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Research Article

Antigenic Determinants of Utrophin from *Dracunculus Medinensis*: New Approach for Synthetic Peptide Vaccines

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Abstract

The synthetic peptide vaccines idea is usually based on identification and chemical synthesis of B-cell and T-cell epitopes which are immunodominant and can induce specific immune responses. Deeper knowledge of antigens of utrophin from *Dracunculus medinensis*, mechanisms of immune response and the development of effective and safe adjuvants give hope that the effective peptide vaccines will be developed in the future. These disadvantages led to the development of subunit vaccines, including synthetic peptides as antigen, which consist of a specific part of the whole antigen which has been demonstrated to stimulate an immune response by eliciting antibodies that neutralize the biological activity of proteins.

Keywords: *Utrophin; Peptide; Antigenicity*

Introduction

Utrophin from *Dracunculus medinensis* are characterized to envision the antigenicity and solvent accessible region that allows potential drug targets to identify active sites against versions reactions. Prediction of antigenicity predicts those segments within utrophin that are antigenic by eliciting an antibody response [1-10]. Antigenic peptides should be located in solvent accessible regions and contain each hydrophobic and hydrophilic residue which believed that majority surface exposed regions of a protein are potential antigenic. Prediction of peptides those are in the N- and C-terminal region of the protein, because the N- and C- terminal regions of proteins are usually solvent accessible and unstructured hence Antibodies against those regions are also likely to recognize the native protein that can help to design of synthetic peptide vaccine and immuno-diagnostic reagents [10-25].

Material and Methods

Antigenic epitopes are determined by exploitation the Hopp and Woods, Welling, Parker, Kolaskar and Tongaonkar antigenicity method and Bepipred Epitope Prediction [16,26-29]. Predictions are on the basis of supported plots that ensure the prevalence of amino acid residues in experimentation notable segmental epitopes [30-34].

Results and Interpretation

Protein Sequence

utrophin, partial [*Dracunculus medinensis*]

DVEVVKAQFKEHEQFMQSLTESQDSVGRVLHRGNVICQKLDDEQNMSLLSQLKLV
NAKWERVREIAMNRQ NLLLEKLNSLQIQQLKKL

Theoretical pI: 8.11
Molecular weight: 10330.9
Number of amino acids: 88

Hopp and Woods antigenicity methods

Hopp and woods method predicts antigenic determinants by searching protein sequences of utrophin from *Dracunculus medinensis* to find the area of greatest local hydrophilicity and the hydrophilic regions in the protein are located on the surface and are potentially antigenic. The point of highest local average hydrophilicity is located in or adjacent to an antigenic determinant. In this scale the amino acid value is starting from -3 (most hydrophobic) to 3 (most hydrophilic).

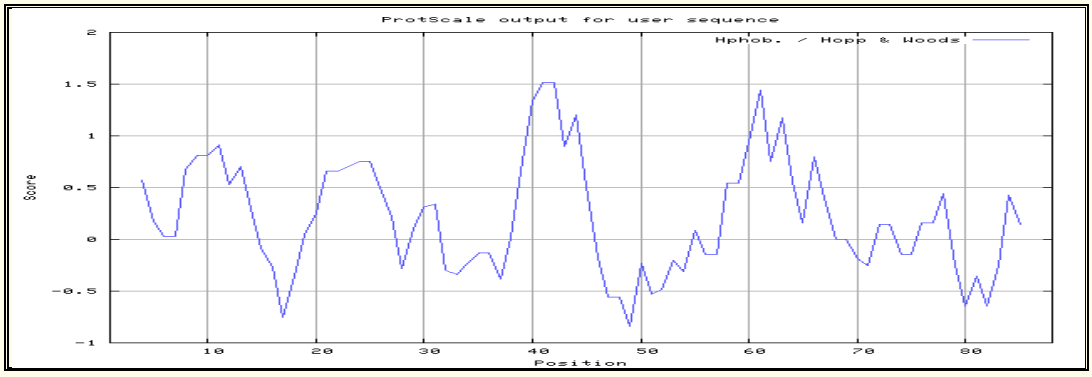

Figure 1: *Hopp and Woods antigenicity plot of utrophin from Dracunculus medinensis. In this scale the amino acid value is starting from -3 are consider (most hydrophobic) to 3 (most hydrophilic). The values greater than 0 are consider to be hydrophilic that are exposed on the surface of the folded protein.*

Min: -0.843, Max: 1.514 score at (position 48-50, 40-42) window-7

Welling antigenicity methods

Welling antigenicity method is based on the percentage of each amino acid present in known antigenic regions (utrophin from *Dracunculus medinensis*) compared to the percentage of the amino acids in the average composition of a protein. Previous strategies are based on the assumption that antigenic regions are primarily hydrophilic at the surface of the protein. This method is better than the Hopp-Woods scale of hydrophobicity which is also used to identify antigenic regions.

