Bioscene

Bioscene
Volume- 22 Number- 03
ISSN: 1539-2422 (P) 2055-1583 (O)
www.explorebioscene.com

Computational Characterization and Validation of Potential Antigenic Peptide Vaccines from the Fasciolopsis Buskii (Lankester, 1857) Tegument Antigen Protein Using Immunoinformatics Approach

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Abstract: Fasciolopsis buskii (Lankester, 1857) is a parasitic trematode, there have been reports of infections with this parasite from many countries of Southeast Asia. In India prevalence can reach up to 60% in the most affected areas, particularly in the southern and eastern regions, with reports of infections mainly concentrated in states like Assam, Bihar, Uttar Pradesh, and Meghalaya. Humans and pigs are susceptible to the neglected zoonotic disease 'fasciolosisasis' due to this foodborne intestinal trematode. It is essential to characterize appropriate antigens for diagnosing and immunising against parasite infections, as this may have implications for the diagnosis and treatment of disease. The MHC class molecules interact with almost every antigen, and activates specific regions. Astern-like red flags known as projected MHC binders are specific to certain antigens and stimulate a chemical reaction of the immune system to the parent antigen. As result, an immune response to just a tiny part of an antigen can be produced against the whole antigen. This concept has been employed in designing synthetic peptide and subunit vaccines against theF. buskii (Lankester, 1857) tegument antigen protein. In current study we predicted the primary structural properties of tegument antigen protein of Fasciolopsis buski using Uniport tool. Physicochemical properties, Secondary structure, amino acid composition and relative positions of the beta-strand and alpha-helix in the amino acid sequence of tegument protein also predicted by using EXPASY server. Immunoinformatics analysis of tegument protein from F. buskiiare important determinant for protection. Current study also projected the binding affinity of tegument antigen protein having 183 amino acids which shows the different conformers. The method also integrates prediction of peptide MHC class I binding, proteasomal C terminal cleavage and TAP transport efficacy of the tegument antigen protein. In this study, we explore the potential of antigen proteins to design a vaccine targetingF. buskii.

Keywords: Antigen protein, Immunoinformatics, Vaccine, F. buskii

Introduction

Fasciola buski generally referred as the liver fluke, which is a parasitic trematode of significant veterinary and human health importance. This parasite primarily infects the bile ducts and liver of various mammalian hosts, including livestock such as sheep and cattle, leading to the disease known as fascioliasis. The life cycle of F. buskii involves a freshwater snail as an intermediate host, with transmission occurring through the ingestion of contaminated vegetation or water. Fascioliasis poses substantial economic and public health challenges. In livestock, it results in decreased productivity, weight gain, and fertility, alongside costs associated with treatment and control (Aleixo, 2015).

Fasciolopsiasis is endemic to parts of Southeast Asia, including China, India, Thailand, and Vietnam, where it poses significant public health and economic challenges. The clinical manifestations of the disease range from mild gastrointestinal discomfort to severe malnutrition, intestinal obstruction, and systemic intoxication. Chronic infections can lead to long-term health complications, particularly in vulnerable populations such as children and the elderly. The pathogenesis of F. buski infection is primarily associated with the mechanical damage caused by the attachment of adult flukes to the intestinal mucosa, as well as the release of toxic metabolic byproducts. Effective control strategies rely on a combination of public health measures, including improved sanitation, health education, and the use of anthelmintic drugs (Hossain, 2022).

In humans, the World Health Organisation categorises fascioliasis as a neglected tropical health issue, with millions of people at risk, particularly in regions with extensive livestock farming. The pathogenesis of fascioliasis is linked to the movement of juvenile flukes through liver parenchyma and their establishment in bile ducts. This can lead to a spectrum of clinical manifestations, ranging from mild symptoms such as fatigue and abdominal pain to severe complications like biliary obstruction and liver cirrhosis (Mas-Coma S, 2022). The emergence of drug-resistant strains of F. buskii has further complicated its management, highlighting the need for innovative control strategies (Aleixo, 2015).Recent research has focused on understanding the biology, immunology, and epidemiology of F. buskii. Advances in genomic and proteomic studies have identified potential targets for vaccine development and therapeutic interventions (Mas-Coma S, 2022).

The tegument, a specialized outer covering of parasitic trematodes like Fasciola buski, plays a pivotal role in host-parasite interactions. This structure acts as a protective barrier against the host's immune defense while facilitating nutrient absorption and secretion of bioactive molecules. Tegumental proteins are integral to these processes, making them essential for the survival and pathogenicity of the parasite.

