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Identification of Antigenic Determinants, Solvent Accessibility and MHC Binders of Peb1a from *Campylobacter jejuni*

Sherkhane AS[1], **Waghmare Somnath**[2] **and Gomase VS**[3*]

[1]*The Global Open University, Nagaland, India*

[2]*Department of Zoology, Nowrosjee Wadia College of Arts and Science, Pune, India*

[3]*Department of Computer Science and IT, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India*

[*]**Corresponding author:** Gomase VS, Department of Computer Science and IT, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India, Tel: 91-9987770696; E-mail: gomase.viren@gmail.com

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Abstract

Campylobacter jejuni is a foodborne, highly mutable in response to antibiotic, pathogen causing gastroenteritis in humans. In this study we summarize the potency of Peb1a from *Campylobacter jejuni* with 259 amino acids. Antigenic peptides of Peb1a from *Campylobacter jejuni* are most suitable for subunit vaccine development because with single epitope, the immune response can be generated in large population. In this research, we used PSSM and SVM algorithms for the prediction of MHC class I & II binding peptide, antigenicity, Solvent accessibility, polar and nonpolar residue to analyses the regions that are likely exposed on the surface of proteins which are potentially antigenic that allows potential drug targets to identify active sites against infection as well as to design effective drug to treat it.

Keywords: *Campylobacter jejuni*; Peb1a; Antigenic peptides; MHC-Binders; TapPred; PSSM; SVM; Nonamers

Introduction

Campylobacter jejuni is a gram-negative, microaerophilic, chemoorganotrophic bacterium, foodborne pathogen, causing gastroenteritis, diarrhea, fever, abdominal cramps and neuromuscular paralysis in humans [1-3]. The organism is transmitted to humans through contaminated water, milk and undercooked poultry meat. To recover the infection of Peb1a from *Campylobacter jejuni* there is need understand the function of Peb1a protein because it is highly mutable in response to antibiotic [4]. Antigenic peptides of Peb1a from from *Campylobacter jejuni* are most suitable for the development of peptide vaccine [4] because a single peptide can generate sufficient immune response. In this research work we have used the phenomenon of cross-protection, whereby an individual undertaken by a mild toxin can have immunity to survive against similar strong toxic effects. MHC molecules are cell surface protein that binds Peb1a from *Campylobacter jejuni* and present them to cell surface for recognition by T-cells. T cell recognition is an important mechanism of the adaptive immune system by which the host identifies and responds to Peb1a from *Campylobacter jejuni* [5,6]. There are two types of MHC molecule and are extremely polymorphic. MHC class I molecules present peptides Peb1a from *Campylobacter jejuni* synthesized within the cell, whereas, MHC class II molecule present peptides derived from endocytosed extracellular proteins. MHC molecules play an important role in immune reactions by taking active part in host immune reactions and involvement of MHC class molecule in response to almost all antigens and it give impacts on specific sites. The involvement of MHC class-I molecule in response to almost all antigens make the study very interesting. They bind to some of the peptide fragments generated after proteolytic cleavage of antigen [7]. Identification of MHC-binding peptides and T-cell epitopes helps improve our understanding of specificity of immune responses [8-11]. Antigenic peptides are most suitable for peptide vaccine development because single epitope can generate large the immune response [12-14].

Methodology

Database searching

The antigenic protein sequence of *Peb1a from Campylobacter jejuni* was retrieved from NCBI databases [15-17].

Prediction of antigenicity

Prediction of antigenicity program predicts those segments of Peb1a from *Campylobacter jejuni* that are likely to be antigenic by eliciting an antibody response. In this research work antigenic epitopes of Peb1a from *Campylobacter jejuni* are determined by using the Hopp and Woods, Welling, Parker, Bepipred , Kolaskar and Tongaonkar antigenicity methods [18-22].

Prediction of MHC binding peptide

The major histocompatibility complex (MHC) peptide binding of Peb1a from *Campylobacter jejuni* is predicted using neural networks trained on C terminals of known epitopes. Rankpep toll predicts peptide binders to MHC-I ligands whose C-terminal end is likely to be the result of proteosomal cleavage using Position Specific Scoring Matrices (PSSMs). Support Vector Machine (SVM) based method for prediction of promiscuous MHC class II binding peptides from protein sequence; SVM has been trained on the binary input of single amino acid sequence [23-27].

Prediction of antigenic peptides by cascade SVM based TAPPred method

We predict cascade SVM based several TAP binders which was based on the sequence and the features of amino acids (Table 3) [28].

Solvent accessible regions

We predict solvent accessible regions of proteins having highest probability that a given protein region lies on the surface of a protein Surface Accessibility, backbone or chain flexibility by Emani et al., [29] and Karplus and Schulz [30]. By using different scale we predict the hydrophobic and hydrophilic characteristics of amino acids that are rich in charged and polar residues i.e. Sweet et al. [31], Kyte and Doolittle [32], Abraham and Leo [33], Bull and Breese [34], Miyazawa, et al. [35], Roseman [36], Wolfenden et al. [37], Wilson et al. [38], Cowan [39], Chothia [40].

Results and Interpretations

Protein sequence of Peb1a from *Campylobacter jejuni* contain long residue of 259 amino acids with 251 nonamers [GI:433552054].

MVFRKSLLKLAVFALGACVAFSNANAAEGKLESIKSKGQLIVG VKNDVPHYALLDQATGEIKGFEVDVAK

LLAKSILGDDKKIKLVAVNAKTRGPLLDNGSVDAVIATFTITPE RKRIYNFSEPYYQDAIGLLVLKEKKYKSLA

DMKGANIGVAQAATTKKAIGEAAKKIGIDVKFSEFPDYPSIKA ALDAKRVDAFSVDKSILLGYVDDKSEILP

DSFEPQSYGIVTKKDDPAFAKYVDDFVKEHKNEIDALAKKWG L

Prediction of antigenic peptides

Antigencity predicted by using Hopp-Woods scale the result found high in position 81, 82, 114-117, and 229-232 in a protein, assuming that the antigenic determinants would be exposed on the surface of the protein and thus would be located in hydrophilic regions (Figure 1). Welling antigenicity plot gives value as the log of the quotient between percentage in a sample of known antigenic regions and percentage in average proteins and Result found high in position 71-72, 138-141 and 189-190 (Figure 2). We also study Hydrophobicity plot of HPLC / Parker Hydrophilicity Result found 98-DNGSVDA-104-(5.371), 228-TKKDDPA-234 (5.829) (maximum), BepiPred predicts the location of linear B-cell epitopes Result found that 24-ANAAEGKLESIK-35, 90-AKTRGPLLDNGSV-102,154-AQAATTKKAIGE-165,209 DDKSEILPDSFEPQSYGIVTKKDDPAFA-236, Kolaskar and Tongaonkar antigenicity methods Predicted peptides result found i.e. 4-RKSLLKLAVFALGACVAFS-22, 37-KGQLIVGVKNDVPHYALLDQ-56, 121-FSEPYYQDAIGLLVLKE-137, 179-FPDYPSIKAALDAKRVDAFSVDKSILLGYVDDK-211 and the predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design (Figures 3-5).

