BioCore Exploring Scientific Community
International Journal of Vaccines and Immune System

ISSN 2475-6326

Research Article Open Access

Study of Solvent Accessible Regions ,Hydrophobicity and Antigenicity of NADH Dehydrogenase subunit 3

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Citation: Sonu Mishra et al. (2016), Study of Solvent Accessible Regions, Hydrophobicity and Antigenicity of NADH Dehydrogenase subunit 3 Int J Vac & Im Sys. 1:1, 7-13. DOI: 10.25141/2475-6326-2016-1.0007

Received: December 12, 2016; **Accepted:** December 16, 2016; **Published:** December 20, 2016

Abstract

In this study NADH dehydrogenase subunit 3 (mitochondrion) protein has been used to investigate its role in antigenicity. NADH dehydrogenase subunit 3 (mitochondrion) protein sequences (111 aa protein) is analyzed through different types B- cell epitope prediction methods. We found that the region of maximal hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because the terminal regions of antigen protein is solvent accessible and unstructured, antibodies against those regions are also likely to recognize the native protein. It was seen that an antigen protein is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility. The predicted antigenic protein segments of NADH dehydrogenase subunit 3 (mitochondrion) can take active part in the host immune reactions. In future study the predicted antigenic protein NADH dehydrogenase subunit 3 (mitochondrion) fragments can be used in the investigation of MHC molecules binding and it can be the first bottlenecks in vaccine design.

Key words : Antigen, Epitope, Protein, NADH dehydrogenase subunit 3 (mitochondrion)

Introduction

NADH dehydrogenase subunit 3 (mitochondrion) protein sequences from *Dracunculus medinensis* has been taken for the investigation of hydrophobicity and the antigenicity. *Dracunculus medinensis* causes Dracunculiasis , transmitted through drinking of contaminated water infected with copepod Cyclops (intermediate host). Dracunculiasis has been known to humankind since antiquity. Guinea worm the largest tissue parasite with unusual life cycle with incubation period of the approximately more than a year with six developmental stages. This one of the most neglected tropic parasite which exhibit clinical importance and needs to be eradicated after small pox [Greenaway C.2004]. Mature and adult female after the copulation produces millions of eggs in its uterus, and is predominantly localized in the lower extremities (80-90%). After an incubation period the female worm release the larvae which induces a painful blister (1 to 6cm diameter) on the skin of lower limbs; the person develop a slight fever , local skin redness , swelling and severe pruritus around the blister . Other symptoms include diarrhoea, nausea, vomiting and dizziness. The severity of the wound infections in the infected individual led to a more complications such as redness and swelling of the skin (cellulitis), boils (abscesses), generalized infection (sepsis), joint infections (septic arthritis) that can cause the joints to lock and deform (contractures), lock jaw (tetanus). The blister burst within 1 to 3 days and female worms one or more slowly comes out from the wounds which causes an excoriating burning sensation and pain [Mullner et al., 1971; Muller,R.1979].The pouring water over the blister provide pain relief . But this the moment that adult female is exposed to the external environment [Ruiz-Tiben et al., 2006]. During emergence of the limbs in open water sources it recognizes the temperature difference and releases the milky white liquid in the water which contains millions of immature larvae, when larvae released in water are ingested by copepods where they mount twice and become infective larvae within two weeks [Iriemenam et al., 2008]. The *D. medinensis* antigen peptides can be most desirable segment for the subunit vaccine development because with the single epitope, the immune response can be generated in large population. This approach is usually based on the phenomenon of cross-protection, whereby infected with the mild strain and is pro-