One of the key functions of tegumental proteins is immune evasion. These proteins release excretory-secretory products that modify the host's immune response, allowing the parasite to begin and continue infection. Additionally,

tegumental proteins contribute to the parasite's ability to adhere to the intestinal mucosa, causing localized inflammation and tissue damage. Studies on related trematodes, such as Fasciola hepatica, have identified glycoproteins and enzymes in the tegument that facilitate host-parasite interactions, suggesting similar mechanisms may be present in F. buski(Ravida, 2016).Proteomic and genomic analyses have provided understandings of the composition in addition to function of tegumental proteins. These studies have highlighted their potential as biomarkers for diagnostic purposes and as targets for vaccine development (Wilson, 2011). Further investigation must be done to comprehensively elucidate the function of tegumental proteins in F. buski, particularly in the framework of its unique host-parasite dynamics. Such studies could pave the way for innovative strategies to control fasciolopsiasis and reduce its impact on public health. By considering F. buski tegument protein as novel and potential candidate for designing the antigenic peptide vaccines, the computational characterization and validations is done by using In Silico approach. This study attempted to develop and evaluate a fasciolopsiasis vaccine for the first time using an in-silico methodology.

Material and Methods

Identification and retrieval of vaccine targets - The tegument antigen protein sequence of F. buskiwere retrieved from National Centre for Biotechnology Information (NCBI) (Gen Bank: KAA0189128.1) for its further analysis and immunoinformatics studies. The retrieved sequence was computed to analyse its physical and chemical parameters with the help of the Prot Pram and its different parameters were recorded.

Analysis of Physico-chemical properties of tegument antigen protein.

The targeted protein sequence of tegument antigen protein from F. buski were retrieved successfully from the NCBI database with GenBank accession number KAA0189128.1 (Table 1) and protein were analyzed for physicochemical properties including molecular weight, theoretical pI, amino acid composition, atomic composition, and extinction coefficient.(Gill SC, 1989)(H., 1967)(Pace CN, 1995) along with estimated half-life(Ciechanover A, Schwartz AL, 1989)(A., 1997), the instability index of protein(Guruprasad K, 1990), the aliphatic index(AJ., 1980) and the grand average of hydropathicity of TCT Protein (GRAVY)(Kyte, 1982) were analysed by the Prot Parammethod (Gasteiger E., 2005). The Prot Param tool were computes various Physico-chemical properties of protein that can be inferred from a protein sequence, these predicted Physico-chemical properties show a very important role in vaccine design and taken into consideration in the process of vaccine development. Predict Protein analysis tool (Rost B, 2004) were used for computing and analysing the secondary structural features of tegument antigen protein sequence. Secondary structure prediction of tegument antigen protein was performed by GOR method (Garnier, 1978) which evaluates the sequences to predict the alpha helix, beta sheets and turn or random coil

secondary structure at each of the position based on 17-amino-acid sequence windows.

The antigenic epitopes of tegument antigen protein from F. bus were identified analysing different hydropathy and antigenicity plots like the, Hopp and Woods, Wellingetc (Welling GW, 1985), Kyte Doolittle Hydropathy (Kyte J, 1982), Kolaskar & Tongaonkar antigenicity plot (Kolaskar AS, 1990) and Parker Hydrophilicity Prediction plot (Parker JM, 1986).Neural networks trained on the C-terminal regions of identified epitopes were applied to predict the binding of antigenic peptides to Major Histocompatibility Complex (MHC) class I and II molecules. In this immune-informatics study, the predicted MHC–peptide binding affinities of antigen proteins were expressed as log-transformed values corresponding to IC50 values in nanomolar (nM) units. RANKPEP was employed to forecast MHC-restricted peptide ligands from protein sequences or sequence alignments using Position-Specific Scoring Matrices (PSSMs). In addition, a Support Vector Machine (SVM)-based approach was used to predict promiscuous MHC class II binding peptides, where the SVM was trained on binary input representations of single amino acid sequences (Gomase VS, 2008),(Buus S, 2003), (Reche PA, 2002),(Nielsen M, 2003). In addition, we assume those MHC ligands from whose C-terminal end will probable to be the outcome of proteosomal cleavage.

Results and Discussion

Protein Sequence Acquisition and Physicochemical Profiling

The sequence of the tegument antigen protein from the F. buski were retrieved and analysed (Table 1)the In silico study of the physiochemical features of tegument antigen protein were analysed by utilising the Prot Param tool, the results of Prot Param analysis exposed that tegument antigen protein contains the 190 amino acids chain, and its molecular weight were21410.44grammes/mole. Whereas total number of atoms in protein the were 2978.

