Journal of Computational Methods in Molecular Design, 2014, 4 (3):84-91

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ISSN : 2231- 3176
CODEN (USA): JCMMDA

Structural and immunogenicity prediction of asp o21 allergen from *Aspergillus Oryzae* – An immunoinformatics approach

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ABSTRACT

Allergy is the immune disorder which occurs due to allergens present in environment. Once when the allergen enters the body, there arise over activation of basophils and mast cells by IgE antibody which results in high inflammatory response leading to varied allergic reactions like asthma, hay fever, eczema etc. Asp O 21 allergen present in common plant pathogen Aspergillus oryzae, which is considered as one of the causative agent of human atopic disorder, can serve as an important target for vaccine development. Hence this study was designed to predict potential B cell and T cell epitopes along with secondary structure, post translational modifications and protein secretion using immune-informatics tools.

Keywords: Allergy, Asp o 21 allergen, B cell & T cell epitope, Secondary structure, Post translational modifications and protein secretion.

INTRODUCTION

Aspergillus oryzae, which belongs to Ascomycota family, is involved in the production of varied soybean products. In spite of its beneficial effects, the same microbe causes allergic bronchopulmonary aspergillosis [1] due to presence of allergen named Asp O21 that codes for amylase. The allergen Asp O21 can serve as an important target for vaccine development.

Use of crude medicinal extracts has got several side effects. Hence using immunoinformatics tools, a systematic attempt has been made to predict potential conserved antigenic determinants due to generation of immune response with more effective and less expensive epitopes obtained. This approach is based on the phenomenon of cross-protection, whereby a host infected with a mild strain of parasite is protected against a more severe strain of the same parasite [2]. This study was also designed to analyze antigenicity, hydrophobicity, surface accessibility and epitopic location of epitopes in *Aspergillus oryzae*. However, there is no report about the epitope of Asp O21 allergen.

MATERIALS AND METHODS

Amino acid sequence

The aminoacid sequence of Isoallergen Asp O21 was retrieved from UniProt database.

Allergenicity prediction

AlgPred [3], a server for predicting allergenicity based on SVM method based amino acid composition of Asp O21 has been used. The server is available from the URL: www.algpred.com.

B cell epitope prediction
Antigenic epitopes of allergen from *Aspergillus oryzae* was also determined using the Gomase in 2007, Hopp and Woods, Welling, Parker and Protrusion Index (Thornton) antigenicity [4-6].

For B cell, epitopes were predicted using ABCpred online tool http://www.imtech.res.in/raghava/abcpred [7]. The predicted B cell epitopes were ranked according to their score obtained by trained recurrent neural network. All predicted epitopes were obtained in the form of small peptide sequences.

T cell epitope prediction
For the prediction of MHC class-I epitopes, ProPred1 online prediction tool http://www.imtech.res.in/raghava/propred1 was used [8]. The sequence was submitted to the ProPred1 server in the FASTA format. The epitopes were predicted for different alleles (HLA-A1, HLA-A2, HLA-A*0201 & HLA-A*0205) of MHC class-I. Only those epitopes having a peptide score above the threshold value (4 %) had been selected.

RankPep http://imed.med.ucm.es/Tools/rankpep.html predicts peptide binders to MHCI and MHCII molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). Support Vector Machine (SVM) based method for prediction of promiscuous MHC class II binding peptides. SVM has been trained on the binary input of single amino acid sequence [9].

Secondary structure prediction
Based on the prediction of turns and loops obtained from analyzing the proteins of known structure, secondary structure including number of helices, sheets and turns were predicted [10].

Prediction of post-translational modifications
Different scales were used to predict glycosylation (Gly), N-myristoylation (Myr), protein kinase C phosphorylation (PKC), and casein kinase II phosphorylation sites (CK2) and disulfide bonds (Dis) [11].