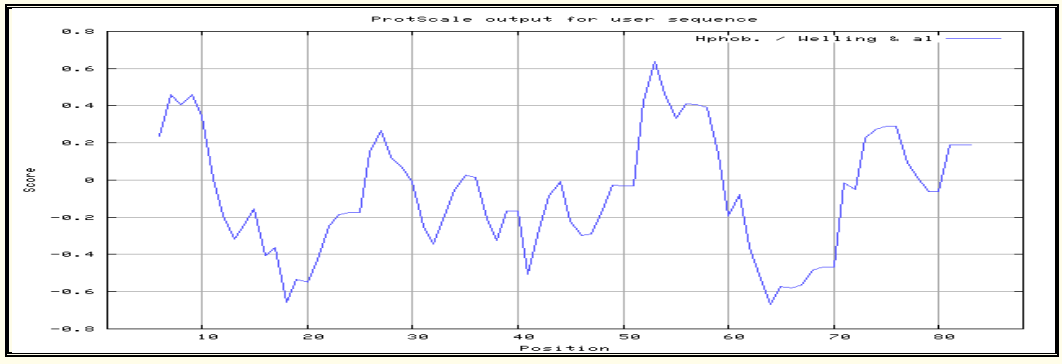

Figure 2: *Welling antigenicity plot of utrophin from Dracunculus medinensis. The Max: 0.636 score which was shown at the Position: 53.*

Min: -0.667, Max: Score: 0.636 (max) score at (position 63-65,51-55) window-11

Parker Hydrophilicity Prediction

Parker scale predicts antigenicity by identifying regions of greatest native hydrophilicity of utrophin from *Dracunculus medinensis*. It was derived from the Hopp-Woods scale however, these uses the HPLC retention times of model peptides to determine hydrophilicity. Parker hydrophilicity scale is sequence-based method that has been shown recently to perform prediction of linear epitopes of utrophin from *Dracunculus medinensis.*

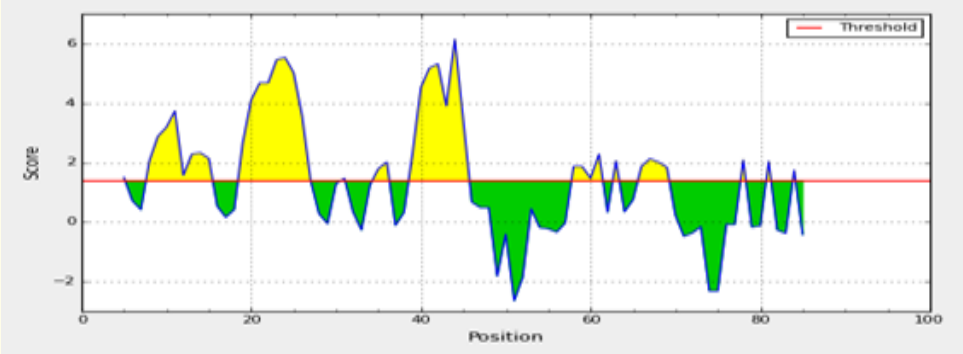

Figure 3: *Parker antigenicity plot of utrophin from Dracunculus medinensis hydrophilic scale based (threshold setting = 1.396). Parker antigenicity scale predicted 88 length peptides this scale predicted maximum score at position 41- DDEQNMS -47, under the threshold value 1.396.*

Center position: 4; Window size: 7; Threshold: 1.396; Average: 1.396, Minimum: -2.657, Maximum: 6.157