Subsequently both the positive and negative charges of protein were equal, the protein's isoelectric point (pI) has no net charge. The theoretical isoelectric point (PI) of tegument antigen protein was 5.45. tegument antigen protein showed the chemical configuration of $C_{939}H_{1482}N_{250}O_{293}S_{14}$. Total number of the negatively charged residues were (Asp + Glu): 28 and total number of the positively charged residues were (Arg + Lys): 24. The extinction coefficient showed how much light a protein absorbs at a certain wavelength. While purifying a protein, it is essential to determine the coefficient with a spectrophotometer, as the tegument antigen protein showed. Extinction coefficients were 29700, Abs is 0.1% (1 g/l) 1.387 assuming all pairs of Cys residues form cystines. Analysis of half-life period is a calculation of the time which takes for half of the volume of protein in a cell to disappear after its formation in the cell target protein, tegument antigen protein showed half-life of 30 hours (mammalian reticulocytes, in vitro) 20 hours (yeast, in vivo) 10 hours (Escherichia coli, in vivo). The

instability index was calculated to be 29.00 the value of the protein was less than 40 so it is confirmed that the protein was more stable. The aliphatic index demarcated as relative volume covered by aliphatic side chain. The aliphatic index of tegument antigen protein was 75.89. Grand average of hydrophobicity of protein (GRAVY) calculated by EXPASY server were -0.383, which is considered as the entirety of hydropathy values of all the studied amino acids, divided by the total number of residues in the sequence.

Analysis Secondary Structure Predictions of Tegument antigen protein

Predict Protein analysis results of tegument antigen protein exhibited that protein is a diverse protein having structure of Helix =40.00%, strand = 14.21%, loop =45.79%, solvent accessibility of tegument antigen protein in F. buski tegument antigen protein shows 08.95%intermediate, buried 36.32% and Exposed 54.74%. amino acids in length of 190 base pairs of proteins, number of align protein in peroxiredoxin are 64, and number of matched PDB structure are 31 (Fig. 01)

Fig 1. Secondary structure prediction and solvent accessibility

GOR secondary structure prediction tool remained used to predict locations of alpha-helix and beta-strand from amino acid sequence tegument antigen protein of F. buski (Fig.02)

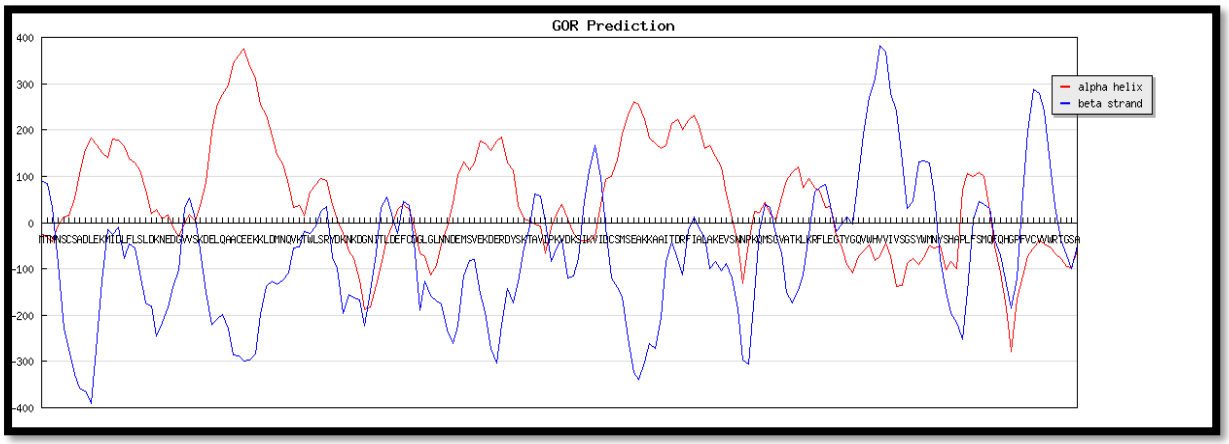

Fig. 2 Quality and reliability assessment of tegument antigen protein structure

The antigenic epitopes of tegument antigen protein from F. buskiare projected by different hydropathy and antigenicity tools (Somnath Waghmare, 2012).