Prediction of protein secretion
Prediction of the secretory or nonsecretory nature of Asp O21 allergen was performed. Protein secretion was categorized as classical or nonclassical. Prediction of classical secretion and detection of the presence of signal sequences were performed using SignalP 3.0 http://www.cbs.dtu.dk/services/SignalP [12]. SecretomeP 2.0 http://www.cbs.dtu.dk/services/SecretomeP was used to predict nonclassical secretion as described previously [13]. Both types of prediction were performed at a default setting score of 0.5. The proteins with predicted scores of 0.5 and above were considered to be secreted.

RESULTS AND DISCUSSION

The aminoacid sequence of Isoallergen Asp O21 have got the sequence length of 499 amino acids which was retrieved from UniProt database and its accession number was P10529.

MMVAWWSLFLYGLQVAAPALAATPADWRSQSIYFLLTDRFARTDGSTTATCNTADQKYCGGTWQGIIDK
LDYIQGMGFTAIWITPVTAQLPQTTAYGDAYHGYWQQDIYSLNENYGTADDLKALSSALHERGMYLMVD
VVANHMGYDGAGSSVDYSVFKPFSSQDYFHPFCLIQNYEDQTQVEDCWLGDNTVSLPDLDTTKDVVKNE
WYDWVGSLVSNYSIDGLRIDTVKHVQKDFWPGYNKAAGVYCIGEVLDGDPAYTCPYQNVMDGVLNYPI
YYPLLNAFKSTSGSMHDLYNMINTVKSDCPDSTLLGTFVENHDNPRFASYTNDIALAKNVAAFIILNDGIPII
YAGQEQHYAGGNDPANREATWASGYPTDSELYKLIASANAIRNYAISKDTGFVTYKNWPIYKDDTTIAMR
KGTDGSQIVTILSNKGASGDSYTLSLSGAGYTAGQQLTEVIGCTTVTVGSDGNVPVPMAGGLPRVLYPTEK
LAGSKICSSS

Fig 1: Sequence of Asp O21 allergen

Prediction by SVM method based on aminoacid composition using AlgPred server detects that Asp O21 is a potential allergen since its score is 1.32 as the threshold value is only 0.4
Using ABCpred online tool, the predicted B cell epitopes are ranked according to their score obtained by trained recurrent neural network (Table 1). Higher score of the peptide means the higher probability to be as epitope. All the peptides shown here are above the threshold value 0.5.

Even, antigenicity plot and hydrophobicity plots were graphically presented as shown in figures 2-7. The region of maximal hydrophilicity is likely to be an antigenic site because terminal regions of antigen protein is solvent

accessible and unstructured, antibodies against those regions are also likely to recognize the native protein (Fig. 2, 3). It was shown that an antigen protein is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility (Fig. 4-7) [9].

