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Identification of B cell epitope from the Intrinsically disordered region of Epstein Barr virus Nuclear antigen.

S.Jeswin Immanuel[1], Sathish Sankar[2*]

[1]Undergraduate, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600 077. India

Email id: 152001031.sdc@saveetha.com

[2]Professor, Department of microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600 077, India

Email id: sathishsankar.sdc@saveetha.com

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ABSTRACT

Background: Epstein–Barr virus (EBV) is a ubiquitous human herpesvirus associated with infectious mononucleosis and several malignancies, including Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma, and gastric cancer. Epstein–Barr Virus Nuclear Antigen 3 (EBNA3) plays a crucial role in viral persistence and host-cell interactions. Intrinsically disordered regions (IDRs) of viral proteins are often enriched in antigenic determinants and may serve as promising targets for immunological studies. **Aim:** The present study aimed to identify potential linear B-cell epitopes within the intrinsically disordered regions of EBNA3 using computational immunoinformatics approaches.

Materials and Methods: The amino acid sequence of EBNA3 was retrieved from a public protein database. Intrinsically disordered regions were predicted using the Predictor of Natural Disordered Regions (PONDR) server, and regions with disorder scores greater than 0.5 were selected for analysis. The identified disordered segments were further screened for linear B-cell epitopes using epitope prediction tools. Predicted epitopes were evaluated based on sequence characteristics, residue positions, and peptide length. The distribution of epitopes within disordered regions was analyzed to assess their potential immunogenicity. **Results:** Multiple intrinsically disordered regions were identified within the EBNA3 protein sequence. Several linear B-cell epitopes were predicted to be localized within these disordered segments, suggesting a strong association between intrinsic disorder and antigenic potential. The identified epitopes demonstrated favorable characteristics for immune recognition and may represent potential targets for vaccine development and diagnostic applications. **Conclusion:** This study highlights the significance of intrinsically disordered regions in EBNA3 as reservoirs of potential B-cell epitopes. The predicted epitopes provide a foundation for further experimental validation and may contribute to the development of EBV-specific immunotherapeutic and diagnostic strategies

Key Words: Epstein–Barr virus (EBV); Epstein–Barr Virus Nuclear Antigen 3 (EBNA3); B-cell epitope; Intrinsically disordered regions (IDRs); PONDR; Immunoinformatics; Antigenicity; Vaccine candidate; Viral proteins; Epitope prediction.

INTRODUCTION

An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells(1). The epitope is the specific piece of the antigen to which an antibody binds. The part of an antibody that binds to the epitope is called a paratope. Although epitopes are usually non-self proteins, sequences derived from the host that can be recognized (as in the case of autoimmune diseases) are also epitopes(2).The epitopes of protein antigens are divided into two categories, conformational epitopes and linear epitopes, based on their structure and interaction with the paratope. Conformational and linear epitopes interact with the paratope based on the 3-D conformation adopted by the epitope, which is determined by the surface features of the involved epitope residues and the shape or tertiary structure of other segments of the antigen(3). A conformational epitope is formed by the 3-D conformation adopted by the interaction of discontiguous amino acid residues. In contrast, a linear epitope is formed by the 3-D conformation adopted by the interaction of contiguous amino acid residues. A linear epitope is not determined solely by the primary structure of the involved amino acids(4). Residues that flank such amino acid residues, as well as more distant amino acid residues of the antigen affect the ability of the primary structure residues to adopt the epitope's 3-D conformation. 90% of epitopes are conformational

In molecular biology, intrinsically disordered proteins (IDPs) are proteins that typically lack a rigid or ordered three-dimensional structure in the absence of macromolecular interaction partners such as other proteins or RNA(5). IDPs range from fully unstructured to partially structured and include random coils, fused bead-like aggregates, or flexible linkers of large multidomain proteins(6). They are sometimes considered a separate class of proteins, along with globular, fibrous, and membrane proteins

Epstein-Barr virus (EBV), formally called human gammaherpesvirus 4, is one of nine known human herpesviruses in the herpes family and is one of the most common viruses in humans. EBV is a double-stranded DNA virus(7). It is best known as the cause of infectious mononucleosis ("mono" or "glandular fever"). It is also associated with various non-, premalignant, and malignant Epstein-Barr virus-associated lymphoproliferative disorders, including Burkitt's lymphoma, hemophagocytic lymphohistiocytosis, and Hodgkin's lymphoma(8). non-lymphocytic malignancies such as gastric and nasopharyngeal cancer; and conditions associated with the human immunodeficiency virus. B. Hairy leukoplakia and lymphoma of the central nervous system. The virus has also been associated with the childhood illnesses Alice in Wonderland syndrome and acute cerebellar ataxia, and on the basis of some evidence has been linked to certain autoimmune diseases, particularly dermatomyositis, systemic lupus erythematosus, and arthritis. Increased risk of developing rheumatoid arthritis and Sjögren's syndrome. About 200,000 cancers annually worldwide are believed to be due to his EBV(9). In 2022, a large study (10 million people over the age of 20) suggested that EBV is the main cause of multiple sclerosis. Her recent EBV infection puts her at increased risk of developing multiple sclerosis by oral infection of saliva and genital secretions 32 times.