Welling antigenicity prediction method

The Welling track computes antigenicity of protein by applying the technique of (Welling GW, 1985). Previous methods assumed that antigenic regions were primarily hydrophilic regions on the facade of a protein. Unlike other approaches, the Welling method determines antigenicity by assessing how frequently each amino acid appears in established antigenic determinants relative to its average representation across protein sequences. The Minimum score at the position: 105 with the score: -1.227and Max score: 0.650(max) at Position: 45 with window size 09 (Fig. 3).

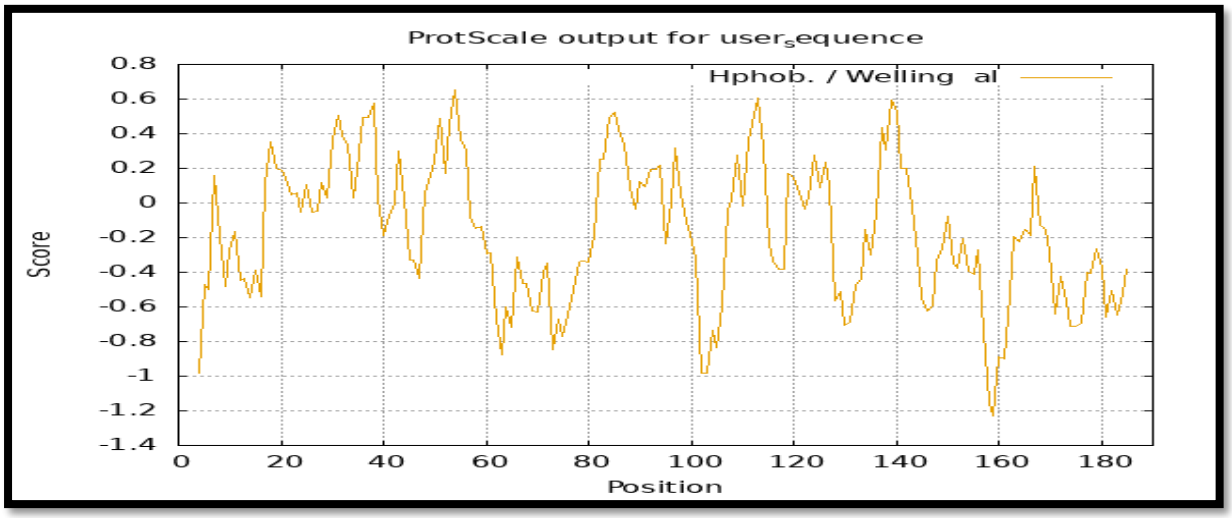

Figure 3: The hydrophobicity plot of tegument antigen protein from F. buskiby Hphob/Wellinget al. scale

Kyte-Doolittle prediction method to predicts the regional hydropathy of proteins

The Kyte–Doolittle hydropathy plot predicted regional hydropathy of the tegument antigen protein of F. buski based on its amino acid sequence, using the approach of Kyte and Doolittle (Kyte J, 1982). Hydropathy values were assigned to each amino acid and averaged across a user-defined sliding window, with the mean value plotted at the center of the window. These residue hydropathy values were derived from water–vapor transfer free energies and the interior–exterior distribution of side chains. The analysis revealed a minimum score of –3.322 at position 84 and a maximum score of 1.700 at position 148, using a window size of 9. (Fig. 4)