Table 1 B cell Epitope Prediction using ABCpred online

Rank	Sequence	Start position	Score
1	TAIWITPVTAQLPQTT	79	0.92
1	TGFVTYKNWPIYKDDT	398	0.92
1	NYPIYYPLLNAFKSTS	272	0.92
2	LSLSGAGYTAGQQLTE	442	0.90
2	EATWASGYPTDSELYK	366	0.90
3	AQLPQTTAYGDAYHGY	88	0.89
3	SQSIYFLLTDRFARTD	29	0.89
3	AYTCPYQNVMDGVLNY	258	0.89
4	DGLRIDTVKHVQKDFW	222	0.88
5	VYCIGEVLDGDPAYTC	246	0.87
6	STSGSMHDLYNMINTV	285	0.86
6	HVQKDFWPGYNKAAGV	231	0.86
6	VSLPDLDTTKDVVKNE	192	0.86
7	GYTAGQQLTEVIGCTT	448	0.85
7	NYAISKDTGFVTYKNW	391	0.85
7	YKLIASANAIRNYAIS	380	0.85
7	FVENHDNPRFASYTND	313	0.85
7	VVKNEWYDWVGSLVSN	203	0.85
7	HGYWQQDIYSLNENYG	101	0.85
8	GSTTATCNTADQKYCG	45	0.84
8	SGDSYTLSLSGAGYTA	436	0.84
8	DGSQIVTILSNKGASG	422	0.84
8	AGQEQHYAGGNDPANR	350	0.84
8	HDLYNMINTVKSDCPD	291	0.84
8	AGSSVDYSVFKPFSSQ	149	0.84
9	AAPALAATPADWRSQS	16	0.82
10	VPVPMAGGLPRVLYPT	472	0.81
10	PGYNKAAGVYCIGEVL	238	0.81
11	TIAMRKGTDGSQIVTI	414	0.80
12	WQGIIDKLDYIQGMGF	63	0.79
13	VGSDGNVPVPMAGGLP	466	0.78
13	GIPIIYAGQEQHYAGG	344	0.78
13	CPDSTLLGTFVENHDN	304	0.78
13	DQTQVEDCWLGDNTVS	178	0.78
13	PFSSQDYFHPFCLIQN	160	0.78
14	FCLIQNYEDQTQVEDC	170	0.77
15	HMGYDGAGSSVDYSVF	143	0.76
15	LHERGMYLMVDVVANH	128	0.76
15	YSLNENYGTADDLKAL	109	0.76
16	CNTADQKYCGGTWQGI	51	0.74
16	YGTADDLKALSSALHE	115	0.74
17	RVLYPTEKLAGSKICS	482	0.73
18	RFASYTNDIALAKNVA	321	0.72
19	VAWWSLFLYGLQVAAP	3	0.71
19	CWLGDNTVSLPDLDTT	185	0.71
20	YAGGNDPANREATWAS	356	0.67
20	AFIILNDGIPIIYAGQ	337	0.67
21	TPADWRSQSIYFLLTD	23	0.61
22	TILSNKGASGDSYTLS	428	0.58