Solvent accessible regions

We also predict solvent accessible regions in proteins; different measurement was performed for the prediction of antigenic activity, surface region of peptides. Emani et al., (Figure 6) predicts the highest probability i.e. found 112-TPERKRI-118,121-FSEPYYQ-127,136-KEKKYKS-142,242-FVKEHKNE-249, that a given protein region lies on the surface of a protein and are used to identify antigenic determinants on the surface of proteins. Karplus and Schulz (Figure 7) High score is found i.e. found 1.075 maximum in 32-ESIKSKGSIKSKGQIKSKGQL-40, 89-NAKTRGPAKTRGPLKTRGPLL-97. Predict backbone or chain flexibility on the basis of the known temperature B factors of the a-carbons. The hydrophobicity and hydrophilic characteristics of amino acids is determined by using different scales that are rich in charged and polar residues i.e. Sweet et al. hydrophobicity Result found high in position 10-13, 108-109,205-206, Kyte and Doolittle result high in position 11-18,104-108,131-134, Abraham and Leo result high in position 10-16, 131-133,205-207, Bull and Breese result high in position 25-27,90-92,157-159, Guy result high in position 114-117,138-141, Miyazawa result high in position 4-6,11-21, 41-4374-75,85-86,106-108, 131-134, Roseman result high in position 14-16,107-108,131-133,204-206, Wolfenden result high in position 132-132, Wilson et 4-5,9-13,40-43,50-54,122-123,131-134,204-207, Cowan 10-16,107-109,131-133, Chothia 13-19,40-43,107-108,131-134,150-154 (Figure 7).

Prediction of MHC binding peptide

We found binding of peptides to a number of different alleles using Position Specific Scoring Matrix. Peb1a *Campylobacter jejuni* sequence is 259 residues long, having 65 nonamers. MHC molecules are cell surface proteins, which actively participate in host immune reactions and involvement of MHC-I and MHC-II in response to almost all antigens. We predict MHC-I peptide binders of Peb1a *Campylobacter jejuni* was tested with on a set of 4 different alleles i.e. H2-Db (mouse) 8mer, H2-Db (mouse) 9mer, H2-Db (mouse) 10mer and H2-Db (mouse) 11mer (Tables 1a, 1b, 1c and 1d) and MHC-II peptide binders for I_Ab.p, I_Ad.p alleles highlighted in red represent predicted binders (Tables 2a, 2b and 2c). Here PSSM-specific binding threshold and is obtained by scoring all the antigenic peptide sequences included in the alignment from which a profile is derived, and is defined as the score value that includes 85% of the peptides within the set. Peptides whose score is above the binding threshold will appear highlighted in red and peptides produced by the cleavage prediction model are highlighted in violet (Figure 8-19). We also use a cascade SVM based TAPPred method which found 80 High affinity TAP Transporter peptide regions which represents predicted TAP binders residues which occur at N and C termini of Peb1a from *Campylobacter jejuni* (Table 3).

MHC-I Allele	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
8mer_H2_Db	104	SVD	AVIATFTI	TPE	816.99	18.033	34.35%
8mer_H2_Db	44	IVG	VKNDVPHY	ALL	953.06	12.117	23.08%

8mer_H2_Db	34	LES	IKSKGQLI	VGV	868.08	6.856	13.06%
8mer_H2_Db	217	ILP	DSFEPQSY	GIV	953.98	6.586	12.55%
8mer_H2_Db	119	KRI	YNFSEPYY	QDA	1064.14	5.626	10.72%
8mer_H2_Db	90	AVN	AKTRGPLL	DNG	837.03	4.556	8.68%
8mer_H2_Db	112	FTI	TPERKRIY	NFS	1044.23	3.653	6.96%
8mer_H2_Db	144	KSL	ADMKGANI	GVA	800.92	2.872	5.47%
8mer_H2_Db	86	IKL	VAVNAKTR	GPL	839.98	1.977	3.77%
8mer_H2_Db	54	YAL	LDQATGEI	KGF	827.89	1.511	2.88%
8mer_H2_Db	76	AKS	ILGDDKKI	KLV	883.05	1.366	2.60%
8mer_H2_Db	175	IDV	KFSEFPDY	PSI	1014.12	1.32	2.51%
8mer_H2_Db	222	FEP	QSYGIVTK	KDD	877.0	1.231	2.35%
8mer_H2_Db	178	KFS	EFPDYPSI	KAA	949.05	1.17	2.23%
8mer_H2_Db	167	GEA	AKKIGIDV	KFS	825.01	1.028	1.96%
8mer_H2_Db	165	AIG	EAAKKIGI	DVK	810.99	0.645	1.23%

Table 1a: Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites of Peb1a from *Campylobacter jejuni*. The binding potential (score) of antigenic peptide to the MHC-1 Allele i.e. 8mer_H2_Db.