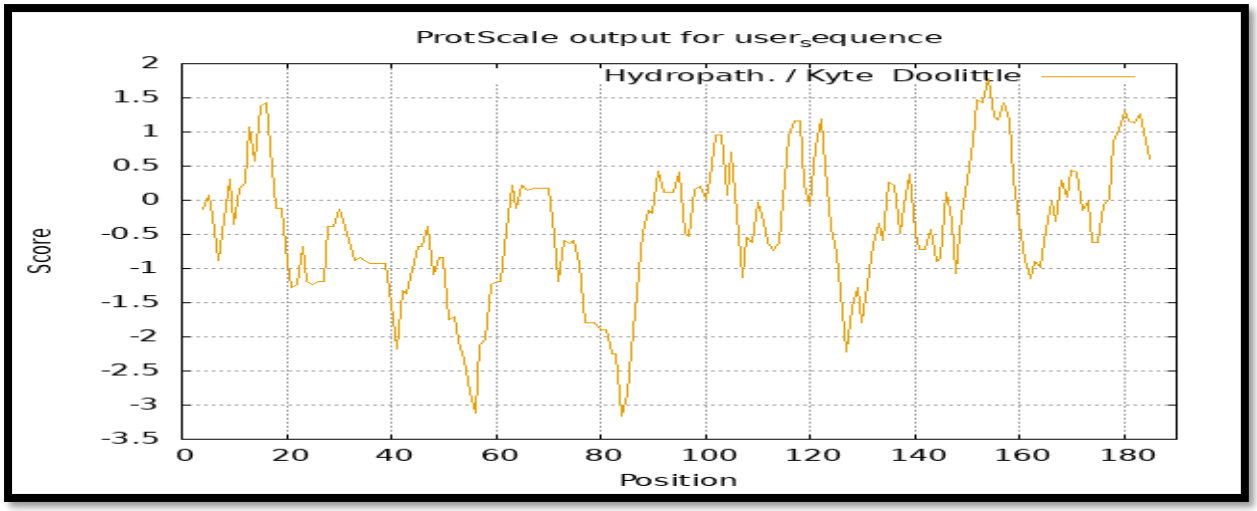

Figure 4: The hydrophobicity plot of tegument antigen protein from F. buski by Hphob. / Kyte & Doolittle

Parker hydrophilicity prediction

The Parker track practices the method of (Parker JM, 1986), which is a hydrophilicity scale created on high-performance liquid chromatography (HPLC) withholding times of model synthetic peptides. Hydrophilicity arrangements have been applied significantly in the prediction of antigenic amino acid deposits. The Parker method applies an altered Hopp-Woods algorithm jointly with a different set of hydrophilicity possibilities. The Min score At Position: 16 with the score: -3.044 and the max score score: 6.178 (max) at position: 85 with the window size 09. (Fig 5)

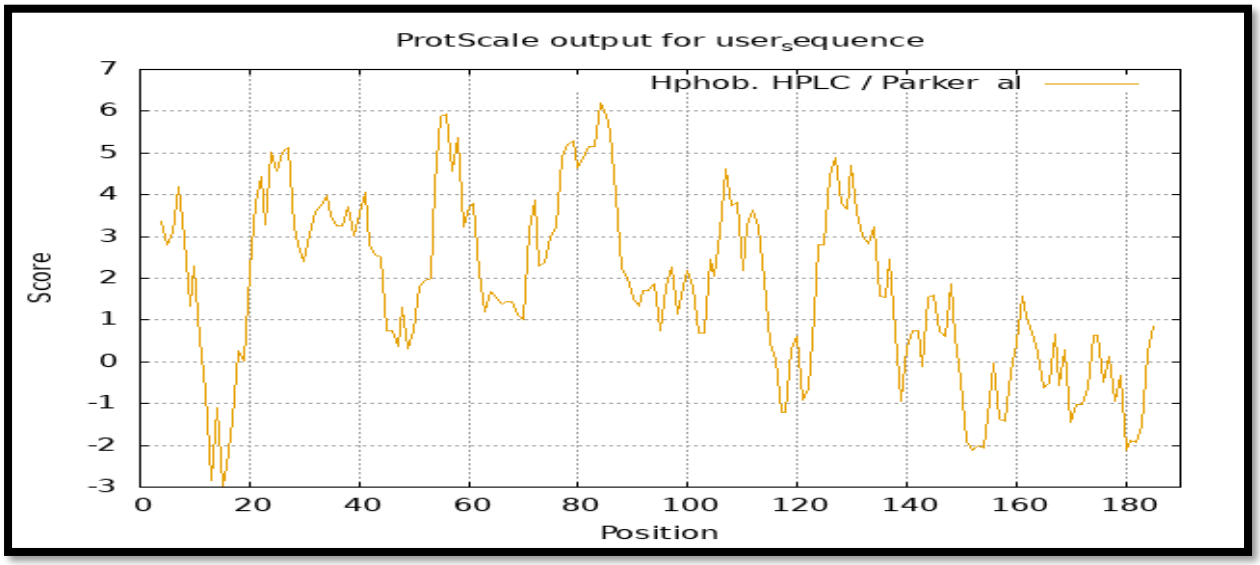

Figure 5: Parker antigenicity plot of tegument antigen protein from F. buski

The Kolaskar and Tongaonkar antigenicity prediction method

This is a semi-empirical approach that utilizes the physicochemical properties of amino acid residues along with their frequency of occurrence in experimentally

validated antigenic regions. Segmental epitopes are identified to estimate antigenic determinants within proteins. Application of this method to a wide range of proteins, as demonstrated by Kolaskar and Tongaonkar, has proven its reliability in predicting antigenic sites with reasonable accuracy.(Kolaskar AS, 1990) with nearly 75% accuracy, this method outperforms most conventional techniques used for antigenicity prediction(Fig.6)