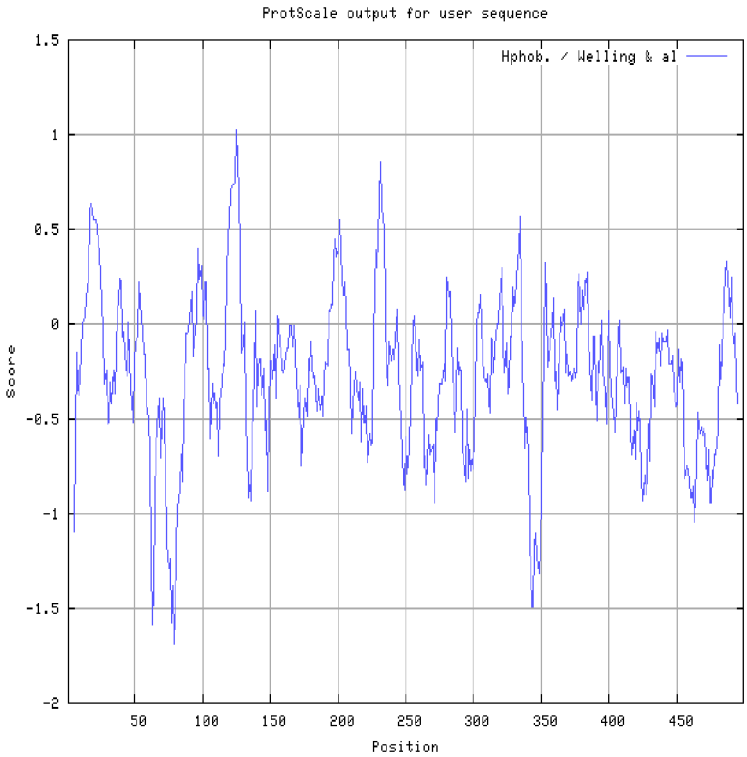

Fig. 2- Antigenicity plot of antigen protein by Welling, et al., scale

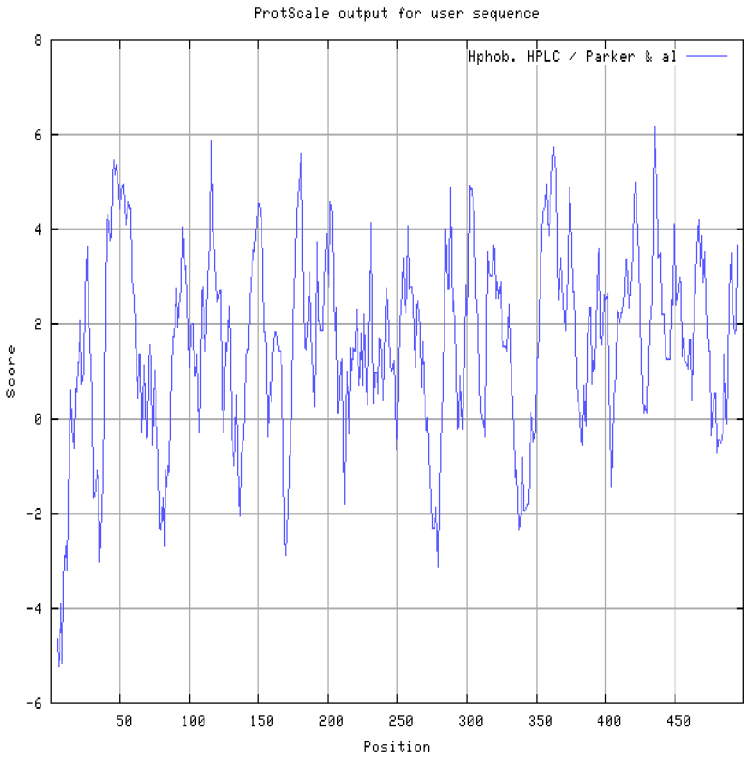

Fig. 3- Antigenicity plot of antigen protein by HPLC / Parker, et al., scale

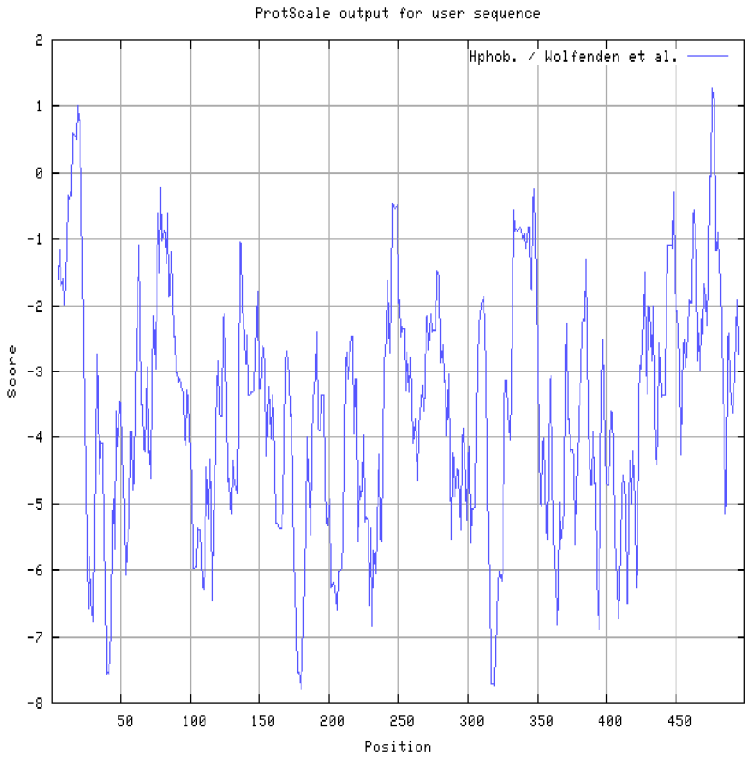

Fig. 4- Hydrophobicity plot of antigen protein by Wolfenden, et al., scale

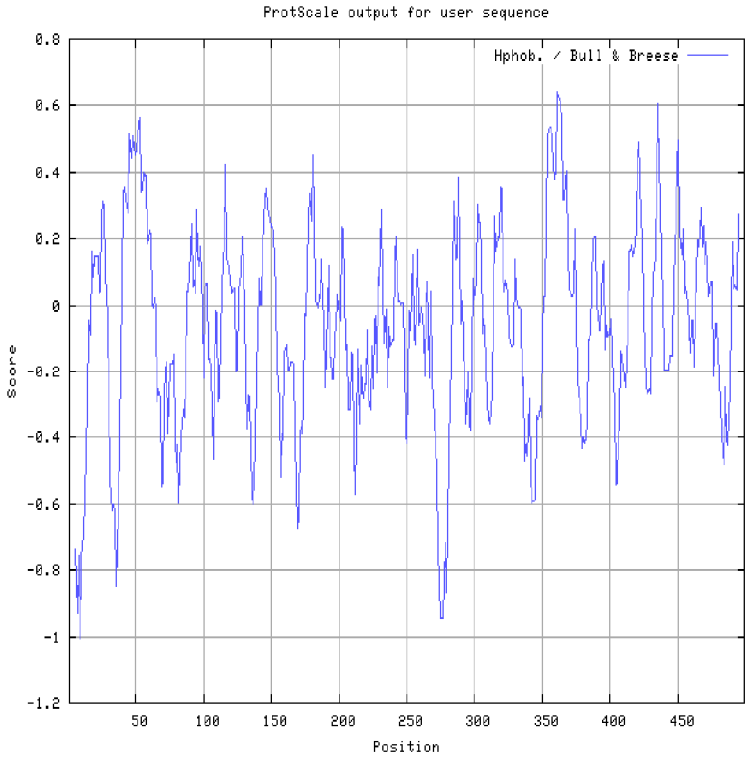

Fig. 5-Hydrophobicity plot of antigen protein by Bull & Breese scale