MHC-I Allele	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
9mer_H2_Db	13	LAV	FALGACVAF	SNA	880.08	9.782	19.42%
9mer_H2_Db	122	YNF	SEPYYQDAI	GLL	1067.14	7.856	15.60%
9mer_H2_Db	46	GVK	NDVPHYALL	DQA	1023.16	7.504	14.90%
9mer_H2_Db	197	VDA	FSVDKSILL	GYV	1003.21	3.668	7.28%
9mer_H2_Db	235	DPA	FAKYVDDFV	KEH	1085.23	3.403	6.76%
9mer_H2_Db	85	KIK	LVAVNAKTR	GPL	953.14	3.346	6.64%
9mer_H2_Db	177	VKF	SEFPDYPSI	KAA	1036.13	3.159	6.27%
9mer_H2_Db	103	GSV	DAVIATFTI	TPE	932.08	3.14	6.23%
9mer_H2_Db	75	LAK	SILGDDKKI	KLV	970.13	2.323	4.61%
9mer_H2_Db	162	TKK	AIGEAAKKI	GID	882.07	1.216	2.41%
9mer_H2_Db	23	AFS	NANAAEGKL	ESI	868.94	0.952	1.89%
9mer_H2_Db	171	KKI	GIDVKFSEF	PDY	1023.16	0.901	1.79%

Table 1b: Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites of Peb1a from *Campylobacter jejuni*. The binding potential (score) of antigenic peptide to the MHC-1 Allele i.e. 9mer_H2_Db.

MHC-I Allele	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
10mer_H2_Db	244	DFV	KEHKNEIDAL	AKK	1178.31	11.229	19.08%
10mer_H2_Db	116	PER	KRIYNFSEPY	YQD	1298.48	10.554	17.93%
10mer_H2_Db	161	TTK	KAIGEAAKKI	GID	1010.24	8.65	14.70%

10mer_H2_Db	42	QLI	VGVKNDVPHY	ALL	1109.24	8.568	14.56%
10mer_H2_Db	170	AKK	IGIDVKFSEF	PDY	1136.32	7.352	12.49%
10mer_H2_Db	151	GAN	IGVAQAATTK	KAI	941.08	4.813	8.18%
10mer_H2_Db	229	IVT	KKDDPAFAKY	VDD	1164.33	4.544	7.72%
10mer_H2_Db	248	EHK	NEIDALAKKW	GL	1146.34	3.871	6.58%
10mer_H2_Db	195	KRV	DAFSVDKSIL	LGY	1076.22	3.654	6.21%
10mer_H2_Db	22	VAF	SNANAAEGKL	ESI	956.02	3.617	6.15%
10mer_H2_Db	45	VGV	KNDVPHYALL	DQA	1151.33	2.711	4.61%
10mer_H2_Db	154	IGV	AQAATTKKAI	GEA	984.15	1.993	3.39%
10mer_H2_Db	138	LKE	KKYKSLADMK	GAN	1193.46	1.857	3.16%
10mer_H2_Db	10	LLK	LAVFALGACV	AFS	945.19	1.677	2.85%
10mer_H2_Db	62	GEI	KGFEVDVAKL	LAK	1087.28	0.783	1.33%
10mer_H2_Db	233	KDD	PAFAKYVDDF	VKE	1154.3	0.665	1.13%
10mer_H2_Db	177	VKF	SEFPDYPSIK	AAL	1164.3	0.417	0.71%

Table 1c: Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites of Peb1a from *Campylobacter jejuni*. The binding potential (score) of antigenic peptide to the MHC-1 Allele i.e. 10mer_H2_Db.

MHC-I Allele	POS.	N	SEQUENCE	C	MW (Da)	SCORE
11mer_H2_Db	116	PER	KRIYNFSEPYY	QDA	1461.66	20.317
11mer_H2_Db	160	ATT	KKAIGEAAKKI	GID	1138.41	19.105
11mer_H2_Db	61	TGE	IKGFEVDVAKL	LAK	1200.44	4.79
11mer_H2_Db	21	CVA	FSNANAAEGKL	ESI	1103.2	4.092
11mer_H2_Db	5	VFR	KSLLKLAVFAL	GAC	1184.53	3.721
11mer_H2_Db	9	SLL	KLAVFALGACV	AFS	1073.36	3.683
11mer_H2_Db	243	DDF	VKEHKNEIDAL	AKK	1277.44	3.405
11mer_H2_Db	197	VDA	FSVDKSILLGY	VDD	1223.44	2.702
11mer_H2_Db	41	GQL	IVGVKNDVPHY	ALL	1222.4	2.646
11mer_H2_Db	32	GKL	ESIKSKGQLIV	GVK	1183.41	2.248
11mer_H2_Db	122	YNF	SEPYYQDAIGL	LVL	1237.35	1.713
11mer_H2_Db	31	EGK	LESIKSKGQLI	VGV	1197.44	1.612
11mer_H2_Db	151	GAN	IGVAQAATTKK	AIG	1069.25	1.048
11mer_H2_Db	141	KKY	KSLADMKGANI	GVA	1129.33	0.812
11mer_H2_Db	214	KSE	ILPDSFEPQSY	GIV	1277.42	0.378

Table 1d: Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites of Peb1a from *Campylobacter jejuni*. The binding potential (score) of antigenic peptide to the MHC-1 Allele i.e. 11mer_H2_Db.

MHC-II Allele	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.

MHC-II I_Ab	179	FSE	FPDYPSIKA	ALD	1019.18	13.645	38.29%
MHC-II I_Ab	146	LAD	MKGANIGVA	QAA	842.01	11.916	33.44%
MHC-II I_Ab	85	KIK	LVAVNAKTR	GPL	953.14	11.351	31.86%
MHC-II I_Ab	19	GAC	VAFSNANAA	EGK	845.91	11.064	31.05%
MHC-II I_Ab	18	LGA	CVAFSNANA	AEG	877.97	10.604	29.76%
MHC-II I_Ab	121	IYN	FSEPYYQDA	IGL	1101.16	9.738	27.33%
MHC-II I_Ab	21	CVA	FSNANAAEG	KLE	861.87	9.738	27.33%
MHC-II I_Ab	179	FSE	FPDYPSIKA	ALD	1019.18	13.645	38.29%

Table 2a: Prediction of MHCII ligands all rows highlighted in red represent predicted binders to the MHC-II Allele i.e. MHC-II I_Ab.

