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RESEARCH ARTICLE

STUDY OF HYDROPHOBICITY, SURFACE ACCESSIBILITY, AND ANTIGENICITY OF PROTEIN FROM DRACUNCULIASIS

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ABSTRACT

To investigate the surface accessisbility , antigenecity and hydrophobicity a protein sequence of 527 amino acids were taken from Dracunculus medinensis and in- depth study were conducted through various B-cell epitopes prediction methods, hydrophobicity prediction methods which provides the highest accurate outcomes. In course of the investigation we detected the maximal hydrophilicity region that could be an antigenic site, which also posses hydrophobic characteristics, because of well studied fact that an antigenic protein's terminal region is likely unstructured and solvent accessible and moreover antibodies against this regions are likely to discern the native protein. We also predicted the antigenicity capacity of 527 amino acid sequence protein and found more antigenic and this protein segment can take active role in immunity of host and can effectively illustrate responses of immune of host. Along with other investigation of protein we also investigated protein's Solvent accessibility, protein residues' polarity to identify the regions which is exposed on the surface of proteins because it is essential point to focus because it can led to detect out the potential target active site and for development of the specific targeted drugs for cure.

INTRODUCTION

Recent proteomics methodology has brought a new path view in arena of protein analysis which has opened a new window to comprehend the cell function and the cellular level changes which are generally involved in disease states, and also contribute for identification of new therapeutic targets. In following study we applied bioinformatics tools with a highest accuracy to identify the hydrophobicity, surface accessibility and the antigenicity of the protein which can contribute to identify the essential targets for the drug targets. As it is known that in the process of the protein folding the primary force is compelled by the hydrophobicity. Preferentially, a hydrophobic residues present in core and the polar residues precisely present at the surface of folded protein (Kauzmann, 1959; Tanford, 1978; Moelbert, Emberly, & Tang, 2004). Study also reveals that, hydrophobic residues tend to be present in the core of protein, where solvent accessibility is high (Rose, Geselowitz, Lesser, Lee, and Zehfus, 1985; Miller, Janin, Lesk, and Chothia, 1987; Lesser, and Rose, 1990; Lins, Thomas, and Brasseur, 2003). On average, there is an existence of the correlation among hydrophobicity and surface exposure (Chothia, 1974; Rose, *et al*., 1985; Miller, Janin, Lesk, and Chothia, C. 1987).

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To study the protein hydrophobicity, surface accessibility and the antigenicity, a protein of 527 amino acid sequences from Dracunculiasis causing agent were considered and analyzed. Dracunculiasis, is nematode caused illness. This infection catches hold by the individuals once they consumed contaminated water with *Dracunculus medinensis*. This organism has an unique way of multiplication, without awareness and asymptomatic way it grow and mature within host and stay for long time to complete its incubation period of one to one and half years or more (Greenaway, 2004). A millions of eggs were released from the female uterus after the copulation whereas male nematode dies. A painful blister triggered by the released larvae and this blister can be seen specifically on lower limbs (Molyneux, D. Hopkins, N. Zagaria., 2004). An infected individual experiences some symptoms after onset of the infection on later on stages like fever, redness of skin, swelling, diarrhoea, nausea, vomiting and dizziness and severe pruritus around the blister . These larvae gets released in the environment after bursting of the blister in very opportunistic way, whenever a infected person comes in contact with water and immerses their infected body parts in water this organisms senses the temperature differences of the environment and comes out and again contribute to the second cycle of the infection (Muller, 1971; Muller, 1979; Ruiz-Tiben, Hopkins, 2006; Iriemenam, Oyibo, Fagbenro-Beyioku, 2008). Based on the fact that a single peptide epitope can trigger immune, *D. medinensis* antigen

peptides can hopefully contribute to develop a subunit synthetic vaccine. The finding of the antigenic peptide from target organism can play essential part in prototype synthetic vaccine development and also helps in validation of target.

MATERIALS AND METHODS

B-cell epitopes are the sites of molecules that are recognized by antibodies of the immune system. Knowledge of B-cell epitopes may be used in the design of vaccines and diagnostics tests. Therefore, it is a prime interest area to develop an improved method for predicting B-cell epitopes (Larsen, Lund, Nielsen, 2006; Mishra Sonu and Virendra S. Gomase 2015; Mishra Sonu and Virendra S. Gomase 2015; Mishra Sonu andVirendra S. Gomase 2015).

Selection and Retrieval of protein sequences

To initiate any investigation the first essential part is data searching and collection. In order to continue the investigation a 527 amino acids protein sequence of NADH dehydrogenase subunit 5 from *Dracunculus medinensis* was retrieve from www.ncbi.nlm.nih.gov, UniProtdatabases (http://www.ncbi.nlm.nih.gov; Sayers, *et al*., 2012; Bairoch, *et al*., 2005).