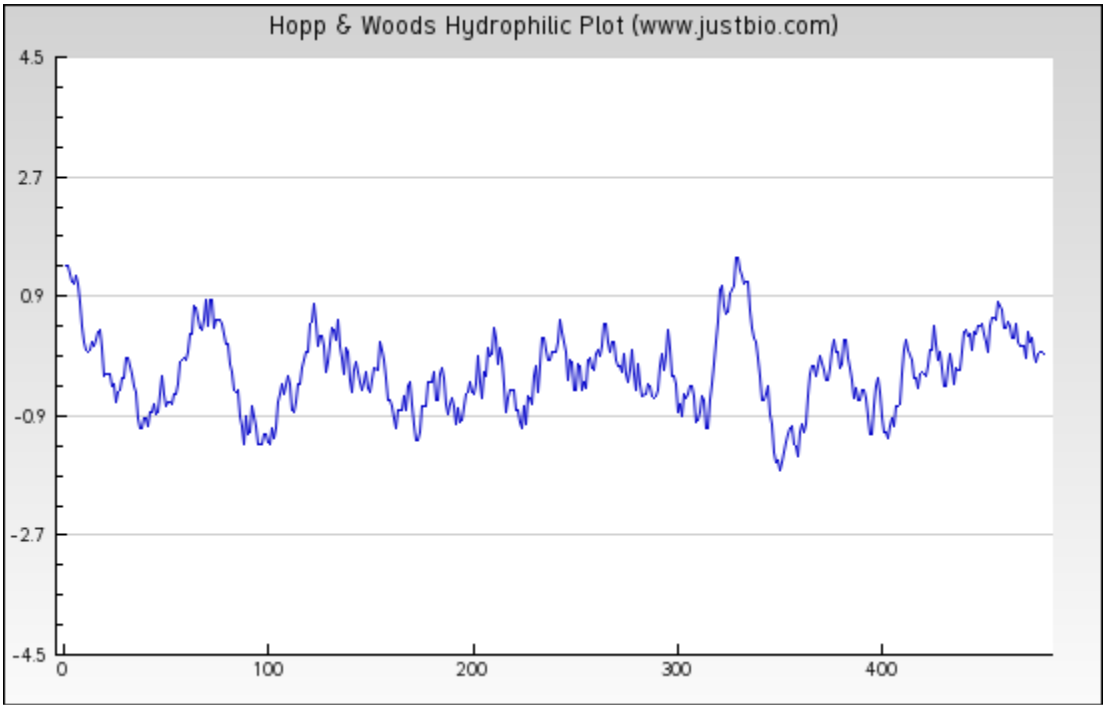

Fig. 6-Hydrophobicity plot of antigen protein by Hopp and Woods

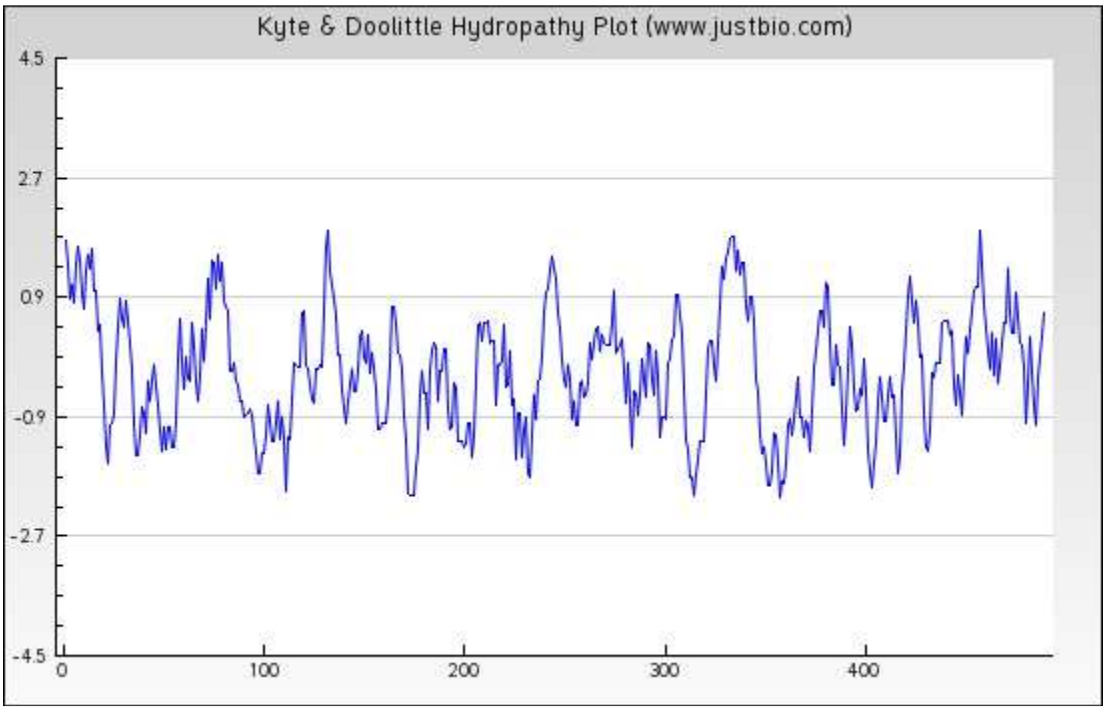

Fig. 7-Hydrophobicity plot of antigen protein by Kyte and Doolittle

T cell epitopes were predicted using ProPred1 and RankPep online tools and were tabulated in Table 2 and Table 3. MHC molecules are cell surface glycoproteins, which take active part in host immune reactions in response to almost all antigens. ProPred1server predict the peptide binders to MHCI molecules of antigen protein sequence are as HLA-A1, HLA-A2, HLA-A*0201 and HLA-A*0205 (Table 2). We also found the SVM based MHCII- IAb peptide regions; MHCII-IAd peptide regions; MHCII-IAg7 peptide regions and MHCII-DQ7 (DQB1*0301) peptide regions, which represented predicted binders from bacterial antigen protein (Table 3).