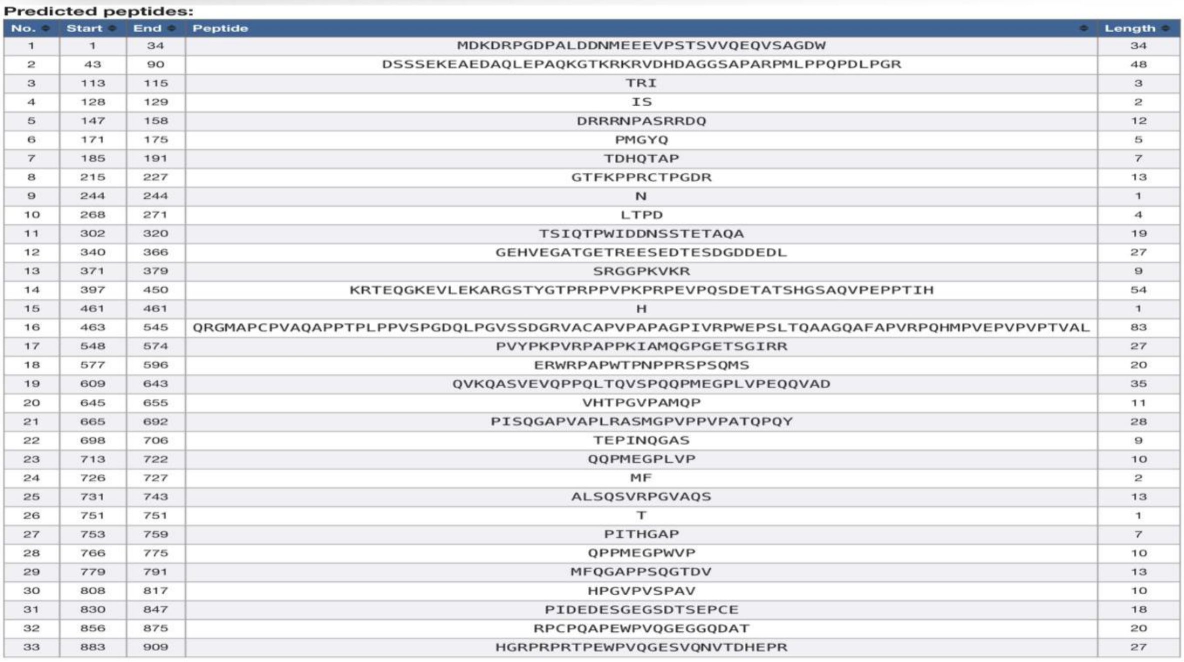
MATERIALS AND METHOD:

The amino acid sequence of Epstein–Barr Virus Nuclear Antigen 3 (EBNA3) was retrieved from a publicly available protein database and used for computational analysis. Intrinsically disordered regions (IDRs) within the protein sequence were identified using the Predictor of Natural Disordered Regions (PONDR) server. Regions exhibiting disorder scores above the threshold value of 0.5 were considered intrinsically disordered and selected for further investigation.

The identified disordered regions were subsequently analyzed for the prediction of linear B-cell epitopes using immunoinformatics-based epitope prediction tools. Predicted epitopes were evaluated based on their amino acid sequence, residue position, and peptide length. Epitopes located within highly disordered regions were shortlisted as potential immunogenic candidates. The disorder profile and epitope distribution were visualized and analyzed to determine the relationship between intrinsic disorder and antigenic potential in EBNA3.

RESULT:

Totally 33 peptides were identified, ranging from 1 to 83. Of these, four peptides were identified between the length 15 to 20 mers that could be highly immunogenic. Among the four peptides three were present in the disordered region.