MHC-II Allele	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
MHC-II I_Ad	15	VFA	LGACVAFSN	ANA	863.0	20.639	38.84%
MHC-II I_Ad	155	GVA	QAATTKKAI	GEA	913.07	12.13	22.82%
MHC-II I_Ad	103	GSV	DAVIATFTI	TPE	932.08	9.318	17.53%
MHC-II I_Ad	170	AKK	IGIDVKFSE	FPD	989.14	8.149	15.33%
MHC-II I_Ad	107	AVI	ATFTITPER	KRI	1017.15	7.964	14.99%
MHC-II I_Ad	147	ADM	KGANIGVAQ	AAT	838.95	7.653	14.40%
MHC-II I_Ad	62	GEI	KGFEVDVAK	LLA	974.12	7.529	14.17%
MHC-II I_Ad	149	MKG	ANIGVAQAA	TTK	795.89	7.296	13.73%

Table 2b: Prediction of MHCII ligands all rows highlighted in red represent predicted binders to the MHC-II Allele i.e. MHC-II I_Ad.

MHC-II Allele	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
MHC-II I_Ag7	186	PSI	KAALDAKRV	DAF	953.15	14.202	34.75%
MHC-II I_Ag7	230	VTK	KDDPAFAKY	VDD	1036.16	14.11	34.52%
MHC-II I_Ag7	237	AFA	KYVDDFVKE	HKN	1124.26	14.021	34.30%
MHC-II I_Ag7	162	TKK	AIGEAAKKI	GID	882.07	13.775	33.70%
MHC-II I_Ag7	92	NAK	TRGPLLDNG	SVD	924.02	13.075	31.99%
MHC-II I_Ag7	39	SKG	QLIVGVKND	VPH	967.12	10.196	24.95%
MHC-II I_Ag7	248	EHK	NEIDALAKK	WGL	983.13	10.021	24.52%
MHC-II I_Ag7	184	DYP	SIKAALDAK	RVD	898.07	9.809	24.00%
MHC-II I_Ag7	85	KIK	LVAVNAKTR	GPL	953.14	9.195	22.50%
MHC-II I_Ag7	192	LDA	KRVDAFSVD	KSI	1018.14	8.875	21.71%

Table 2c: Prediction of MHCII ligands all rows highlighted in red represent predicted binders to the MHC-II Allele i.e. MHC-II I_Ag7.

Peptide Rank	Start Position	Sequence	Score	Predicted Affinity
1	143	LADMKGANI	8.646	High
2	92	TRGPLLDNG	8.639	High

3	100	GSVDAVIAT	8.632	High
4	235	FAKYVDDFV	8.627	High
5	63	GFEVDVAKL	8.626	High
6	62	KGFEVDVAK	8.624	High
7	153	VAQAATTKK	8.613	High
8	225	GIVTKKDDP	8.610	High
9	142	SLADMKGAN	8.595	High
10	17	ACVAFSNAN	8.591	High
11	233	PAFAKYVDD	8.590	High
12	159	TKKAIGEAA	8.588	High
13	67	DVAKLLAKS	8.587	High
14	122	SEPYYQDAI	8.584	High
15	193	RVDAFSVDK	8.584	High
16	83	IKLVAVNAK	8.563	High
17	61	IKGFEVDVA	8.539	High
18	134	VLKEKKYKS	8.529	High
19	79	DDKKIKLVA	8.527	High
20	41	IVGVKNDVP	8.526	High
21	212	SEILPDSFE	8.524	High
22	184	SIKAALDAK	8.523	High
23	23	NANAAEGKL	8.512	High
24	75	SILGDDKKI	8.463	High
25	66	VDVAKLLAK	8.446	High
26	158	TTKKAIGEA	8.443	High
27	226	IVTKKDDPA	8.434	High
28	74	KSILGDDKK	8.422	High
29	198	SVDKSILLG	8.407	High
30	50	HYALLDQAT	8.388	High
31	181	DYPSIKAAL	8.358	High
32	206	GYVDDKSEI	8.352	High
33	115	RKRIYNFSE	8.324	High
34	34	IKSKGQLIV	8.314	High
35	147	KGANIGVAQ	8.304	High
36	156	AATTKKAIG	8.276	High
37	33	SIKSKGQLI	8.249	High
38	157	ATTKKAIGE	8.231	High

39	125	YYQDAIGLL	8.231	High
40	201	KSILLGYVD	8.190	High
41	65	EVDVAKLLA	8.140	High
42	32	ESIKSKGQL	8.077	High
43	35	KSKGQLIVG	8.073	High
44	126	YQDAIGLLV	8.043	High
45	141	KSLADMKGA	8.038	High
46	223	SYGIVTKKD	7.936	High
47	180	PDYPSIKAA	7.915	High
48	139	KYKSLADMK	7.901	High
49	131	GLLVLKEKK	7.845	High
50	247	KNEIDALAK	7.790	High
51	29	GKLESIKSK	7.779	High
52	245	EHKNEIDAL	7.745	High
53	161	KAIGEAAKK	7.585	High
54	129	AIGLLVLKE	7.560	High
55	12	VFALGACVA	7.482	High
56	216	PDSFEPQSY	7.478	High
57	160	KKAIGEAAK	7.283	High
58	96	LLDNGSVDA	7.125	High
59	40	LIVGVKNDV	7.119	High
60	146	MKGANIGVA	7.044	High
61	228	TKKDDPAFA	7.033	High
62	249	EIDALAKKW	7.029	High
63	102	VDAVIATFT	6.991	High
64	44	VKNDVPHYA	6.974	High
65	52	ALLDQATGE	6.965	High
66	171	GIDVKFSEF	6.913	High
67	207	YVDDKSEIL	6.895	High
68	200	DKSILLGYV	6.890	High
69	10	LAVFALGAC	6.875	High
70	91	KTRGPLLDN	6.862	High
71	140	YKSLADMKG	6.776	High
72	149	ANIGVAQAA	6.694	High
73	57	ATGEIKGFE	6.693	High
74	183	PSIKAALDA	6.633	High

75	82	KIKLVAVNA	6.629	High
76	86	VAVNAKTRG	6.582	High
77	188	ALDAKRVDA	6.578	High
78	169	KIGIDVKFS	6.515	High
79	145	DMKGANIGV	6.477	High
80	119	YNFSEPYYQ	6.437	High

Table 3: cascade SVM based High affinity TAP Binders of Peb1a from *Campylobacter jejuni*