Prediction of antigenic peptide

Antibodies are most powerful tool because of its multiple applications in clinical research. Antibodies recognizes the antigens in linear or native three-dimensional epitopes, but due to few technical constraint a whole native protein availability for Abs to identify 3D epitopes as immunogens are not possible .Henceforth, peptide induced immunization is one of the suitable alternative techniques and the Abs produced through this methods identify the linear epitopes. To detect the antigenic peptide we used Kolaskar and Tongaonkar (1990) prediction tool that predicts specific segments from protein sequences that can illustrate Abs response and likely to be antigenic and the accuracy level of this approach is 75 percentage which is quite high accuracy that can be used for prediction. In course of investigation we applied various approaches like Hopp and Woods, Welling, Parker, Bepipred, Kolaskar and Tongaonkar antigenicity methods (Hoop, &Woods, 1978; Welling, Weijer, van der Zee Welling,-Wester 1985; Parker, Bednarek, Coligan, 1994; Jens,Erik., Pontoppidan, Larsen, Ole Lund and Morten, Nielsen., 2006; Kolaskar, Tongaonkar, 1990).

Solvent Accessible Regions

A protein or peptide sequence to be antigenic the condition it needs to meet that an antigenic peptides should be located in solvent accessible region and should posses both hydrophilic and hydrophobic residue .Therefore, it's important to find the solvent accessible region of the protein or peptide segments. To analyzed the solvent accessible regions of proteins that posses richest probability that a provided protein region lies in on the surface of a protein. Surface Accessibility, backbone or chain flexibility is predicted through Emini *et al*., (Emini, Hughes, Perlow, D.S., Boger, 1985) and Karplus and Schulz (Karplus, and Schulz, 1985). 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*. (1983), Kyte & Doolittle (1982), Abraham & Leo (19987), Bull and Breese (1974), Guy (1985), Miyazawa, *et al* (1985), Roseman (1988), Wolfenden *et al*. (1981), Wilson *et al*. (1981), Cowan (1990), Chothia (1976) (Sweet and Eisenberg, 1983; Kyte, Doolittle, 1982; Abraham, Leo, 1987; Bull, Breese, 1974; Miyazawa, Jernigen, 1985; Roseman, 1988; Wolfenden, Andersson, Cullis, Southgate, 1985; Wilson, Honegger, Stotzel, Hughes, 1981; Cowan. Whittaker, 1990; Chothia, 1976).

RESULTS AND INTERPRETATIONS

The *Dracunculus medinensis* protein, contain a long residue of 527amino acids with 519nonamers.

MDVYIYWFGVILLCFLLLFVFFYDDFVFSLDFSSLELLQFQFRL
DWFSFSFFCLLVMVVGSVVVYSGFYMMEDYNFNYFCVVLSIF
VFSMVGVVFSNNCISMLIFWDLLGVSSYFLVLYYGNWDSCSG
SMNTVMMNRVGDVCVFLVFCGLFFMGIDFISLELVVSVALFF
FILSTFTKSAQYPFSSWLPKAMSAPTPVSALVHSSTLVTAGLFL
GMCFSEVMFLDFVLDFMFFVGLFTMFSSGLMAYFEFDIKKLV
ALSTLSQIGFCFFGLGLGLVYFSFIHMLSHAVFKSCLFMQMGYI
IHLCGGQQDSRGYVGVGGLSSVVYIQTFVSLMCLCGLFFLGGS
VSKEILLEHYFFCNWSLFLVFLFFFSILLTYLYCYRLMKGFYYY
CSSSLFYSGGSLVFSFVSLVLVVFSIVFLWWLNYNSFVISSSLL
YSDYYSLFYLFILGLVLCVVFFKFGSFDVKYKFYGDLLPKVIIR
GNYVVKWSDCMVDYSIMKFGDFSFYVSKIFVMGFSGKEINFL
FLCLFLLLMI

Prediction of Antigenic Peptides

In this study, we found the antigenic determinants by finding the area of greatest local hydrophilicity. The Hopp-Woods scale Hydrophilicity Prediction Result Data found high in position: 308 with maximum score: 0.633 and sequence is 305- GGQQDSR-311 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 (Fig.: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 prediction result data found high in Position: 257 with high Score: 0.742 (max) and the sequence is 254-FFCNWSL-260 (Fig.:2).We also predicted the hydrophobicity plot of HPLC / Parker hydrophilicity prediction scale and the result data found i.e., the maximum predicted residues at the position: 308 (Residue : Q) with maximum score: 6.3 and sequence is 305-GGQQDSR-311; and the other highest peak found at position:309 (Residue: D) with maximum score:6.3 and sequence is 306- GQQDSRG-312(Fig.: 3), BepiPred predicts the location of linear B-cell epitopes and predicted result found at position 309(Residue: D) with Max Score:1.369 and sequence is found 12 amino acid long that is 306-GQQDSRGYVGVG-317(Fig.4), Kolaskar and Tongaonkar antigenicity methods (Fig.: 5 & 5a) predicted peptides and the result found are listed in table:1(Refer table for predicted antigen sequences). The predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design.