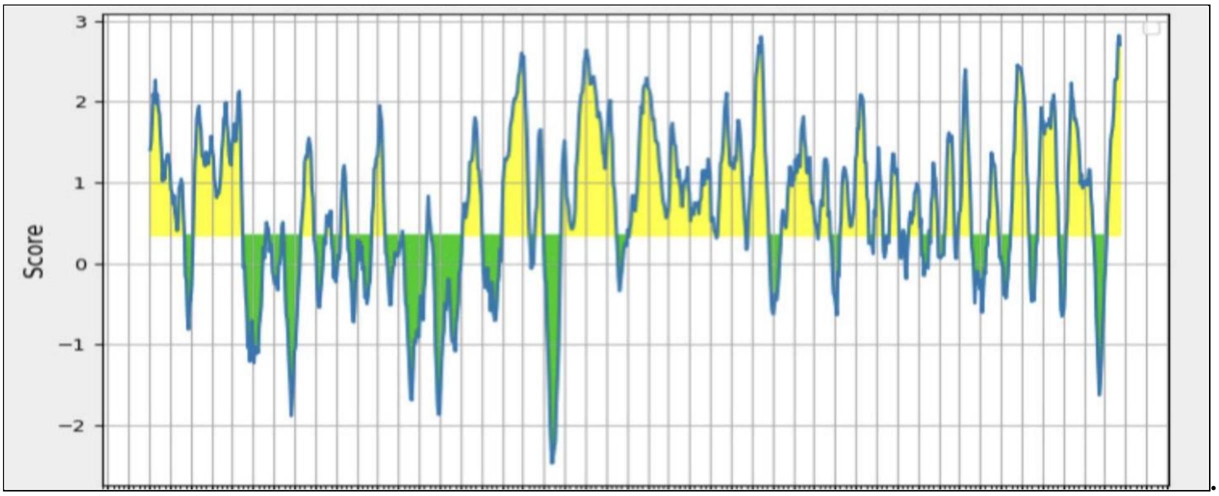
Predicted peptides:

No.	Start	End	Peptide	Length
1	1	34	MDKDRPGDPALDDNMEEEVPSTSVVQEQVSAGDW	34
2	43	90	DSSSEKEAEDAQLEPAQKGTKRKRVDHDAGGSAPARPMLPPQPDLPGR	48
3	113	115	TRI	3
4	128	129	IS	2
5	147	158	DRRRNPASRRDQ	12
6	171	175	PMGYQ	5
7	185	191	TDHQTAP	7
8	215	227	GTFKPPRCTPGDR	13
9	244	244	N	1
10	268	271	LTPD	4
11	302	320	TSIQTPWIDDNSSTETAQA	19
12	340	366	GEHVEGATGETREESEDTESDGDDEDL	27
13	371	379	SRGGPKVKR	9
14	397	450	KRTEQGKEVLEKARGSTYGTPRPPVPKPRPEVPQSDETATSHGSAQVPEPPTIH	54
15	461	461	H	1
16	463	545	QRGMAPCPVAQAPPTPLPPVSPGDQLPGVSSDGRVACAPVPAPAGPIVRPWEPSLTQAAGQAFAPVRPQHMPVEPVPVPTVAL	83
17	548	574	PVYPKPVRPAPPKIAMQGPGETSGIRR	27
18	577	596	ERWRPAPWTPNPPRSPSQMS	20
19	609	643	QVKQASVEVQPPQLTQVSPQQPMEGPLVPEQQVAD	35
20	645	655	VHTPGVPAMQP	11
21	665	692	PISQGAPVAPLRASMGPVPPVPATQPQY	28
22	698	706	TEPINQGAS	9
23	713	722	QQPMEGPLVP	10
24	726	727	MF	2
25	731	743	ALSQSVRPGVAQS	13
26	751	751	T	1
27	753	759	PITHGAP	7
28	766	775	QPPMEGPWVP	10
29	779	791	MFQGAPPSQGTDV	13
30	808	817	HPGVPVSPAV	10
31	830	847	PIDEDESGEGSDTSEPCE	18
32	856	875	RPCPQAPEWPVQGEGGQDAT	20
33	883	909	HGRPRPRTPEWPVQGESVQNVTDHEPR	27

Figure 1. Predicted linear B-cell epitopes identified within the intrinsically disordered regions of EBNA3.

Disordered segment	Average strength
1-87	0.908
140-165	0.8753
337-514	0.9222
525-649	0.9062
658-663	0.5803
665-698	0.8478
706-713	0.6375
791-794	0.5176
804-819	0.7705
826-879	0.7674
889-906	0.6534
924-935	0.8989

Figure 2. Predicted intrinsically disordered regions of EBNA3 with their corresponding disorder