Position	Residue	Start	End	Peptide	Score
36	I	33	39	GNVICQK	2.014
37	C	34	40	NVICQKL	-0.114
38	Q	35	41	VICQKLD	0.314
39	K	36	42	ICQKLDD	2.271
40	L	37	43	CQKLDDE	4.529
41	D	38	44	QKLDDEQ	5.186
42	D	39	45	KLDDEQN	5.329
43	E	40	46	LDDEQNM	3.914
44	Q	41	47	DDEQNMS	6.157(Maximum)
45	N	42	48	DEQNMSL	3.414
46	M	43	49	EQNMSLL	0.671
47	S	44	50	QNMSLLS	0.486
48	L	45	51	NMSLLSQ	0.486
49	L	46	52	MSLLSQL	-1.829
50	S	47	53	SLLSQLK	-0.414
51	Q	48	54	LLSQLKL	-2.657(Minimum)
52	L	49	55	LSQLKLV	-1.871

53	K	50	56	SQLKLVN	0.443
54	L	51	57	QLKLVNA	-0.186
55	V	52	58	LKLVNAK	-0.229

Table 1: *Parker Hydrophilicity Prediction Result Data.*

Average: 1.396 Minimum: -2.657 Maximum: 6.157

Predicted residue scores

Kolaskar & Tongaonkar Antigenicity

Kolaskar & Tongaonkar Antigenicity is a semi-empirical method for the prediction of antigenic regions including information of surface accessibility and flexibility. The method was able to predict antigenic determinants with an accuracy of 75%.

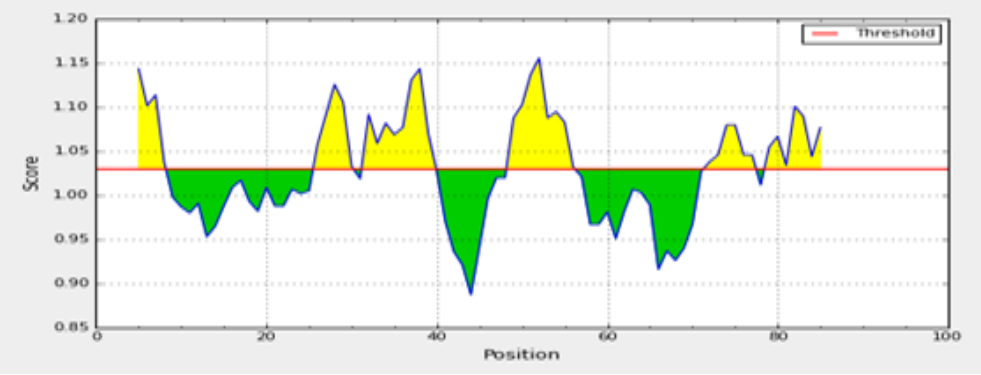

Figure 4: *Kolaskar and Tongaonkar antigenicity prediction plot of utrophin from Dracunculus medinensis predicts those segments among a protein sequence that are to be antigenic by eliciting an antibody response (threshold setting = 1.030). This scale predicted peptide in the positions 49-LSQLKLV-55.*

Center position: 4; Window size: 7; Threshold: 1.030.

No.	Start Position	End Position	Peptide	Peptide Length
1	49	55	LSQLKLV	7

Table 2: *Predicted peptides of utrophin from Dracunculus medinensis.*

Position	Residue	Start	End	Peptide	Score
38	Q	35	41	VICQKLD	1.144
39	K	36	42	ICQKLDD	1.07
40	L	37	43	CQKLDDE	1.027
41	D	38	44	QKLDDEQ	0.97
42	D	39	45	KLDDEQN	0.936
43	E	40	46	LDDEQNM	0.921
44	Q	41	47	DDEQNMS	0.887(Minimum)
45	N	42	48	DEQNMSL	0.942
46	M	43	49	EQNMSLL	0.997

47	S	44	50	QNMSLLS	1.02
48	L	45	51	NMSLLSQ	1.02
49	L	46	52	MSLLSQL	1.088
50	S	47	53	SLLSQLK	1.103
51	Q	48	54	LLSQLKL	1.137
52	L	49	55	LSQLKLV	1.156(Maximum)
53	K	50	56	SQLKLVN	1.088
54	L	51	57	QLKLVNA	1.095
55	V	52	58	LKLVNAK	1.083