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. Emini *et al*., (Fig.:6) predicts the highest probability i.e. found in position:

308 (Residue:Q) i.e 306- GQQDSR-311, and in the position :309 (Residue:D) i.e., 307- QQDSRG -312, that a given protein region lies on the surface of a protein and are used to identify antigenic determinants on the surface of proteins.

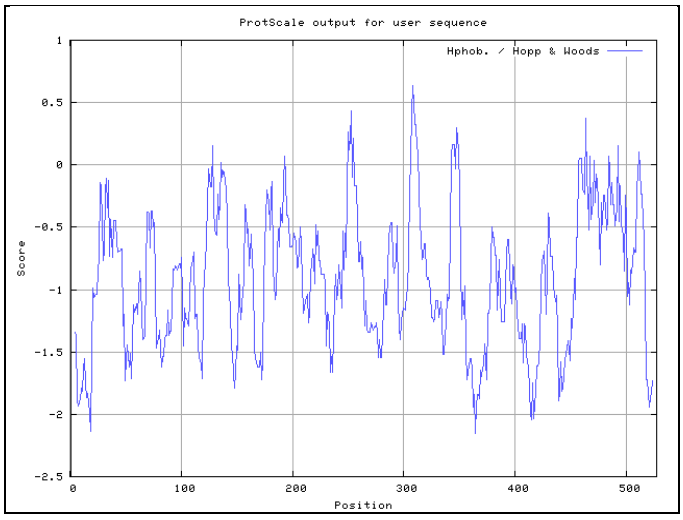

Fig. 1. Hydrophobicity plot of Hopp and Woods (1981) of protein

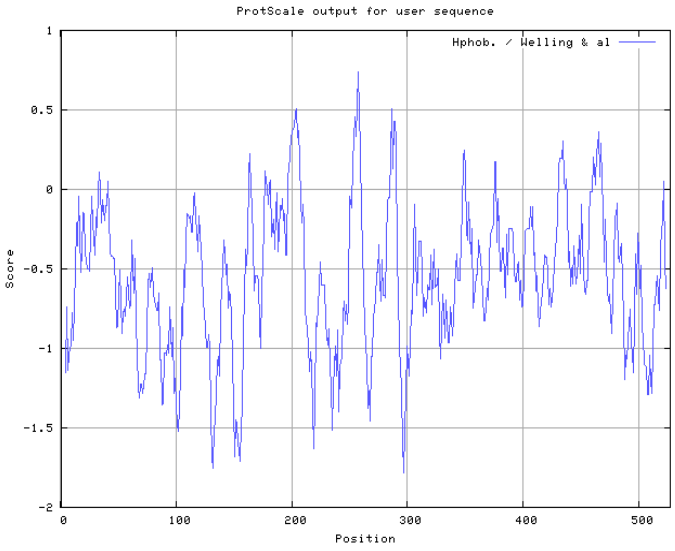

Fig. 2. Hydrophobicity plot of Welling *et al.* (1985) of protein

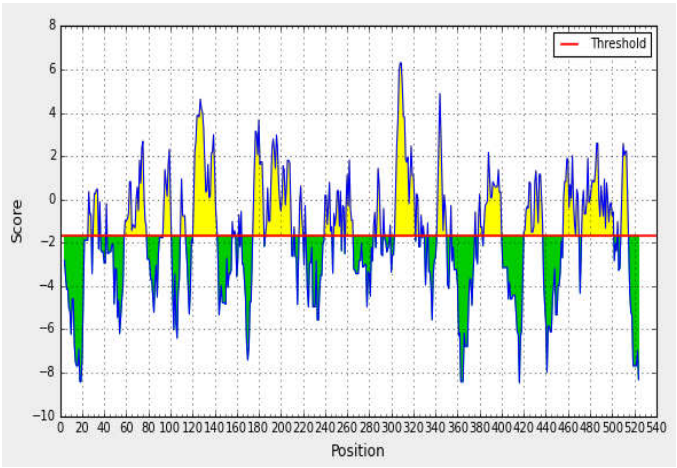

Fig. 3. Hydrophobicity plot of Parker *et al.* (1986) of protein

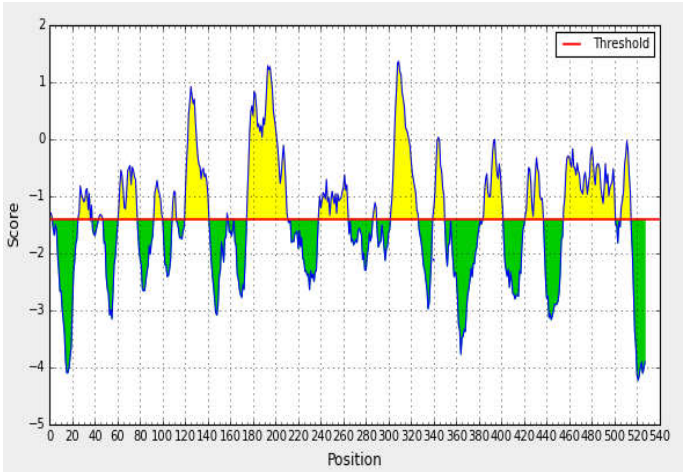

Fig. 4. Bepipred Linear Epitope Prediction plot of protein

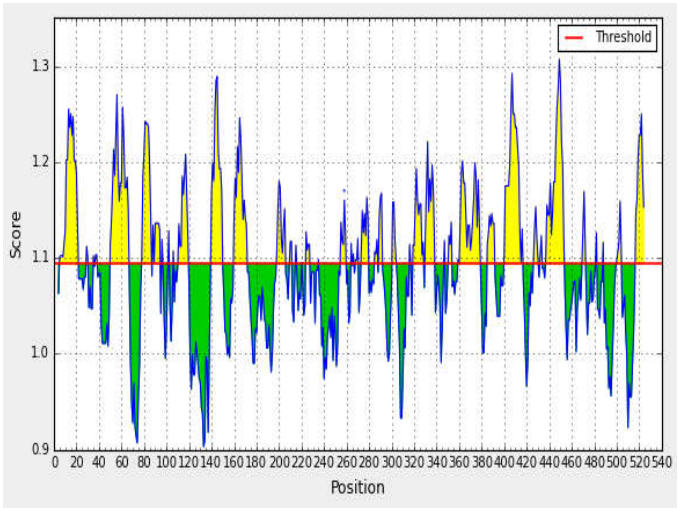

Fig. 5. Kolaskar and Tongaonkar antigenicity plot of protein

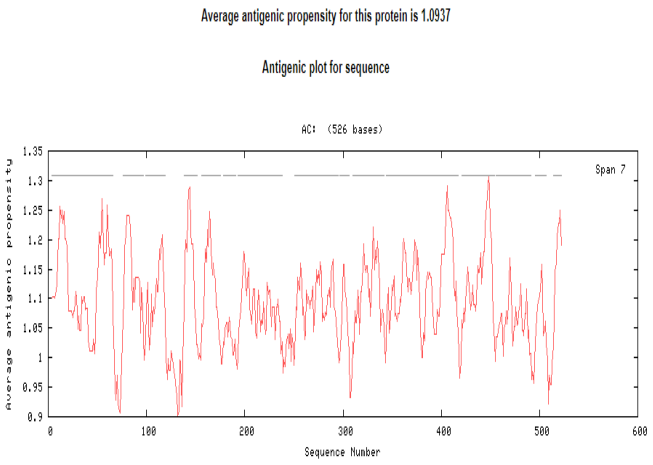

Fig. 5a. Kolaskar and Tongaonkar antigenicity plot, the average antigenic propensity for protein is 1.0937