tected against a more severe strain of the same. The phenotype of the resistant transgenic hosts includes fewer centers of initial infection, a delay in symptom development and low accumulation. In this study NADH dehydrogenase subunit 3 (mitochondrion) 111amino acid sequence protein has been used to investigate its role in antigenicity. NADH:ubiquinone oxidoreductase (complex I) is a respiratory-chain enzyme. This catalyses and transfer two electrons from NADH to ubiquinone in a reaction which is associated with proton translocation across the membrane (NADH + ubiquinone = NAD+ + ubiquinol).The major source of reactive oxygen species (ROS) is Complex. The occurrence of the Complex I is observed in bacteria, cyanobacteria (as a NADH-plastoquinone oxidoreductase), archaea, mitochondria, and in the hydrogenosome, a mitochondria-derived organelle. The researcher found that defects in the mitochondrial structural and molecular are involved in the progression of hepatic steatosis pathogenesis which is due to the accumulation of an excess amount of triglycerides and other fats inside liver cells which results in abnormal hepatic lipid metabolism. The mitochondrial-encoded NADH dehydrogenase (MT-ND), and the hepatic methylation and transcriptional activity play a critical role in the non-alcoholic fatty liver disease (NAFLD) progression. Furthermore, the influence expression of MT-ND3 can also be observed for hypoxia, oxidative stress, and lipotoxicity and thus may play a role in the progression of hepatic steatosis [Wang et al., 2014]. The inherited neurometabolic disorders is the one of the most common disorder found in childhood, minimum 1 in 7500 live births in case of the mitochondrial respiratory chain diseases. Whereas, the isolated complex I deficiency is also the most common respiratory chain disorder observed in children, which interns results in organ-specific or multisystem disease, but most frequently present as Leigh syndrome, and the cause is due to mitochondrial DNA mutations. It has been identified that the pathogenic point mutations in the MTND3 gene - including m.10191T>C (p.Ser45Pro) [Nesbitt et al., 2012]. The mutation in complex I subunit genes in the respiratory chain also leads to the disorder like “Mitochondrial Encephalopathies” [Werner et al., 2009]. The exhaustive investigation indicates that the Leber hereditary optic neuropathy and dystonia (LDYT) is generally associated with the mitochondrial disorder, occasionally noticed as variable combinations of vision loss and progressive generalized dystonia. The unique oxidative phosphorylation disorder is LDYT, caused by mutations in mitochondrial ND6 or ND4 gene [Wang et al., 2009; Li YJ et al., 2014] .Antigen protein prediction from *D. medinensis* is necessary for few paradigms of synthetic vaccine development and target validation.

II. METHODOLOGY

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. It is therefore of interest to develop improved methods for predicting B-cell epitopes [Larsen, et al.,2006]. In this research work, antigenic epitopes of antigen protein NADH dehydrogenase subunit 3 (mitochondrion) from *D.medinensis* is determined using the Gomase in 2007, Bepipred Linear Epitope Prediction, Emini Surface Accessibility Prediction, Karplus & Schulz Flexibility Prediction, Kolaskar & Tongaonkar Antigenicity, Parker Hydrophilicity Prediction,Rose & al, Kyte & Doolittle method,Bull & Breese,Welling &et al.,Eisenberg, et al., Parker & et al., Rao & Argos, Manavalan et al., [Gomase, et al.,2007; Gomase, et al., 2008; Gomase, et al.,2008a; Gomase & Chitlange, 2012; Gomase & Chitlange, 2012; Mishra and Gomase, 2015].

1. Database searching

The antigenic protein sequence of NADH dehydrogenase subunit 3 from *Dracunculus medinensis* was retrieved from www.ncbi.nlm.nih.gov, UniProt databases are initially the most important [http://www.ncbi.nlm.nih.gov; Sayers, E.W.et al., 2012; Bairoch, A., Apweiler, R., Wu, CH., Barker, W.C., Boeckmann, B., Ferro, S., Gasteiger, E., Huang, H., Lopez, R., Magrane, M., Martin, M.J., Natale, D.A., O’Donovan, C., Redaschi, N., Yeh, L.S. 2005].

2. Prediction of antigenicity

Prediction of antigenicity program predicts those segments from antigen NADH dehydrogenase subunit 3 protein that are likely to be antigenic by eliciting an antibody response. In this research work antigenic epitopes of *Dracunculusmedinensis a*ntigen NADH dehydrogenase subunit 3 (mitochondrion) are determined by using the Hopp and Woods, Welling, Parker, Bepipred ,Kolaskar and Tongaonkar antigenicity methods [Welling, G.W., Weijer, W.J., van der Zee R, Welling,-Wester S., 1985; Parker, K.C.,Bednarek, M.A., Coligan, J.E. ,1994; Jens,Erik., Pontoppidan, Larsen, Ole Lund and Morten, Nielsen., 2006; Kolaskar, A.S., Tongaonkar, P.C., 1990].