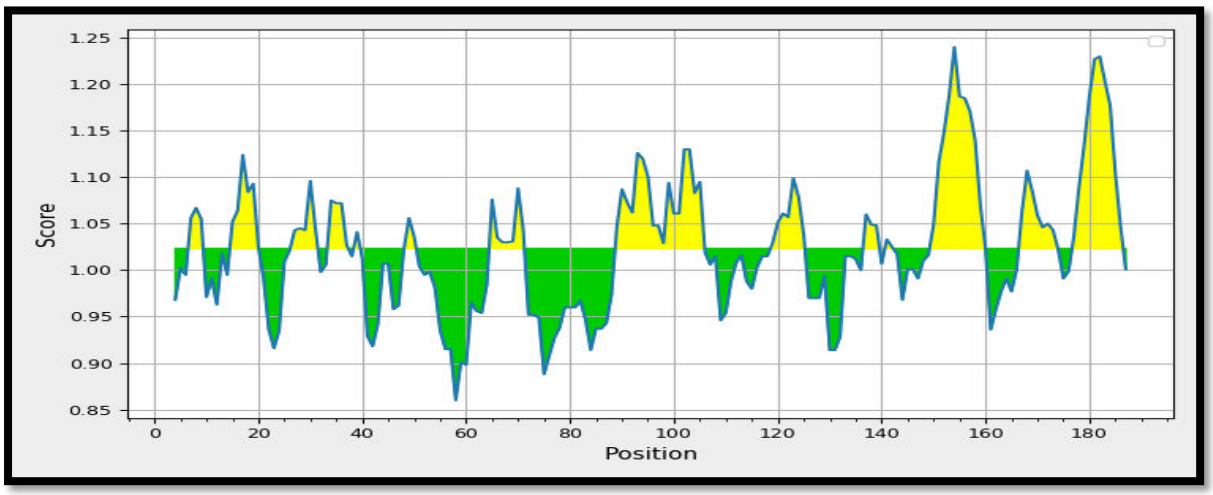

Figure 6: The Kolaskar and Tongaonkar antigenicity plot

MHC class I and MHC class II binding peptides prediction

By using Position Specific Scoring Matrix (PSSM) RANKPEP study of MHC class I and MHC class II binding peptides shows the binding of peptides to a number of different alleles. To almost all antigens MHC molecules are cell surface receptors proteins, which actively participate in host immune responses. From of F. buskiwe able to guess MHC-I peptide binders of tegument antigen protein. We found predicted MHC-I peptide binders of protein for Matrix: 8mer_H2_Db.p.mtx, Consensus: QNWNCCTI, with optimal score of: 52.494 and binding threshold was 33.04: Matrix: 9mer_H2_Db.p.mtx, the predicted consensus: FCIHNCDYM, optimal Score: 50.365and binding threshold 17.96; Matrix: 10mer_H2_Db.p.mtx, with consensus: SGYYNFFWCL, along with optimal Score: 58.858, binding threshold 41.32; matrix: 11mer_H2_Db.p.mtx, the consensus: CGVYNFYYCCY, with optimal Score of: 79.495, the binding threshold 56.96 (Table 1) in addition the MHC-II peptide binders intended for matrix of: I_Ad.p.mtx, with consensus: QMVHAAHAE, along with optimal score of: 53.145, the binding threshold 7.10; the matrix of:I_Ag7.p.mtx, along with the consensus of: WYAHAFKYV, with optimal score: 40.873, the binding threshold is 7.54; the matrix: I_Ak.p.mtx the consensus: DFWCWECCC with optimal score of: 39.9 with the binding threshold is : 14.17 for the MHC II allele (Table 2) were confirmed and selected for the result.