Karplus and Schulz (Fig.: 7) High score i.e. found highest score: 1.122 at position: 308 (Residue:Q) i.e 305-GGQQDSR-311. Predict backbone or chain flexibility on the basis of the known temperature B factors of the alpha-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

Table 1. List of 15 antigenic determinants of protein

There are 15 antigenic determinants in protein sequence			
n	Start Position	Sequence	End Position
1	4	YIYWFGVILLCFLLLFVFFYDDFVFSLDFSSLELLQFQFRLDWFSFSFFCLLVMVVGSVVVYS	66
2	76	FNYFCVVLSIFVFSMVGVVFSN	97
3	99	CISMLIFWDLLGVSSYFLVLY	119
4	138	VGDVCVFLVFCGLFF	152
5	156	DFISLELVVSVALFFFILST	175
6	178	KSAQYPFSSWLPKA	191
7	193	SAPTPVSALVHSSTLVTAGLFLGMCFSEVMFLDFVLDFMFFVGLFT	238
8	251	DIKKLVALSTLSQIGFCFFGLGLGLVYFSFIHMLSHAVFKSCLFM	295
9	297	MGYIIHLCGG	306
10	310	SRGYVGVGGLSSVVYIQTFVSLMCLCGLFFLGG	342
11	344	VSKEILLEHYFFCNWSLFLVFLFFFSILLTYLYCYRLMKGFYYYCSSSLFYSGGSLVFSFVSLVLVVF SIVFLW	417
12	421	YNSFVISSSLLYSDYYSLFYLFILGLVLCVVFFK	454
13	456	GSFDVKYKFYGDLLPKVIIRGNYVVKWSDCMVDYSI	491
14	496	DFSFYVSKIFVM	507
15	514	INFLFLCLF	522

