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Predicting B-Cell Epitopes in Heminecrolysin Protein Sequences Using Bioinformatics

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ABSTRACT

As the first hemolytic fraction of Hemiscorpius lepturus venom, heminecrolysin is an important venom protein, the effects are mediated by the phospholipase D activity. The paramount features of HL-PLD1 protein consider its physicochemical characteristics, secondary structure, tertiary structure, position, and potential epitopes of B cell in its definition. This article employed bioinformatic analytic approaches for identifying potential epitopes in order to obtain a highly effective antivenom. The results of Bcepred, ABCpred and BCPREDS online software showed that GHMVNAVKQI and VTFDDNGNPKWTYHGTPCDC peptides had the maximum safety scores 0.80 and 0.963, respectively. The data also showed that these peptides are properly applicable in vaccine and diagnostic clinical assay development studies. The basic theoretical information regarding HL-PLD1 can be used for further studies on designing a highly efficient antivenom against HL-PLD1.

Keywords

Heminecrolysin, Bioinformatics, *Hemiscorpius lepturus*, Epitope, Antivenom

INTRODUCTION

Due to its climatic conditions, Iran has a large number of scorpions including 32 species of 3 families of scorpions. *Hemiscorpius lepturus*, belonging to the *Hemiscorpidus* family, as a highly dangerous scorpion in Iran due to its pain-free bites and associated complications (1, 2), has already led to the highest number of deaths as a result of envenomation in Khuzestan province, southwest Iran (3). Hemorrhagic venom is reported to have systemic hemolytic, neurotoxic and cytotoxic effects on internal organs and tissues (1, 2). Likewise, containing various enzymes

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such as heminecrolysin, hemicalcin, hemiotoxin, hemilipin, phospholipase, metalloprotease, hyaluronidase and protease, scorpion venom is known to cause major effects on different body organs (4-9). Among the components of the *Hemicascorpius leptorus* venom, Heminecrolysin is the main toxin protein with hemomolytic and dermonecrotic effects, which exerts its function through phospholipase D (5-7). Phospholipases are indeed the most abundant enzymes in scorpion venom which are divided into four groups A, B, C and D, including anti-inflammatory, neuronal, hemolytic activity and platelet aggregation inhibitory effects (10). Phospholipase D activity is known to be responsible for the mortality caused by *Hemicascorpius leptorus* venom and its sphingomyelinase functioning is probably a trigger for this to occur (11). In effect, Phospholipase D hydrolyzes sphingomyelin and releases choline and ceramide-1 phosphate, leading to cellular damage (12-16). Using heminecrolysin as an immunogen with a high potential for antidote production against the *Hemicascorpius leptorus* venom, Borchani et al. (2011) reported that heminecrolysin produced high levels of high neutralizing antibodies compared to the raw venom of *Hemicascorpius leptorus*. Given that sphingomyelinase D has been found in the venom of spiders and even pathogenic strains of Corynebacterium (6, 7), various studies have identified sphingomyelinase as a suitable substitute for raw toxin aimed at antidote production and treatment (17, 18). The application of antidotes and antibodies produced in the body of animals exposed to venom is indeed the most widely used treatment for scorpion bites (19, 20). Although the application of antidotes for treatment of scorpion envenoming neutralizes the venom, it can lead to anaphylactic shock and even death in some scorpion envenomation victims (21). New biotechnology tools using peptide epitopes or DNA strands are currently used for the production of high-potential neutralizing antibodies, making them safe and cost effective by minimizing the effects of toxin administration and antidote acquisition (22-25). Immuneinformatics is widely used to identify and predict antigenic areas for epitopes of B and T cells (26). B and T cells as well as molecules of the host immune system identify epitope as an antigenic part. Given that even a limited number of amino acid residues with epitope (not the protein entirely) are adequate to form protective responses, it is of great importance to predict or identify such a substantial segment of amino acid residues for recognizing the immune pathogenesis mechanisms of the pathogens and designing vaccines and immunodiagnostic tests based on epitope (27). Heminecrolysin (HNC) is the only SMaseD of scorpion, which is purified from *Hemiscorpius lepturus* venom (6, 7). Pathophysiologically and structurally viewed, it has been assumed to be a venom PLD (vPLD), with its physiopathological impacts being correlated with the lysoPLD activity (5). In addition, it has been found that HNC as a highly immunogenic-raising antibody neutralizes both in-vitro and in-vivo impacts of the venom efficiently (6, 7). However, most of the anti-HL antibodies might not be directed against the PLD proteins (28). This study was thus intended to introduce efficient epitopes against phospholipase D, considering the immunogenic potential of heminecrolysin and its sphingomyelinase activity through phospholipase D using bioinformatics methods.

MATERIALS AND METHODS

Sequence aligning of phospholipase D Hemiscorpius lepturus venom proteins

4 phospholipase-D sequences (KX932446, KX932445, KX932447 and KX932448) from *H.lepturus* venomous gland transcriptome were identified (10). The selected accession number KX932445.1 for the highest homology with the common sequence was chosen for epitope prediction.