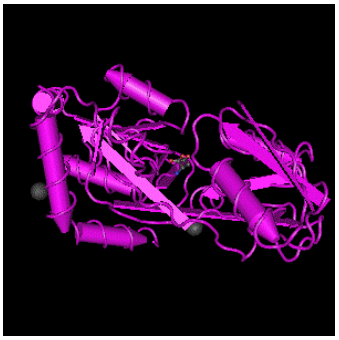

Figure 1: X-Ray Diffraction with Resolution 1.49 Å 3D Structure of the Peb1a from *Campylobacter jejuni*

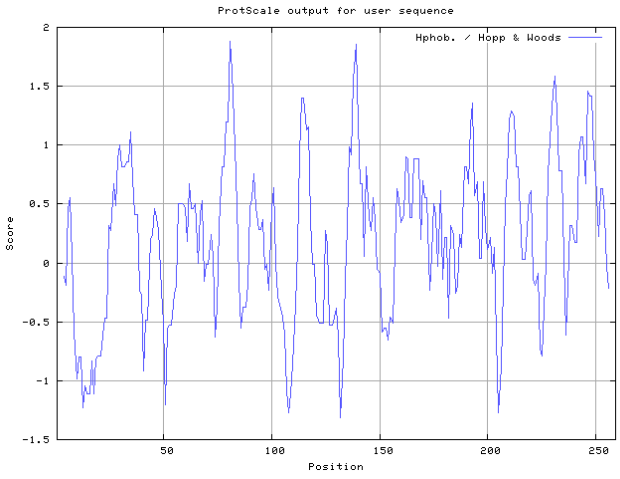

Figure 2: Hydrophobicity plot of Hopp and Woods [17] of Peb1a from *Campylobacter jejuni*

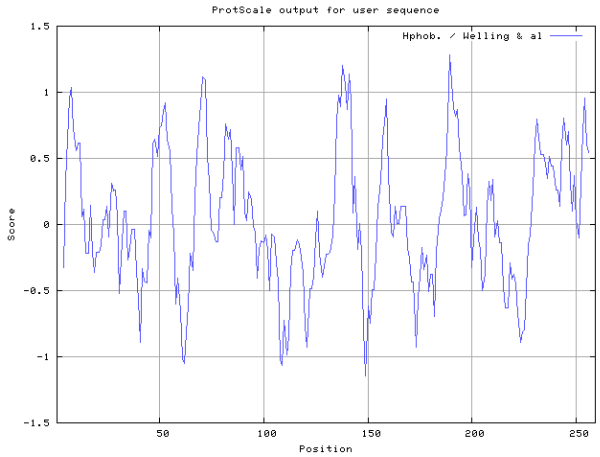

Figure 3: Hydrophobicity plot of Welling et al. [18] of Peb1a from *Campylobacter*

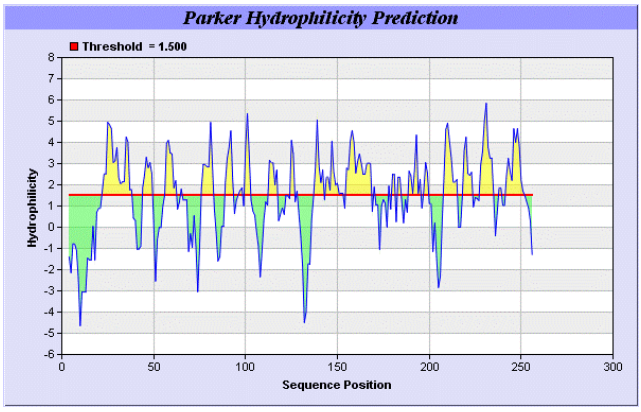

Figure 4: Hydrophobicity plot of HPLC / Parker et al. [19] of Peb1a from *Campylobacter jejuni*

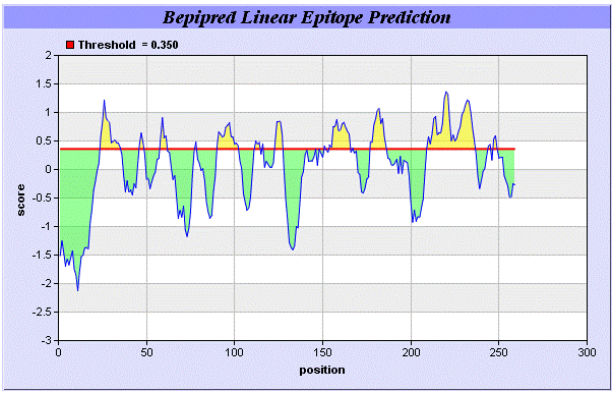

Figure 5: Bepipred Linear Epitope Prediction plot showing antibody recognized B-cell epitopes of Peb1a from *Campylobacter jejuni*

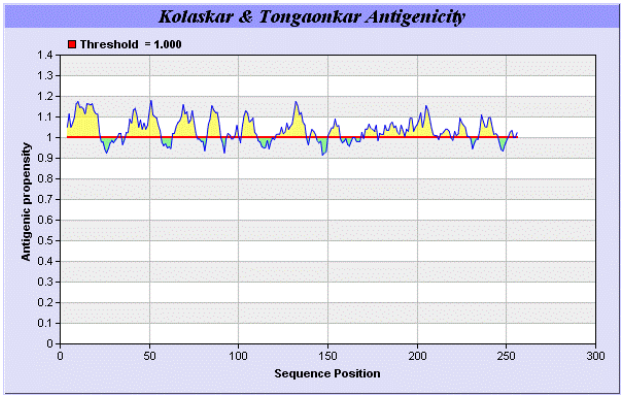

Figure 6: Kolaskar and Tongaonkar [21] antigenicity plot of Peb1a from *Campylobacter jejuni*

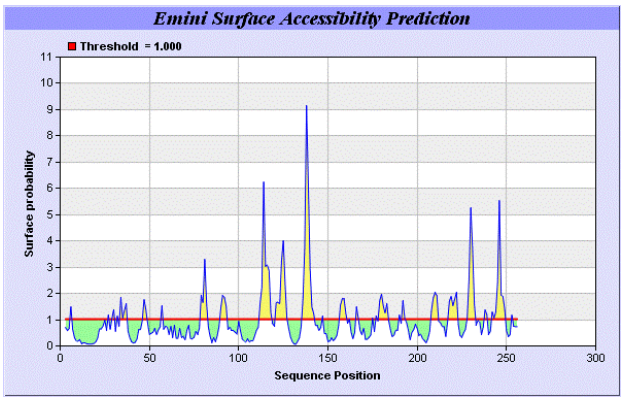

Figure 7: Emini Surface Accessibility Prediction plot of Peb1a from *Campylobacter jejuni*