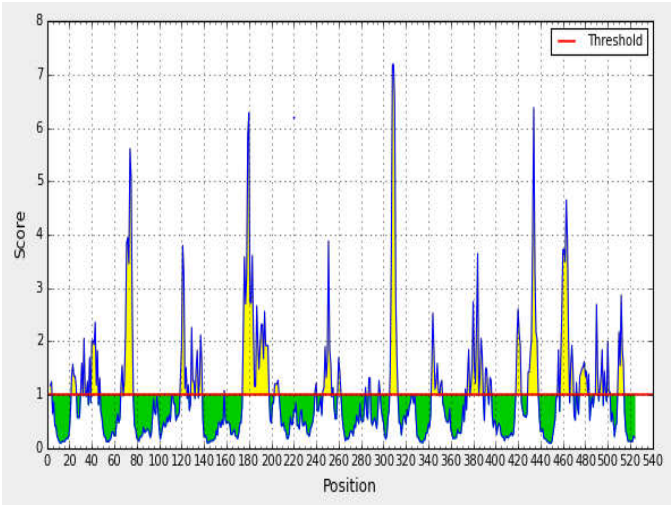

Fig. 6. Emini Surface Accessibility Prediction plot of protein

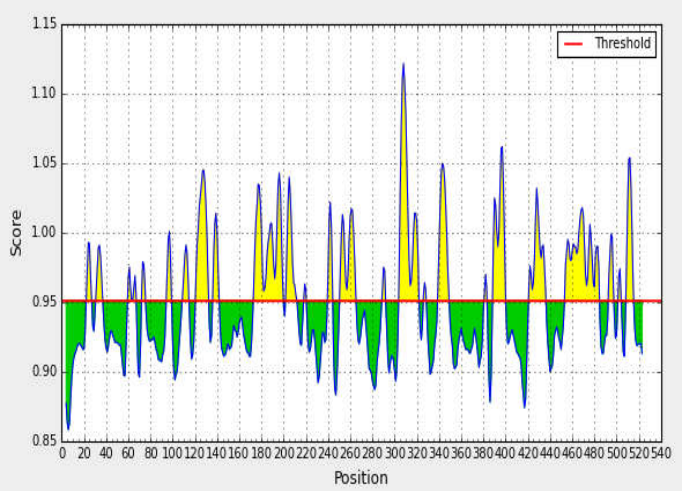

Fig. 7. Karplus & Schulz Flexibility Prediction of protein

prediction result data found high at position: 364, with Max score:1.574 (Sequence is 361-FLVFLFF-367) (Fig. 8), Kyte & Doolittle result high at Position: 14, Score: 3.667 (max) (Sequence is 11- ILLCFLL-17)(Fig. 9), Abraham & Leo result high at Position: 364 ; Score: 2.423 (max) (Sequence is 361-FLVFLFF-367) (Fig.10), Bull and Breese result high at Position: 308; Score: 0.717 (max) (Sequence is 305-GGQQDSR -311) (Fig.11), Miyazawa result high at

