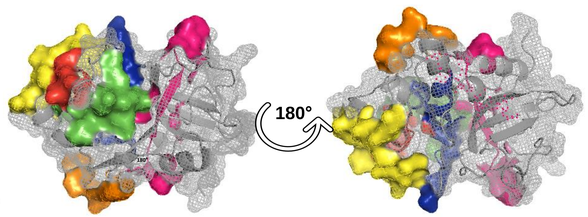
Before loop refinement:



After loop refinement:



Representation of B & T-cell Epitopes on Model Structure



MPSMLIPTLLLLSFTFSHATAMSIINYSENEVMDMYEEWLVKHRKVYNGLDEKEKRFQVFKDNLGFIQDHNAQNNTYTLGLNKFA

B-cell Epitope 1
DITNKEYRAMYLGTRTDAKRRVMKTQNTGHRYAYNSGDQLPVHVDWRLKGAVGPIKDQGNCGSCWAFSTVAAVEGINNIVTGE

B-cell Epitope 2 | T-cell Epitope 1 | B-cell Epitope 3
FVSLSEQELVDCDREYDEGCNGGLMDYAFQFIIQNGGIDTEEDYPYQGIDGTCDETKKKTKVVQIDGYEDVPSNNENALKKAVSHQ

T-cell Epitope 2
PVSVAIEASGRALQLYQSGVFTGKCGTALDHGVVVVGYGTENGVDYWLVRNSWGTGWGEDGYFKMERNVRSTSEGKCGIAMDC

T-cell Epitope 3
SYPVKYGLNSAVPSSVYESTEASISSA

In-silico Allergenicity of Predicted Epitopes

Predicted Epitopes	Allergen online	SDAP PD Index (<10)	Algpred	VexiJen Score (> 0.5)
B-cell Epitopes	All epitopes showed allergenicity			

Predicted Epitopes	Allergen online	SDAP PD Index (<10)	Algpred	VexiJen Score (> 0.5)
T-cell Epitopes				
LLSFTFSHATAMSII	-	-	-	-
GGLMDYAFQF	✓	-	✓	-
VSHQPVSVAIEASGR	✓	-	-	✓
KYGLNSAVPSSVYES	-	-	-	-

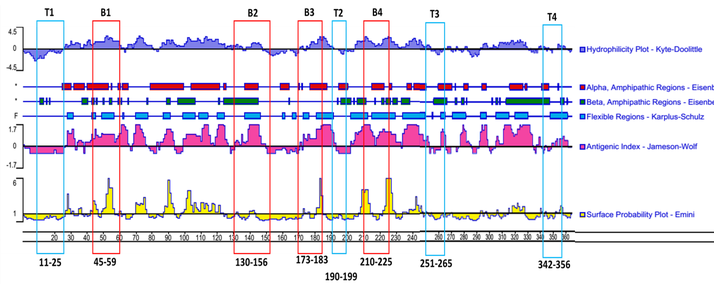
B-cell Epitopes Conservancy & Properties

Predicted B-cell epitopes	IEDB conservancy analysis (≥50%)	Water Solubility (PepCalc server)	Secondary structure prediction (PEP-FOLD server)
KVYNGLDEKEKRFQV	69.57% (16/23)	Hydrophilic	Helical
DWRLKGAVGPIKDQGNC	73.91% (17/23)	Hydrophilic	Coil
SEQELVDCDRE	95.63% (22/23)	Hydrophilic	Helical-coil
EDYPYQGIDGTCDETK	52.17% (12/23)	Hydrophilic	Coil

T-cell Epitopes Conservancy & Properties

Predicted T-cell epitopes	Promiscuous epitope in 27 HLA alleles	IEDB conservancy analysis (≥50%)	Water Solubility (PepCalc server)	Secondary structure prediction (PEP-FOLD server)
LLSFTFSHATAMSII	92%	26.09% (6/23)	Hydrophobic	Helical
GGLMDYAFQF	85%	69.57% (16/23)	Hydrophilic	Helical-Coil-helical
VSHQPVSVAIEASGR	66%	69.57% (16/23)	Hydrophilic	Helical-strand-helical
KYGLNSAVPSSVYES	70%	4.35% (1/23)	Hydrophobic	Coil

Physicochemical Properties of B & T-cell Epitopes on DNAstar

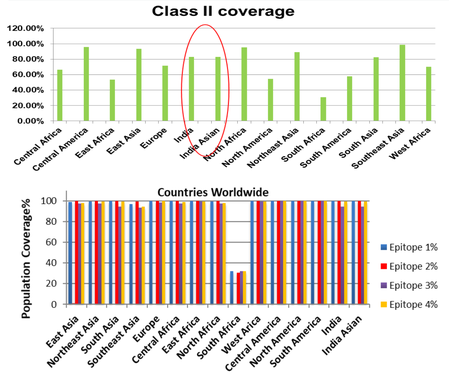


Property Distance (PD) on SDAP database for Cross-reactivity Prediction

B-cell Epitope	Allergen	PD seq similarity Index	Matching region
KVYNGLDEKEKRFQV	*Ananas comosus* (Ana c 2)	6.69	RVYKDDDEKMRRFQI
	Actinidia deliciosa (Act d 1)	6.72	KSYNSLGEWERRFEI
	Carica papaya (Car p papain)	7.27	KIYKNIDEKIYRFEI
	Glycephagus domesticus (Gal d 3)	7.60	KIRDLLERQEKRFGV
	Glycine max (Gly m 1)	7.78	RVYHNHEEEAKRLEI
DWRLKGAVGPIKDQGNC	*Carica papaya* (Car p papain)	3.26	DWRQKGAVTPVKNQGSC
	Blomia tropicalis (Blo t 1)	5.66	DWRQKTHVNPIRNQGGC
	Actinidia deliciosa (Act d 1)	6.69	DWRSAGAVVDIKSQGEC
	Glycine max (Gly m 1)	7.35	DWRKKGVITQVKYQGGC
	Ananas comosus (Ana c 2)	7.60	DWRDYGAVNEVKNQNPC
	Blomia tropicalis (Blo t 1)	7.84	DWRQKARLTRIRQQGSC

B-cell Epitope	Allergen	PD seq similarity Index	Matching region
SEQELVDCDRE	*Carica papaya* (Car p papain	2.28	SEQELLDCDRR
	Actinidia deliciosa (Act d 1)	3.83	SEQELIDCGRT
	Glycine max (Gly m 1)	4.04	SEQELVDCVEE
	Dermatophagoides farina (Der f 1)	4.20	SEQELVDCASQ
	Blomia tropicalis (Blot t 1)	4.37	SEQELVDCTYN
	Dermatophagoides pteronyssinus (Der p 1)	5.58	AEQELVDCASQ
EDYPYQGIDGTCDETK	*Ananas comosus* (Ana c 2)	7.52	ENYPYLAVQGTCNANS
	Aspergillus flavus (Asp f 5)	8.18	ESYVFKGVSGTVSDPK

Population Coverage Analysis on IEDB



Conclusion

- Four B & T-cell Epitopes identified and validated by *in-silico* approach.
- B-cell epitopes showed allergenic properties and potential cross-reactivity with known allergens.
- T-cell epitopes showed significant MHC class II population coverage in Indian and world population.

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