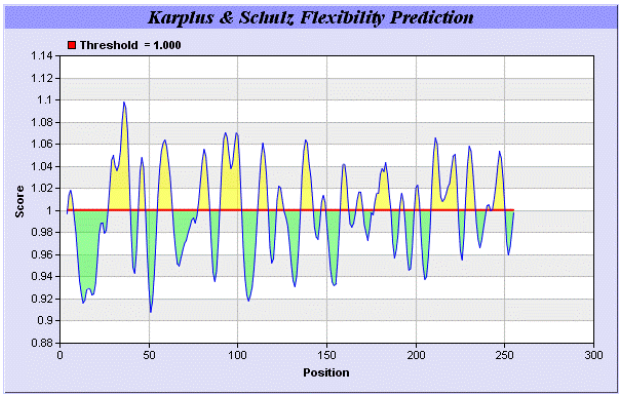

Figure 8: Karplus and Schulz [28] Flexibility Prediction of Peb1a from *Campylobacter jejuni*

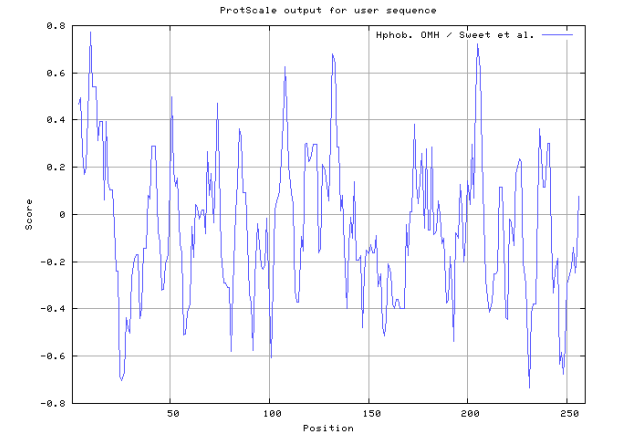

Figure 9: Hydrophobicity plot of Sweet et al. [29] of Peb1a from *Campylobacter jejuni*

Discussion

Antigenecity is predicted by using Hopp and Woods ilicity scale to identify potentially antigenic sites in proteins by analyzing amino acid sequences in order to find the point of greatest hydrophilic. The Window size of 5-7 is good for finding hydrophilic regions, greater than 0 values are consider as hydrophilic which is consider as antigenic. Welling used information on the relative occurrence of amino acids in antigenic regions to make a scale which is useful for prediction of antigenic regions. Welling antigenicity plot gives value as the log of the quotient between percentage in sample of known antigenic regions and percentage in average proteins. Hydrophobicity plot of HPLC / Parker Hydrophilicity. BepiPred predicts the location of linear B-cell. There are 3 antigenic determinant sequences is found by Kolaskar and Tongaonkar antigenicity scales.

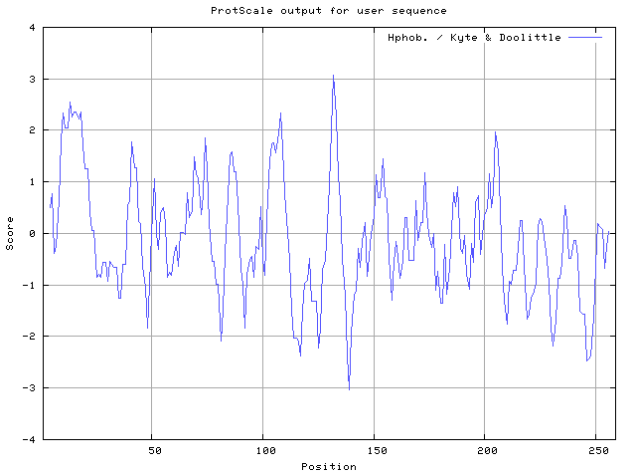

Figure 10: Kyte and Doolittle [30] hydrophobicity plot of Peb1a from *Campylobacter jejuni*

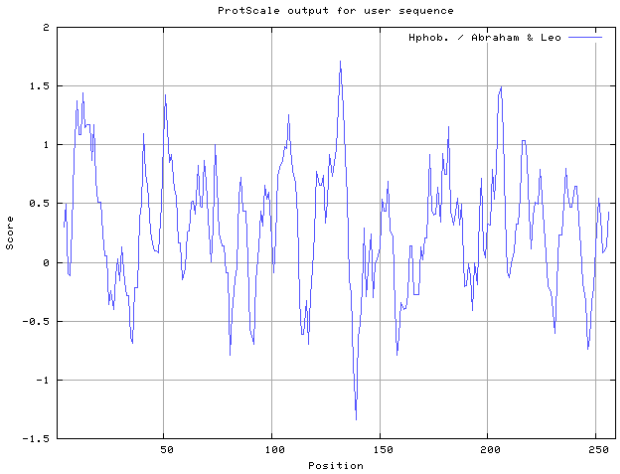

Figure 11: Abraham and Leo [31] hydrophobicity plot of Peb1a from *Campylobacter jejuni*

Result of determined antigenic sites on proteins has revealed that the hydrophobic residues if they occur on the surface of a protein are more likely to be a part of antigenic sites. This method can predict antigenic determinants with about 75% accuracy and also gives the information of surface accessibility and flexibility. Further this region form beta sheet which show high antigenic response than helical region of this peptide and shows highly antigenicity. X-Ray Diffraction with Resolution 1.49 Å 3D Structure of the Peb1a from *Campylobacter jejuni* is predicted by PDB vive. Sequence analysis of the chosen target and then structure determined the target experimentally to evaluate their similarity to known protein structures and to determine possible relationships that are identifiable from protein sequence alone.