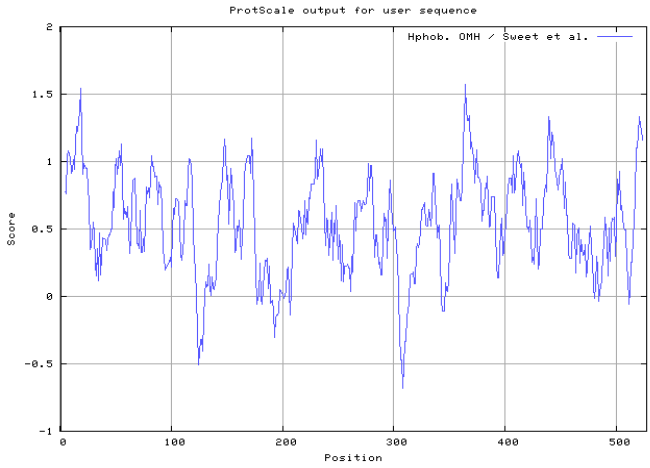

Fig. 8. Hydrophobicity plot of Sweet *et al.* (1983) of protein

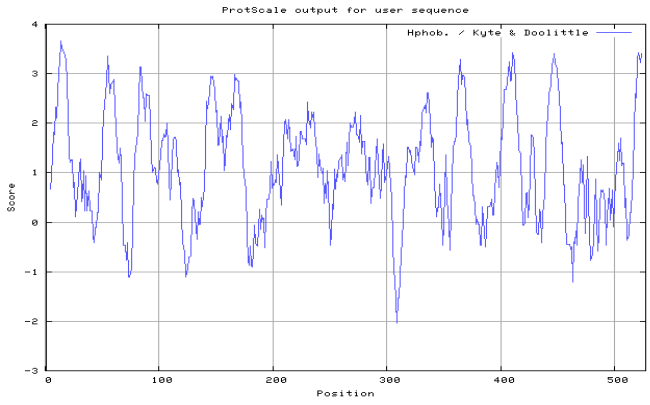

Fig. 9. Kyte & Doolittle hydrophobicity plot of protein

Position: 364; Score: 8.688 (max) (Sequence is 361-FLVFLFF -367) (Fig. 12), Guy result high at position: 309 Score: 0.654 (max) (Sequence is 306- GQQDSRG -312) (Fig. 13), Wolfenden result high at Position: 447; Score: 1.790 (max) (Sequence is 444- LGLVLCV -450) (Fig.14), Roseman result high at Position: 364; Score: 2.012 (max) (Sequence is 361- FLVFLFF -367) (Fig. 15), Wilson et Position: 364; Score: 7.022 (max) (Sequence is 361- FLVFLFF -367) (Fig. 16), Cowan at Position: 364; Score: 1.716 (max) (Sequence is 361- FLVFLFF -367(Fig.17), Chothia at Position: 145 ; Score: 0.492 (max) (Sequence is 142- CVFLVFC -148) (Fig. 18).