Table 3: *Kolaskar & Tongaonkar Antigenicity Result Data.*

Average: 1.030 Minimum: 0.887 Maximum: 1.156

Discussion

Antigenic determinants of utrophin from *Dracunculus medinensis* are determined by finding the area of greatest local hydrophilicity using the Hopp-Woods method. This method has a high success rate than other methods. The success of this method is its cautious approach to charge interactions that gives equal weight to positive and negative charged residues, whereas other methods tend to favor one or the other. The sites chosen by this method is to be highly exposed and charged regions of the protein's surface therefore, have ample opportunity to contact other proteins. Here we found high peaks at position: 41 with max score: 1.514 and the minimum found at the position: 49 with minimum score: -0.843 using window-7. Welling Method used to locate hydrophilic regions in a protein since, it is assumed that antigenic determinants are located on surface which contain charged and polar residues. These methods are used to obtain a rough idea for estimation of potentially antigenic regions. However, as shown by Hopp and Woods not all antigenic regions are hydrophilic and not all hydrophilic regions are antigenic. Therefore, welling developed a method based on the percentage of each amino acid present in known antigenic determinants compared with the percentage of the amino acids in the average composition of a protein. Here we found the Position: 53 with Score: 0.636 (max) and at Position: 64 Score: -0.667 (min) by using window-11. Parker used three parameters - hydrophilicity, accessibility and flexibility to calculate the antigenic propensity using a composite plot. This method has improved to predict antigenic determinants as compared to Hopp and Woods' method. Parker antigenicity plot is based on (threshold setting = 1.396) and this scale predicted maximum score at position: 44(residue: Q) 41-DDEQNMS-47 with the score: 6.157 (Maximum) under the threshold value 1.396. Kolaskar Tongaonkar antigenicity methods and predict location of antigenic determinants within utrophin from *Dracunculus medinensis* that are antigenic by eliciting an antibody response. This plot predicts those segments among a protein sequence that are to be antigenic by eliciting an antibody response (threshold setting = 1.030). This scale predicted a 7 length peptide in the position: 52 (Residue: L) and the sequence: 49- LSQLKLV-55 with score:1.156 (Maximum) . A typical profile show characteristic peaks and troughs, corresponding to the most hydrophobic and most hydrophilic parts of the protein respectively. Different residues which are rankings are commonly used hydrophobicity scales. While the scales differ in detail, there is a general consensus regarding the types of residue that appear at the most hydrophobic end (S, L, V and M) and those that appear at the most hydrophilic end (N, Q, E, D and K) (Figure 1 to Figure 4). We also find the location in solvent accessible regions in protein by using the hydrophobic scale Emini surface accessibility. This prediction revealed an epitope with 6 amino acid residues maximum (2.755) in the sequence positions:40 i.e 38- QKLDDE-43 and at position:41 with sequence 39- KLDDEQ-44 of utrophin from *Dracunculus medinensis* (Table 3). Hydropathy scale is a physiochemical property that quantifies the hydrophobicity of an amino acid. A window size is suggested to be 7-9 residues for predicting surface sites. The most of used scales are hydrophobicity scales which are derived on the basis of experimental studies on partitioning of peptides in apolar and polar solvents to predict membrane-spanning segments that are highly hydrophobic and secondary structure conformational parameter scales. The maximum region of hydrophilicity is to be considered as an antigenic site having hydrophobic characteristics.

Conclusion

Peptide fragments of utrophin from *Dracunculus medinensis* involved multiple antigenic components to direct and empower the immune system to protect the host. From the above result, it is concluded that antigenicity methods predict the location of antigenic determinants utrophin from *Dracunculus medinensis* that are antigenic by eliciting an antibody response. Hence, the region spanning the sequence positions will be of greater importance for epitope-based vaccine design. The amino acids making up the epitope are usually charged and hydrophilic in nature. From the study of physicochemical properties, it was found that, the region of maximal hydrophilicity is likely to be antigenic site, having hydrophobic characteristics because c- terminal region of utrophin from *Dracunculus medinensis* is solvent accessible. The mobility of protein segments those are located on the surface of a protein due to an entropic energy potential which seem to correlate well with known antigenic determinants. These antigenic peptides can be used as their identifiers. Therefore, these antigenic determinants are also important for synthetic peptide vaccine production.

Conflict of Interest

None.

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