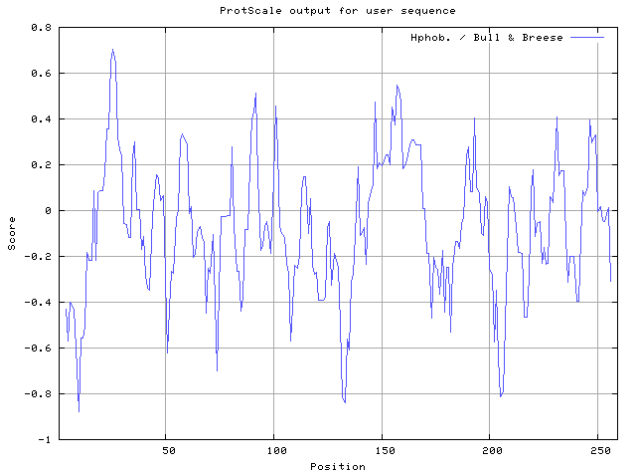

Figure 12: Bull and Breese [32] use surface tension to measure hydrophobicity and also uses negative values to describe the hydrophobicity of Peb1a from *Campylobacter jejuni*

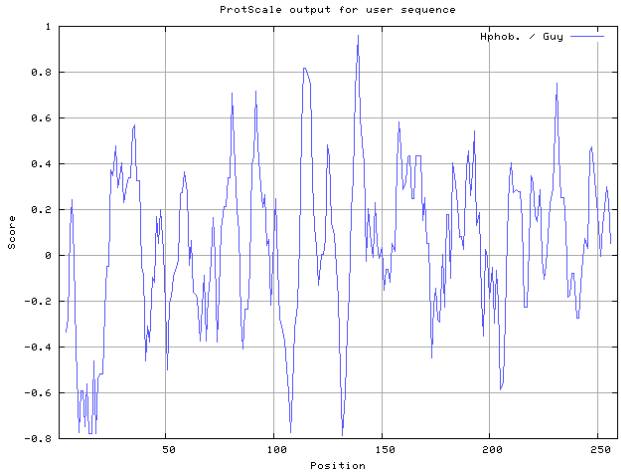

Figure 13: Hydrophobicity plot of Miyazawa et al. [33] of Peb1a from *Campylobacter jejuni*

The target structure will also serve as a detailed model for determining the structure of peptide within that protein structure. We predict Solvent accessibility by using Emini et al. [27], the result found the highest probability that a given protein region lies on the surface of a protein and are used to identify antigenic determinants on the surface of proteins. This algorithm also used to identify the antigenic determinants on the surface of proteins and Karplus and Schulz [28] predict backbone or chain flexibility on the basis of the known temperature B factors of the a-carbons.

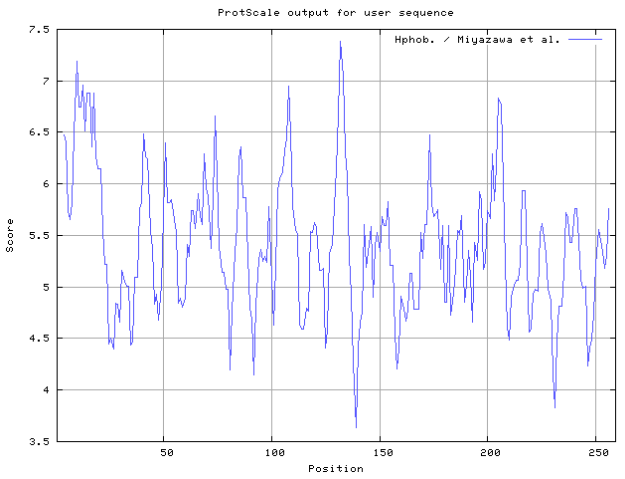

Figure 14: Hydrophobicity plot of Roseman [34] of Peb1a from *Campylobacter jejuni*

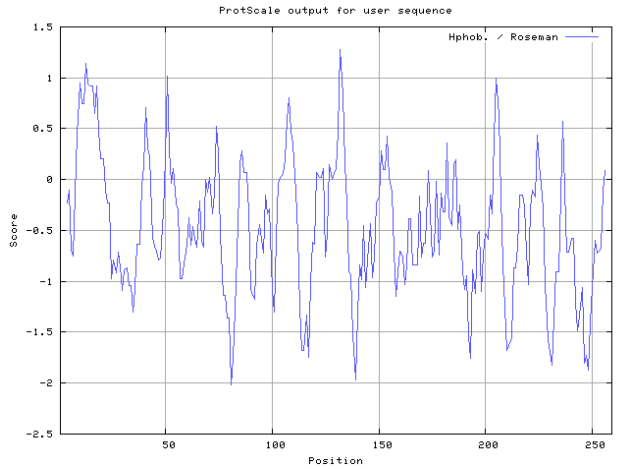

Figure 15: Hydrophobicity plot of Wolfenden et al. [35] of Peb1a from *Campylobacter jejuni*

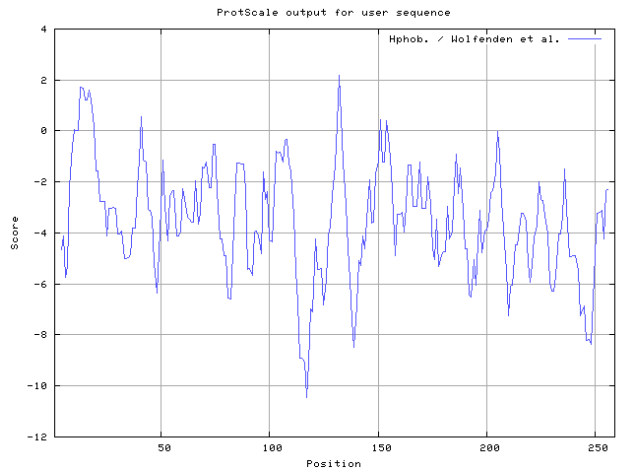

Figure 16: Hydrophobicity plot of Roseman MA [34] of Peb1a from *Campylobacter jejuni*