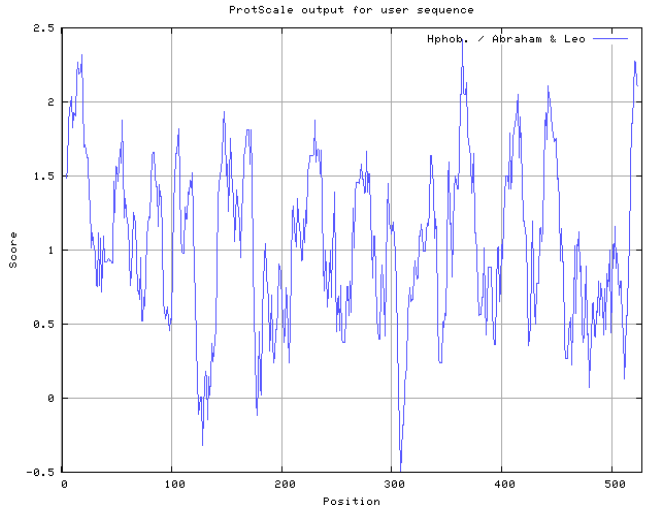

Fig. 10. Abraham & Leo hydrophobicity plot of protein

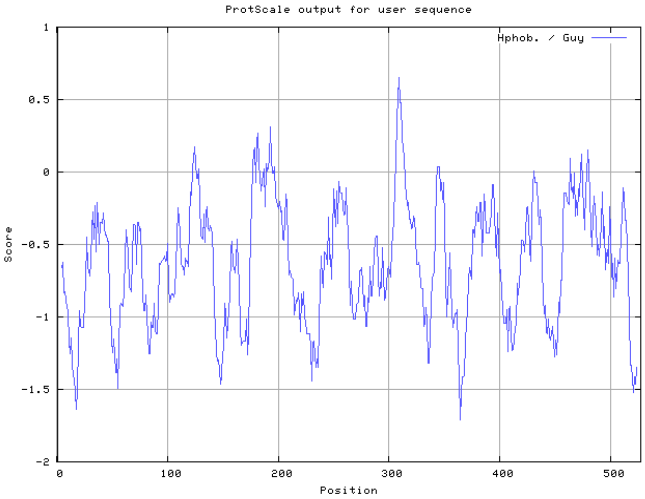

Fig. 13. Hydrophobicity plot of Guy (1988) *of protein*

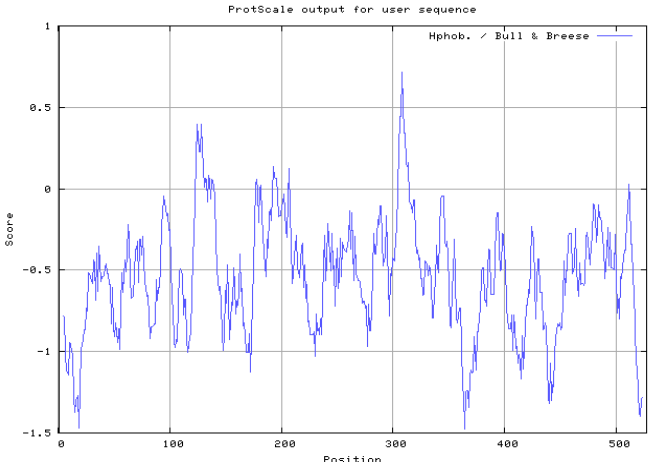

Fig. 11. Bull & Breese use surface tension to measure of protein

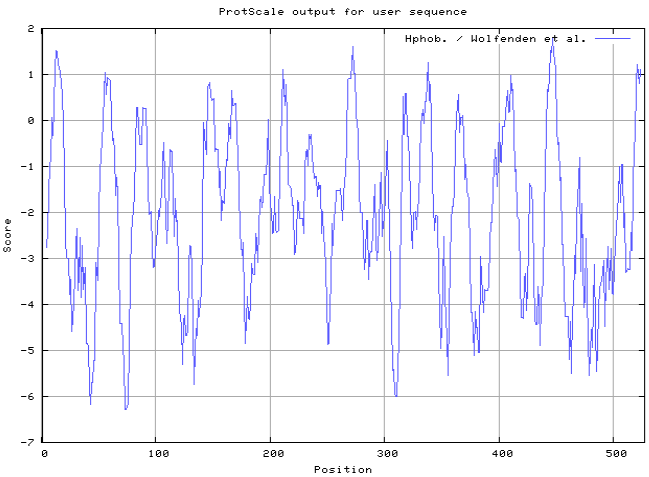

Fig. 14. Hydrophobicity plot of Wolfenden *et al.* (1981)

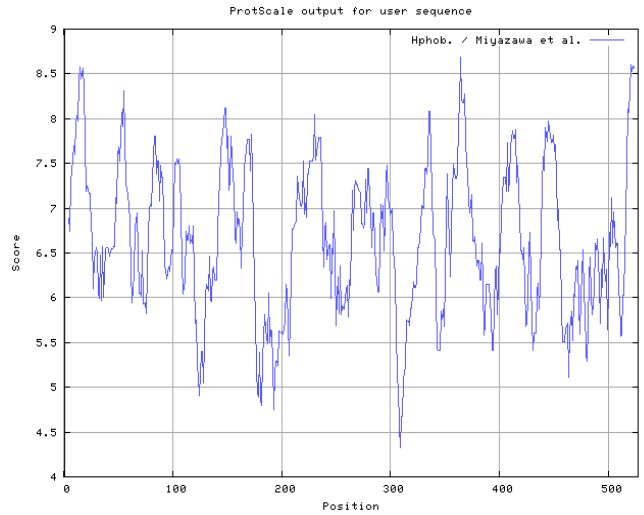

Fig. 12. Hydrophobicity plot of Miyazawa *et al.* (1985) of protein

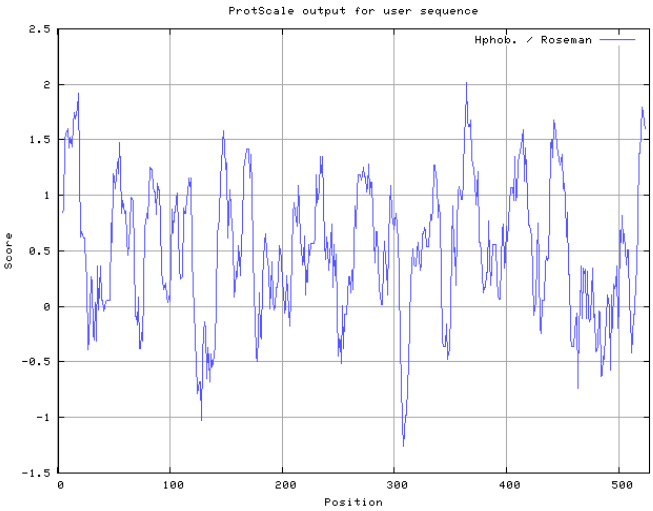

Fig. 15. Hydrophobicity plot of Roseman M.A. (1988) of protein

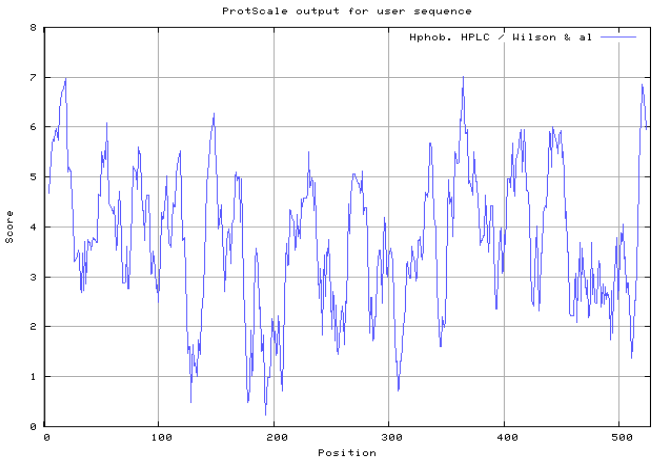

Fig. 16. Hydrophobicity/HPLC plot of Wilson & al (1981)