Table 1: Promiscuous of MHC ligands showing C-terminal ends are proteasomal cleavage sites of the tegument antigen protein of F. buski

MHC-I Allele	Rank	POS.	N	Sequence	C	MW (Da)	Score	% OPT.
8mer_H2_Db.	1	174	FSM	QFQHGPFV	CVV	941.06	18.356	34.97 %
8mer_H2_Db.	2	176	MQF	QHGPFVCV	VWR	868.02	14.547	27.71 %
8mer_H2_Db.	3	126	AKE	VSNNPKQM	SGV	899.02	13.185	25.12 %
8mer_H2_Db.	4	150	TYG	QVWHVVIV	SGS	938.16	12.585	23.97 %
8mer_H2_Db.	5	113	AKK	AAITDRFI	ALA	888.04	12.296	23.42 %
9mer_H2_Db	1	70	FCD	GLGLNNDEM	SVE	944.02	16.927	33.61 %
9mer_H2_Db	2	125	LAK	EVSNNPKQM	SGV	1028.14	13.061	25.93 %
9mer_H2_Db	3	19	LFL	SLDKNEDGV	VSK	957.99	12.141	24.11 %
9mer_H2_Db	4	162	GSY	WMNYSHAPL	FSM	1077.26	8.46	16.80 %
9mer_H2_Db	5	175	SMQ	FQHGPFVCV	VWR	1015.2	7.473	14.84 %
10mer_H2_Db	1	131	NNP	KQMSGVATKL	KRF	1044.26	15.799	26.84 %
10mer_H2_Db	2	124	ALA	KEVSNNPKQM	SGV	1156.31	13.916	23.64 %
10mer_H2_Db	3	90	YSK	TAVIPKVDKS	IKV	1039.23	7.958	13.52 %
10mer_H2_Db	4	79	DEM	SVEKDERDYS	KTA	1209.25	7.942	13.49 %
10mer_H2_Db	5	145	RFL	EGTYGQVWHV	VIV	1134.24	6.396	10.87 %
11mer_H2_Db	1	124	ALA	KEVSNNPKQMS	GVA	1243.39	16.398	20.63 %
11mer_H2_Db	2	112	EAK	KAAITDRFIAL	AKE	1200.45	8.345	10.50 %
11mer_H2_Db	3	70	FCD	GLGLNNDEMSV	EKD	1130.23	6.031	7.59 %
11mer_H2_Db	4	155	WHV	VIVSGSYWMNY	SHA	1277.49	2.968	3.73 %
11mer_H2_Db	5	25	KNE	DGVVSKDELQA	ACE	1142.23	2.74	3.45 %

Table 2: Prediction of the MHC-II Ligands shows all rows highlighted predicted binders to the MHC-II Allele

MHC-II Allele	Rank	POS.	N	Sequence	C	MW (Da)	Score	% OPT.
MHC-II I_Ad	1	132	NPK	QMSGVATKL	KRF	916.09	15.374	28.93 %
MHC-II I_Ad	2	49	QVK	TWLSRYDKN	KDG	1141.28	11.974	22.53 %
MHC-II I_Ad	3	106	IDC	SMSEAKKAA	ITD	904.05	9.724	18.30 %
MHC-II I_Ad	4	150	TYG	QVWHVVIVS	GSY	1025.24	8.58	16.14 %
MHC-II I_Ad	5	85	KDE	RDYSKTAVI	PKV	1034.18	8.474	15.95 %
MHC-II I_Ag7	1	135	QMS	GVATKLKRF	LEG	1001.23	12.429	30.41 %
MHC-II I_Ag7	2	133	PKQ	MSGVATKLK	RFL	916.13	11.456	28.03 %
MHC-II I_Ag7	3	50	VKT	WLSRYDKNK	DGN	1168.35	8.929	21.85 %
MHC-II I_Ag7	4	106	IDC	SMSEAKKAA	ITD	904.05	8.508	20.82 %
MHC-II I_Ag7	5	111	SEA	KKAAITDRF	IAL	1031.22	7.355	17.99 %
MHC-II I_Ak	1	97	PKV	DKSIKVIDC	SMS	1002.19	13.889	34.81 %
MHC-II I_Ak	2	59	KNK	DGNITLDEF	CDG	1005.05	12.954	32.47 %

MHC-II I_Ak	3	158	VIV	SGSYWMNYS	HAP	1053.15	10.394	26.05 %
MHC-II I_Ak	4	174	FSM	QFQHGPFVC	VVW	1044.2	10.158	25.46 %
MHC-II I_Ak	5	65	ITL	DEFCDGLGL	NND	950.04	9.09	22.78 %

Conclusion

Nonameric peptides of the Fasciolopsis buski tegument antigen protein are implicated in antigenic components that can directly stimulate and modulate the immune system. These peptides, aligned within the protein sequence, are known to bind specific MHC molecules and thus serve as predictors of MHC–peptide interactions. Both MHC class I and class II molecules recognize and bind their respective epitopes with high specificity, playing a crucial role in host immune responses by presenting antigens and providing protection against infections. Importantly, it is well established that an entire protein is not required to elicit an immune response; instead, small antigenic fragments are often sufficient to activate immunity against the whole antigen. This highlights that enhancing the affinity of MHC-binding peptides may improve the immunogenicity of the F. buski tegument antigen protein. Consequently, in silico drug design approaches may aid in the development of advanced computational tools for more precise identification of T-cell epitopes. Accurate prediction of antigenic proteins remains vital for the rational design of synthetic peptide-based vaccines and holds great promise for future vaccine development.