Table 2: Prediction of MHC Class-I Binding Peptides

ALLELE: HLA-A1				
Analysis at 4 % threshold [Numerical Value = -0.693]				
Rank	Sequence	Peptide Position	Peptide Score	Binder/Non-Binder
1	VAWWSLFLY	2	0.050	Predicted Binder
2	MMVAWWSLF	0	0.050	Predicted Binder
3	MVAWWSLFL	1	0.000	Predicted Binder

ALLELE: HLA-A2				
Analysis at 4 % threshold [Numerical Value = 1.553]				
Rank	Sequence	Peptide Position	Peptide Score	Binder/Non-Binder
1	MVAWWSLFL	1	5.646	Predicted Binder
2	MMVAWWSLF	0	0.113	Predicted Non-Binder
3	VAWWSLFLY	2	0.039	Predicted Non-Binder

ALLELE: HLA-A*0201				
Analysis at 4 % threshold [Numerical Value = 1.143]				
Rank	Sequence	Peptide Position	Peptide Score	Binder/Non-Binder
1	MVAWWSLFL	1	17.477	Predicted Binder
2	VAWWSLFLY	2	0.156	Predicted Non-Binder
3	MMVAWWSLF	0	0.001	Predicted Non-Binder

ALLELE: HLA-A*0205				
Analysis at 4 % threshold [Numerical Value = 0.519]				
Rank	Sequence	Peptide Position	Peptide Score	Binder/Non-Binder
1	MVAWWSLFL	1	23.800	Predicted Binder
2	MMVAWWSLF	0	0.009	Predicted Non-Binder
3	VAWWSLFLY	2	0.000	Predicted Non-Binder

Table 3: SVM based prediction of promiscuous MHC class II binding peptides from Asp O21

Allele	Sequence	Residue No.	Peptide score
I-Ab	FTAIWITPV	78	16.151
I-Ab	MVDVVANHM	136	15.472
I-Ab	STTATCNTA	46	15.229
I-Ab	FARTDGSTT	40	14.589
I-Ad	QEQHYAGGN	352	21.243
I-Ad	KALSSALHE	122	16.607
I-Ad	PALAATPAD	18	13.269
I-Ad	YGLQVAAPA	11	12.088
I-Ag7	AYTCPYQNV	258	19.377
I-Ag7	AYGDAYHGY	95	17.88
I-Ag7	ASANAIRNY	384	16.682
I-Ag7	NDIALAKNV	327	14.101
DQ7(DQB1*0301)	NREATWASG	364	21.996
DQ7(DQB1*0301)	WPGYNKAAG	237	15.753
DQ7(DQB1*0301)	ATPADWRSQ	22	15.419
DQ7(DQB1*0301)	IIYAGQEQH	347	12.799

Apart from epitope studies, prediction of secondary structure, post translational modifications and protein secretion studies were performed and the results are tabulated in Table 4-6.

Since the threshold has been fixed as 0.5, the readings above that value were considered as positive for protein secretion.

Table 4: Secondary structure Prediction using CFSSP server

No. of helices	No. of sheets	No. of turns	Helices % value	Sheets % value	Turns % value
246	253	67	49.3	50.7	13.4

Table 5: Prediction of post translational modifications

Character	Gly sites	PKC sites	CK2 sites	Myr sites	Dis sites
Number	7	19	5	0	9

Table 6: Prediction of protein secretion

Non classical secretion score	Classical secretory protein
0.941084	0.713

CONCLUSION

Thus, the predicted antigen peptide to varied MHC class molecules enhance the immunogenicity thereby playing an important initiative to develop effective vaccines from *Aspergillus oryzae* infections. Further wet laboratory experiments can be carried out to verify the possibility of above predicted epitopes.

Acknowledgments
We are thankful to authorities of Nirmala College for Women, Coimbatore, Tamil Nadu for providing necessary facilities. The first author is sincerely grateful to University Grants Commission, Government of India, for providing the award of Junior Research Fellowship in Science to pursue the research work.

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