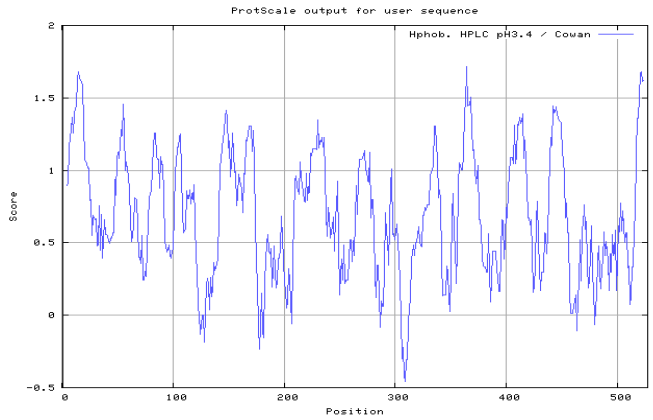

Fig. 17. Hydrophobicity/HPLC pH 3.4/ plot of Cowan (1990)

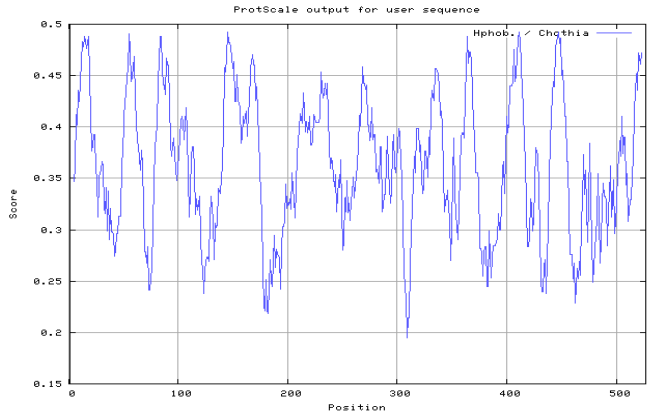

Fig. 18. Hydrophobicity plot of Chothia (1976)

DISCUSSION

The major gal of this study was to in depth study of the protein hydrophobiclity, Hydrophilicity, surface accessibility and the protein antigenicity. Diagnosis of the disease is the not the accurate treatment of any disease. The treatment methods that are used should be efficient enough to cure the disease completely and should not posses any side- effect to patients.

In order to develop any drug or vaccine it's essential to study the chemical properties and activity in proper way. To identify the potential antigenic site with the highest hydrophilic point in protein Hopp and woods hydrophobicity scale were used and the obtained score found to be high in position: 308 with maximum score: 0.633 and sequence is 305- GGQQDSR-311 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. Welling *et al*., utilizes information on the relative occurrent of amino acids in antigenic site to attain a scale which is useful for prediction of antigenic point and the predicted data found high in Position:257 with high Score:0.742 (max) and the sequence is 254-FFCNWSL-260.

We also study Hydrophobicity plot of HPLC / Parker Hydrophilicity prediction result data found i.e., the maximum predicted residues at the Position: 308(Residue:Q) With maximum score:6.3 and sequence is 305- GGQQDSR-311; and the other highest peak found at position:309 (Residue: D) with maximum score:6.3 and sequence is 306- GQQDSRG-312(Fig.: 3), BepiPred predicts the location of linear B-cell epitopes result found at position 309(Residue: D) with Max Score:1.369 and sequence is found 12 amino acid long that is 306-GQQDSRGYVGVG-317 (Fig.4), Kolaskar and Tongaonkar antigenicity methods (Fig.: 5) predicted peptides result found are listed in table:1(Refer table for predicted antigen sequences). The predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design. The obtained outcomes of analysis determined probable the antigenic site in protein and this also infer that the hydrophobic residues occurrence probability on the surface of a protein are most probably to be a portion of the site of antigenicity .

Conclusion

The purpose of the above investigation concludes that the predicted antigenic peptide fragments posses the antigenicity properties and this can play an essential part in vaccine development process. Overall, the obtained outcomes are encouraging, both the 'sites of action' and 'physiological functions' can be predicted with very high accuracies helping minimize the number of validation experiments. Furthermore, this protein antigenic peptide fragment can be further utilizes to investigate nonamer for use in rational vaccine design and can help to develop the understanding of roles in the immune system in infectious disease.

Abbreviations

GWD: Guinea worm disease
UniProt: The Universal Protein Resource
NCBI: National Center for Biotechnology Information
Abs: Antibodies

Conflicts of Interest

The authors declare no conflict of interest.

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