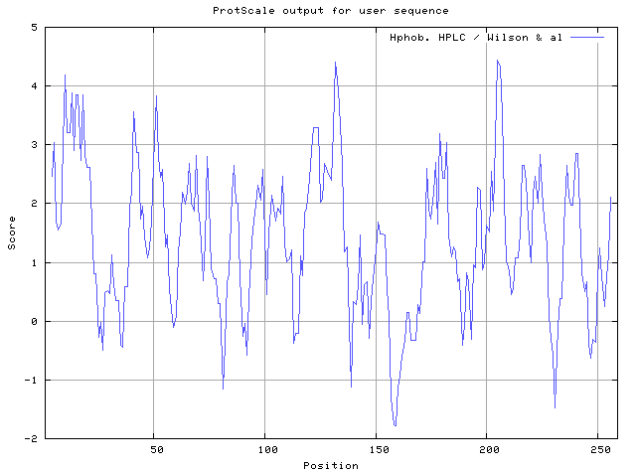

Figure 17: Hydrophobicity/HPLC plot of Wilson et al. [36] of Peb1a from *Campylobacter jejuni*

We predict Solvent accessibility of Peb1a from *Campylobacter jejuni* for delineating hydrophobic and hydrophilic characteristics of amino acids. Solvent accessibility used to identify active site of functionally important residues in membrane proteins. Solvent-accessible surface areas and backbone angles are continuously varying because proteins can move freely in a three-dimensional space. The mobility of protein segments which are located on the surface of a protein due to an entropic energy potential and which seem to correlate well with known antigenic determinants. We also found hydrophobicity by Sweet et al. [29], Kyte and Doolittle [30], Abraham and Leo [31], Bull and Breese [32], Guy Miyazawa [33], Roseman [34], Wolfenden [35], Wilson et al. [36], Cowan [37], Chothia [38].

These scales are a hydrophilic with a polar residues assigned negative value. Because the N- and C- terminal regions of proteins are usually solvent accessible and unstructured, antibodies against those regions recognize the antigenic protein. In this study, we found predicted MHC-I peptide binders of Peb1a from *Campylobacter jejuni* for 8mer_H2_Db alleles, 9mer_H2_Db, 10mer_H2_Db with, 11mer_H2_Db and I_Ab, I_Ad, MHC-II I_Ag7 for MHC II allele was tasted. We also use a cascade SVM based TAPPred method which found 80 High affinity TAP Transporter peptide regions which represents predicted TAP binders residues which occur at N and C termini Peb1a from *Campylobacter jejuni*. TAP is an important transporter that transports antigenic peptides from cytosol to ER. TAP binds and translocate selective antigenic peptides for binding to specific MHC molecules. The efficiency of TAP-mediated translocation of antigenic peptides is directly proportional to its TAP binding affinity. Thus, by understanding the nature of peptides, that

bind to TAP with high affinity, is important steps in endogenous antigen processing. The correlation coefficient of 0.88 was obtained by using jackknife validation test.

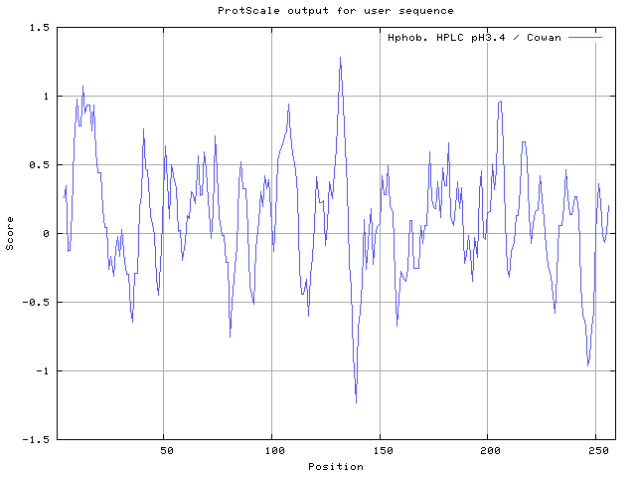

Figure 18: Hydrophobicity/HPLC pH 3.4/ plot of Cowan [37] of Peb1a from *Campylobacter jejuni*

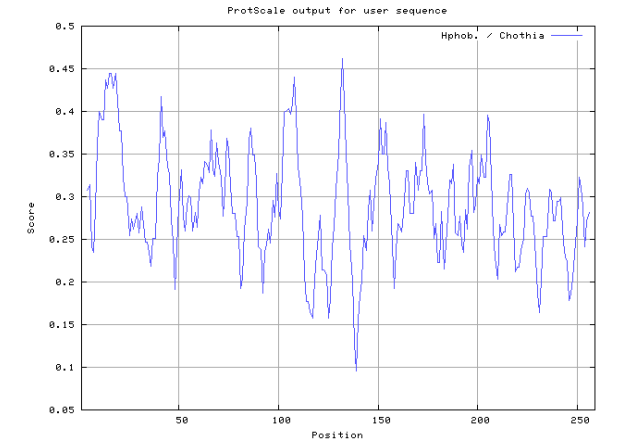

Figure 19: Hydrophobicity plot of Chothia [38] of Peb1a from *Campylobacter jejuni*

The MHCI and MHCII binding regions

T cell immune responses are derived by antigenic epitopes hence their identification is important for design synthetic peptide vaccine. T cell epitopes are recognized by MHCI molecules producing a strong defensive immune response against Peb1a from *Campylobacter jejuni*. Therefore, the prediction of peptide binding to MHCI molecules by appropriate processing of antigen peptides occurs by their binding to the relevant MHC molecules. Because, the C-terminus of MHCI-restricted epitopes results from cleavage by the proteasome and thus, proteasome specificity is important to determine T-cell epitopes. Consequently, RANKPEP also focus on the prediction of conserved epitopes. C-terminus of MHCI-restricted peptides is generated by the proteasome, and thus RANKPEP also determines whether the C-terminus of the predicted MHCI-peptide binders is the result of proteasomal cleavage. Moreover, these sequences are highlighted in purple in the output results. Proteasomal cleavage predictions are carried out using three optional models obtained applying statistical language models to a set of known epitopes restricted by human MHCI molecules as indicated here.

Conclusion

From the above result and discussion it is concluded that the increase in affinity of MHC binding peptides may result in enhancement of immunogenicity of Peb1a from *Campylobacter jejuni* and are helpful in the designing of synthetic peptide vaccine. This approach can help reduce the time and cost of experimentation for determining functional properties of Peb1a from *Campylobacter jejuni*. Overall, the results are encouraging, both the 'sites of action' and 'physiological functions' can be predicted with very high accuracies helping minimize the number of validation experiments.

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