5. Solvent Accessible Regions

We also analyzed the 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 Emini et al., [Emini, E.A., Hughes, J.V., Perlow, D.S., Boger, J., 1985] and Karplus and Schulz [Karplus, P.A and Schulz, G.E., 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. Kyte& Doolittle (1982), Bull and Breese (1974), Roseman (1988), Wilson et al. (1981) [Kyte, J., Doolittle, R.F., 1982; Bull, H.B., Breese, K.,1974; Roseman, M.A. 1988; Wilson, J., Honegger, A., Stotzel, R.P., Hughes, G.J., 1981].

III. RESULTS AND INTERPRETATIONS

The *Dracunculus medinensis* antigen NADH dehydrogenase subunit 3 (mitochondrion), contain a long residue of 111amino acids with

MSVLLMMGFVCFFFVFIFYLLVLLLSVKIEYYVKLSSFECGFNSLGFICSSFSVHFFIMMLMFVIFDLEV

I M F L S V V V S S Y S S V F S Y A V L L F F V V F G F Y M E W WYGKLVWVV

1. Prediction of Antigenic Peptides

In this study, we found the antigenic determinants by finding the

area of greatest local hydrophilicity. The 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:24 with high score:0.588 (Fig.1). NADH dehydrogenase subunit 3 (mitochon-

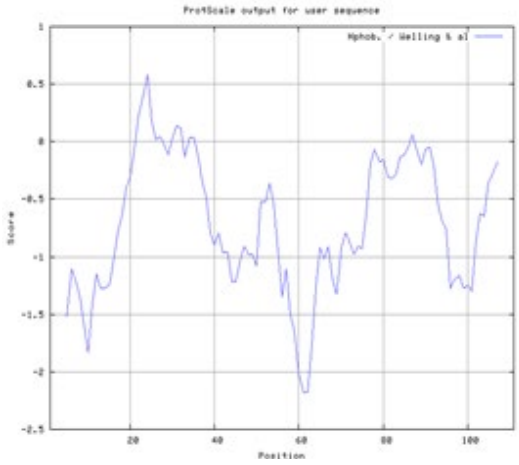

Fig.1. Hydrophobicity plot of antigen by Hphob/Welling & al., scale

drion) protein sequences (111 aa protein) is analyzed through different types B- cell epitope prediction methods. The highest score for the residue indicates the probability to be a part of the epitope (Residue coloured in yellow).We also study Hydrophobicity plot of HPLC / Parker Hydrophilicity prediction result data found i.e., the maximum predicted residues in the position: 80(Residue:S) i.e 77-VVSSYSS-83(Maximum score:2.386) and in position 81(Residue:Y) i.e.,78-VSSYSSV-84(Maximum score:2.386 (Fig. 2 & Table-1),

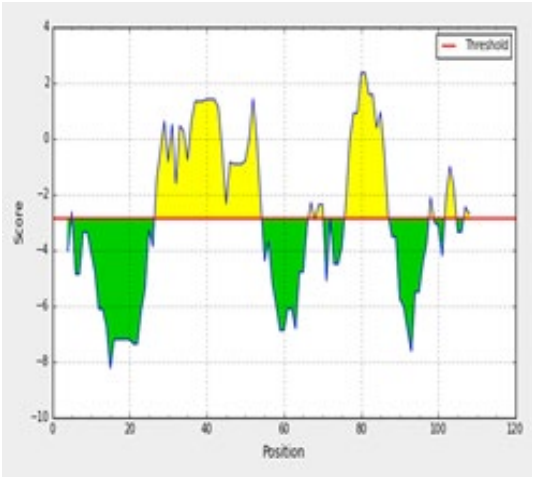

Fig.2- Hydrophobicity plot of HPLC / Parker et al. (1986)

BepiPred predicts the location of linear B-cell epitopes Result found at position the highest peak with high score is found in position:83(residue:S) with maximum score:-0.137(Fig.3), Kolaskar and Tongaonkar antigenicity prediction(Fig.4) the result data found are