References

1. A., V. (1997). The N-end rule pathway of protein degradation. Genes Cells,, 2, 13-28.
2. AJ., I. (1980). Thermostability and aliphatic index of globular proteins. JBiochem, 1980,(88) 1895-, 88, 1895-1898.
3. Aleixo, M. A. (2015). Fasciola hepatica: epidemiology, perspectives in the diagnostic and the use of geoprocessing systems for prevalence studies. Recuperado de: Semina: Ciências Agrárias,.
4. Buus S, L. S. (2003). Sensitive quantitative predictions of peptide-MHC binding by a ‘Query by Committee’ artificial neural network approach. Tissue Antigens,, ,(62): , 378-384.
5. Ciechanover A, Schwartz AL. (1989). How are substrates recognized by the ubiquitin-mediated proteolytic system? Trends Biochem Sci,, 14, 483-488.
6. Garnier, J. O. (1978). Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. . J. Mol. Biol., 97-120.
7. Gasteiger E., H. C. (2005). Protein Identification and Analysis Tools on the Expasy Server;. In J. M. (ed), The Proteomics Protocols Handbook, (pp. 571-607). Humana Press .
8. Gill SC, v. H. (1989). Calculation of protein extinction coefficients from amino acid sequence data. Anal. Biochem,, 182, 319-326.

9. Gomase VS, K. K. (2008). Computer Aided Multi Parameter Antigen Design: Impact of Synthetic Peptide Vaccines from Soybean Mosaic Virus. ICETET, (pp. 629-634). California,: IEEE Xplore, Los Alamitos,.
10. Guruprasad K, R. B. (1990). Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. Protein Eng,, 4, 155-161.
11. H., E. (1967). Spectroscopic determination of tryptophan and tyrosine in proteins. Biochemistry,, 6, 1948-1954.
12. Hossain, M. S. (2022). "Food- and Vector-borne Parasitic Zoonoses: Global Burden and Impacts.". Advances in Parasitology, 87-136.
13. Kolaskar AS, T. P. (1990). A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett., 276(1-2), 172-84.
14. Kyte J, D. R. (1982). A simple method for displaying the hydropathic character of a protein. J. Mol Biol,, 157, 105-132.
15. Kyte, J. a. (1982). A simple method for displaying the hydropathic character of a protein. J. Mol. Biol., 157, 105-132.
16. Mas-Coma S, V. M. (2022). Human and Animal Fascioliasis: Origins and Worldwide Evolving Scenario. Clinical Microbiology Reviews, 35(04), 1-96.
17. Nielsen M, L. C. (2003). Reliable prediction of T cell epitopes using neural networks with novel sequence representations. Protein Sci., (12) , 1007-1017.
18. Pace CN, V. F. (1995). How to measure and predict the molar absorption coefficient of a protein. Protein Sci,, 11, 2411-2423.
19. Parker JM, G. D. (1986). New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. Biochemistry., 25(19), 5425-32.
20. Ravida, A. C. (2016). Fasciola hepatica surface tegument: glycoproteins at the interface of parasite and host. Molecular and Cellular Proteomics, 1-43.
21. Reche PA, G. J. (2002). Prediction of MHC Class I Binding Peptides Using Profile Motifs. Hum Immunol., (63) , 701-709.
22. Rost B, Y. G. (2004). The PredictProtein server. Nucleic Acids Res,, 1(32), 1-5.
23. Somnath Waghmare, V. G. (2012). Immunoproteomics Approach for Development of Synthetic Peptide Vaccine from Thioredoxin Glutathione Reductase. Metabolomics, 4-9.
24. Welling GW, W. W.-W. (1985). Prediction of sequential antigenic regions in proteins. FEBS Letters,, 188(02), 215-218.
25. Wilson, R. A.-B.-M. (2011). Exploring the Fasciola Hepatica Tegument Proteome. International Journal for Parasitology, 41(14), 1347-1359