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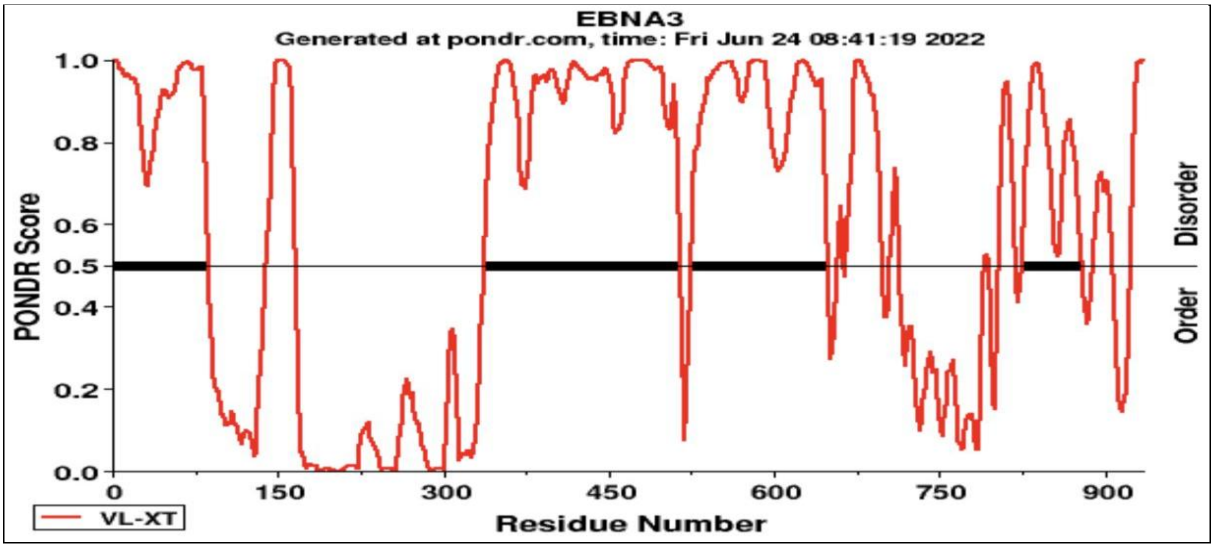

Figure 3. PONDR disorder profile showing residue-wise intrinsic disorder distribution across the EBNA3 protein sequence.

DISCUSSION:

Epstein–Barr Virus Nuclear Antigen 3 (EBNA3) plays a crucial role in viral latency, host-cell transformation, and immune modulation. Identification of B-cell epitopes within intrinsically disordered regions (IDRs) is particularly important because these regions lack a fixed three-dimensional structure, making them highly flexible and accessible to immune recognition. In the present study, computational analysis revealed multiple disordered segments throughout the EBNA3 protein, indicating that a substantial portion of the antigen may participate in host–virus interactions and immune responses.(10)

The disorder prediction analysis identified several highly disordered regions, notably residues 1–87, 337–514, 525–649, and 924–935, with average disorder scores exceeding the accepted threshold. These regions are enriched in polar and charged amino acids, characteristics commonly associated with intrinsically disordered proteins. Such regions are known to facilitate molecular recognition, signaling, and regulatory interactions, thereby contributing to viral persistence and pathogenesis.(11)

Prediction of linear B-cell epitopes from these disordered segments yielded several peptide candidates with varying lengths and sequence compositions. The localization of predicted epitopes within highly disordered regions supports previous observations that intrinsically disordered domains often harbor immunodominant antigenic determinants. Their structural flexibility enhances solvent exposure, increasing the likelihood of antibody binding and recognition by the host immune system.(10,12)

The identified peptides may serve as promising candidates for peptide-based diagnostics and vaccine development against Epstein–Barr virus infection. Computational epitope prediction offers a rapid and cost-effective approach for screening large viral proteins and narrowing down regions of immunological significance before experimental validation. Nevertheless, in vitro and in vivo studies are necessary to confirm the antigenicity, specificity, and immunogenic potential of the predicted epitopes.(13)

Overall, the findings suggest that intrinsically disordered regions of EBNA3 contain several potential B-cell epitopes that may contribute to immune recognition of Epstein–Barr virus. The overlap between highly disordered segments and predicted antigenic peptides highlights the importance of structural disorder in shaping viral immunogenicity. These results provide a foundation for future experimental studies aimed at developing epitope-based diagnostic tools and immunotherapeutic strategies targeting EBV-associated diseases.

CONCLUSION:

This study successfully identified several potential B-cell epitopes within the intrinsically disordered regions of Epstein–Barr Virus Nuclear Antigen 3 (EBNA3) using computational prediction tools. The overlap between highly disordered segments and predicted antigenic peptides suggests that these regions play an important role in immune recognition. The identified epitopes may serve as promising candidates for the development of peptide-based diagnostic and vaccine strategies against Epstein–Barr virus. Further experimental validation is required to confirm their immunogenicity and evaluate their potential applications in EBV-associated disease management.

Our study has identified important and highly immunogenic B cell epitopes within the intrinsically disordered regions. Such peptides could have profound effects on the cellular metabolism and gene expression.

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