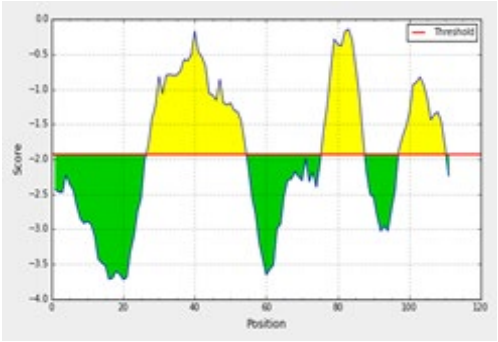

Fig. 3- Bepipred Linear Epitope Prediction plot)

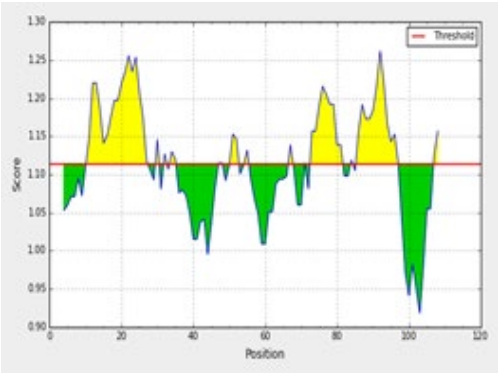

Fig. 4- Kolaskar and Tongaonkar antigenicity plot

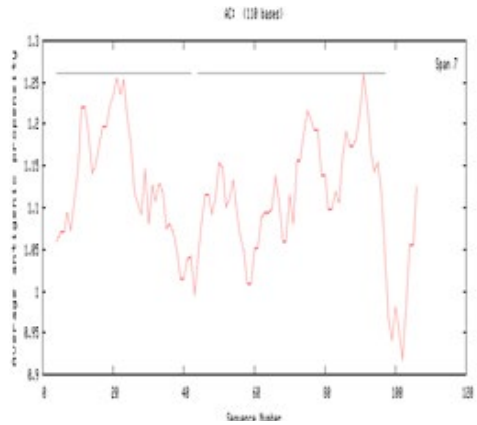

Fig 4a- Kolaskar and Tongaonkar antigenicity plot, the a verage antigenic propensity for proteinis 1.1143

Table-1: Parker Hydrophilicity Prediction Prediction Residue Scores Table

		Predicted residue scores					
		Position	Residue	Start	End	Peptide	Score
2	Parker Hydrophilicity Prediction Prediction	80	S	77	83	VVSSYSS	2.386
		81	Y	78	84	VSSYSSV	2.386
		82	S	79	85	SSYSSVF	1.6
		83	S	80	86	SYSSVFS	1.6
		84	V	81	87	YSSVFSY	0.4
		85	F	82	88	SSVFSYA	0.971

There are 2 antigenic determinants in Protein sequence			
n	Start Position	Sequence	End Position
1	4	LLMMGFVCFFFVFIFYLLVLLLSVKIEYYVKLSSFECGF	42
2	44	SLGFICSSFSVHFFIMMLMFVIFDLEVIMFLSVVVSSYSSVFSYAVLLFFVVFG	97

Fig. 4b- The 2 antigenic determinants of protein

4-LLMMGFVCFFFVFIFYLLVLLLSVKIEYYVKLSSFECGF-42,
44-SLGFICSSFSVHFFIMMLMFVIFDLEVIMFLSVVVSSYSSVFSYAVLLFFVVFG-97 and there is the possibility that the predicted antigenic fragments can bind to MHC molecule.

2. 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. 5) predicts

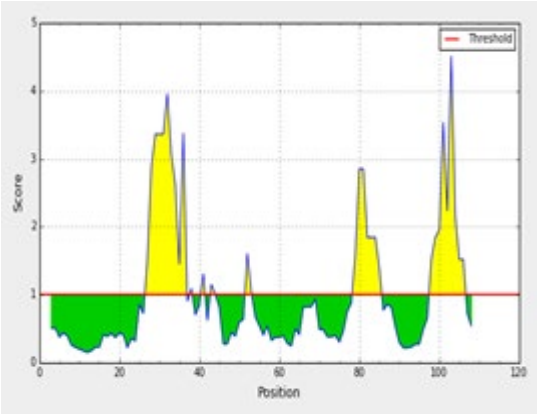

Fig. 5- Emini Surface Accessibility Prediction plot

the highest probability i.e. found in position :103 (Residue:W) i.e., 101-EWWYGK-106 and the maximum score:4.516 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 High score is found i.e. found at position:81(Residue:Y) i.e 78-VSSYSSV-84 and the maximum score:1.011(Fig.6 & Table-2). 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. Kyte& Doolittle result high in position:92,Max score:3.378(-

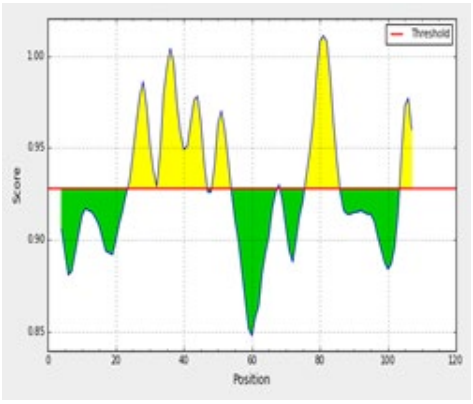

Fig. 6- Karplus& Schulz Flexibility Prediction

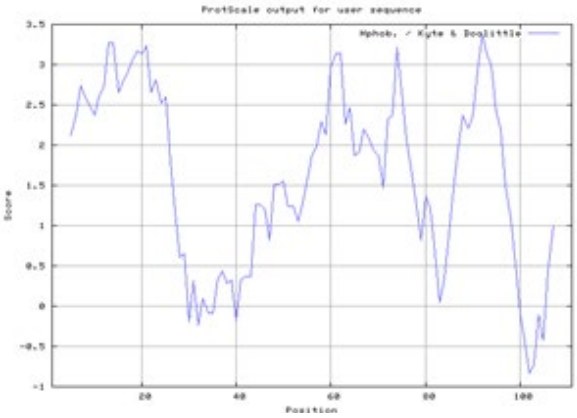

Fig. 7- Kyte& Doolittle hydrophobicity plot

Fig. 7), Bull and Breese result high in position:40,Max score:0.088 (Fig.8), Roseman result high in position:16, Max score:1.973

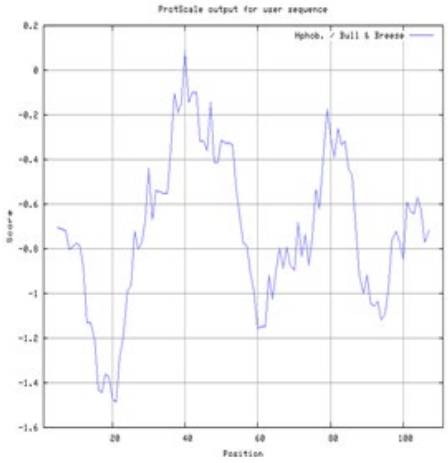

Fig. 8- Bull & Breese use surface tension to measure

(Fig. 9), Wilson & al results found in position:16,Max score:6.822(-Fig. 10).

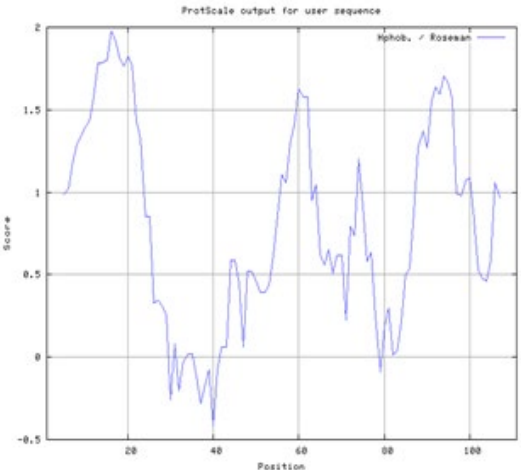

Fig. 9- Hydrophobicity plot of Roseman M.A.. (1988)

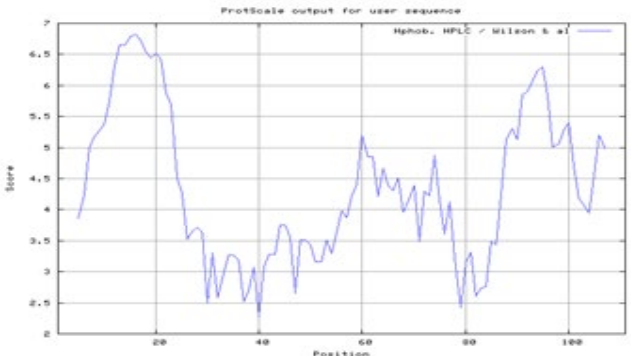

Fig. 10- Hydrophobicity/HPLC plot of Wilson & al (1981)

IV. DISCUSSION

NADH dehydrogenase subunit 3 (mitochondrion) protein sequences (111 aa protein) is analyzed through different types B-cell epitope prediction methods. The highest score for the residue indicates the probability to be a part of the epitope (Residue coloured in yellow). In this study, we found the antigenic determinants by finding the area of greatest local hydrophilicity. Welling et al., used information on the relative occurrence of amino acids in antigenic regions to make a scale which is useful for prediction of antigenic regions and the predicted result data found high in sequence in position:24 with high score:0.588. Welling et al., antigenicity plot gives value as the log of the quotient between percentage in asample of known antigenic regions and percentage in average proteins. We also study Hydrophobicity plot of HPLC / Parker Hydrophilicity Prediction Result Data found i.e., the maximum predicted residues in the position: 80(Residue:S) i.e 77-VVSSYSS-83(Maximum score:2.386) and in position 81(Residue:Y) i.e.,78-VSSYSSV-84(Maximum score:2.386 . BepiPred predicts the location of linear B-cell epitopes Result found at position:83(residue:S) with maximum score:-0.137. There are 10 antigenic determinant sequences is found by Kolaskar and Tongaonkar antigenicity scales the results show highest pick at 4-LLMMGFVCFFFVFIFYLLV LLLSVKIEYYVKLSSFECGF-42,44-SLGFICSSFSVHFFIM MLMFVIFDLEVIMFLSVVVSSYSSVFSYAVLLFFVVFG-97. 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. We predict Solvent accessibility by using Emini et al., (Fig. 5) predicts the highest probability i.e. found in position :103 (Residue:W) i.e., 101-EWWYGK-106 and the maximum score:4.516 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 predict backbone or chain flexibility on the basis of the known temperature B factors of the a-carbons here we found the result with High score found i.e., position:81(Residue:Y) i.e 78-VSSYSSV-84 and the maximum score:1.011. We predict Solvent accessibility of *Dracunculus medinensis a*ntigen NADH dehydrogenase subunit 3 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. The hydrophobicity and hydrophilic characteristics of amino acids is determined by using different scales that are rich in charged and polar residues i.e. Kyte& Doolittle result high in position:92,Max

Table-2: Karplus & Schulz Flexibility Prediction Residue Scores Table

		Predicted residue scores					
		Position	Residue	Start	End	Peptide	Score
1	Karplus & Schulz Flexibility Prediction Prediction	80	S	77	83	VVSSYSS	1.008
		81	Y	78	84	VSSYSSV	1.011
		82	S	79	85	SSYSSVF	1.008
		83	S	80	86	SYSSVFS	0.99
		84	V	81	87	YSSVFSY	0.964
		85	F	82	88	SSVFSYA	0.943

score:3.378(Fig. 7), Bull and Breese result high in position:40,Max score:0.088 (Fig.8), Roseman result high in position:16, Max score:1.973(Fig. 9), Wilson & al results found in position:16,Max score:6.822(Fig. 10).

V. CONCLUSION

An antigenic protein NADH dehydrogenase subunit 3 (mitochondrion) from *D. medinensis*can plays an important role in vaccine development. The peptide fragments of antigen protein can be used to select nonamer for use in rational vaccine design and can 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

Conflicts of Interest

The authors declare no conflict of interest.

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