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Analysis of secondary structures and the designed 3D model

Garnier-Osguthorpe-Robson, GOR, service for secondary structure forecast (https://npsa-prabi.ibcp.fr/cgi-bin/ npsa_automat.pl? page=npsa_gor4.html) was adopted for HLPLD1 secondary structure. This service forecasts alpha-helix, random coil and extended strand as 3 important motif classes forming the secondary structure of proteins. Besides, a homology modeling technique (i.e., the SWISS MODEL available at https://swissmodel.expasy.org/) was utilized to construct the 3-D models of the HL-PLD1 sequence.

Chemical and physical characteristics

From the GenBank of National Centre for Biotechnology Information (NCBI), at https://www.ncbi.nlm.nih.gov/nuccore/262479636, gene and amino acid sequences of HLPLD1 were provided. Subsequently, the analysis of the basic physicochemical traits of HLPLD1, including the number and the composition of amino acid, theoretical isoelectric point (pI), molecular weight (MW), total number of positively and negatively charged residues, extinction coefficients, and atomic composition, mammal reticulocytes' estimated half-life, Escherichia coli and yeast, indices of instability and aliphatic, and hydropathicity's grand average (GRAVY) was carried out.

Predicting B cell epitopes

The well-known bioinformatic tools such as B cell epitope prediction (Bcepred), ANN-based B cell epitope prediction (ABCpred) accessible at http://crdd.osdd.net/raghava/abcpred/ABC_submission.html, server for predicting epitopes of B cells (BCPREDS) at http://ailab.ist.psu.edu/bcpred/predict.html, and database of immune epitope (IEDB) at http://tools.iedb.org/bcell/ were consulted to forecast and analyze the epitopes of B-cell on HLPLD1 protein.

RESULTS

Analysis of the secondary structure and the designed 3D model

The forecast of a protein's secondary structure significantly affects its biological capacity. The information extricated from the GOR server for forecasting secondary structures showed that the ratios of alpha-helix, expanded strand, and irregular curl in HLPLD1 succession were 123 (37.96%), 72 (22.22%) and 129 (39.81%), respectively (Figures 1 and 2). Besides, the SWISS MODEL online service was utilized to forecast and form the HL-PLDI protein's 3D structure. Based on the SWISS-MODEL results for the HL-PLDI protein, 27 templates matched the target sequence. In this context, the model that had the best coverage and sequence identity was selected. Figure 3 represents the SWISS-MODEL outputs involving the predicted 3D model for the HL-PLDI protein, the estimated global quality of the protein, coverage and sequence identity, alignment of model-template, and the estimation of the local quality.

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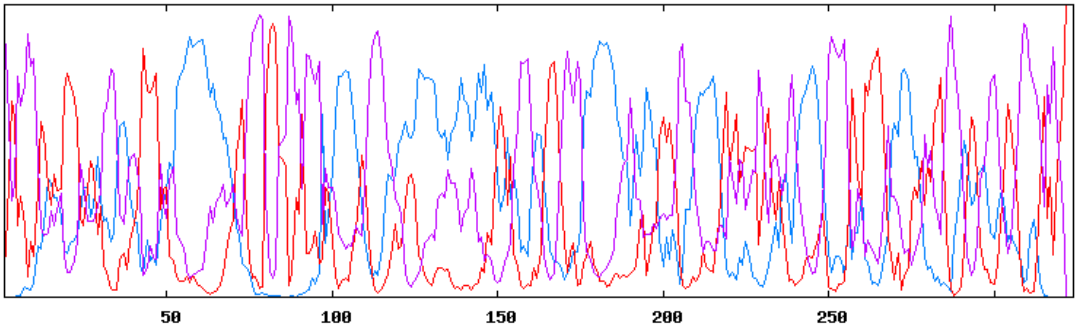

Figure 1. The GOR IV-based analyzing of HLPLD1 secondary structure. Graphic schema of the secondary structure.

```
        10        20        30        40        50        60        70
        |         |         |         |         |         |         |
MAHCYYNSKRGCNRVMKTVALVVLISTVMVEESRGDSQEDKKRPIWNIGHMVNAVKQIEEFLDLGANALE
ccccceeccccccceeeeeeeeeeeeccceeeeccccchhhhccceeeeeeccccchhhhhhhhhhhhhhh
ADVTFDDNGNPKWTYHGTPCDCFRDCLRWEYVDEYLKRIRELTSPGSSKFRKGFILLMLDLKISKLSDNA
hceeeccccceeeeccccccccccccccchhhhhhhhhhhhcccccccchhhhhhhhhhhhhhhhhhhh
KSKAGKEIADMIIKRLWSGSGEKAQLYIVLSFPYVNDIEFVRAFRERVKSKGFASEAEKRIGWDISGNED
hhhhhhhhhhhhhhhhcccccceeeeeeeeccccccchhhhhhhhhhhhhccchhhhhhheeeeccccc
LGKIRDAYQKLGITDNVWQSDGITNCLTRSHDRLAEAVCKRDSDKEWPSLKKVYYWTVDKQSSMKEALKV
chhhhhhhhhhceeccceeeecceccccccccchhhhhhhhhcccccccceeeeeeeeeeechhhhhhhhhh
GVDGMITNDPDDLVAVLNEFSGTHRLANINDSPWQKIPRPKSNC
ccceeeccccccchhhhhhhccccceeeeccccccccccccceec
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Figure 2. The GOR IV-based analyzing of HLPLD1 secondary structure. The secondary structure predicted (c: coil, e: extended strand, and h: helix).

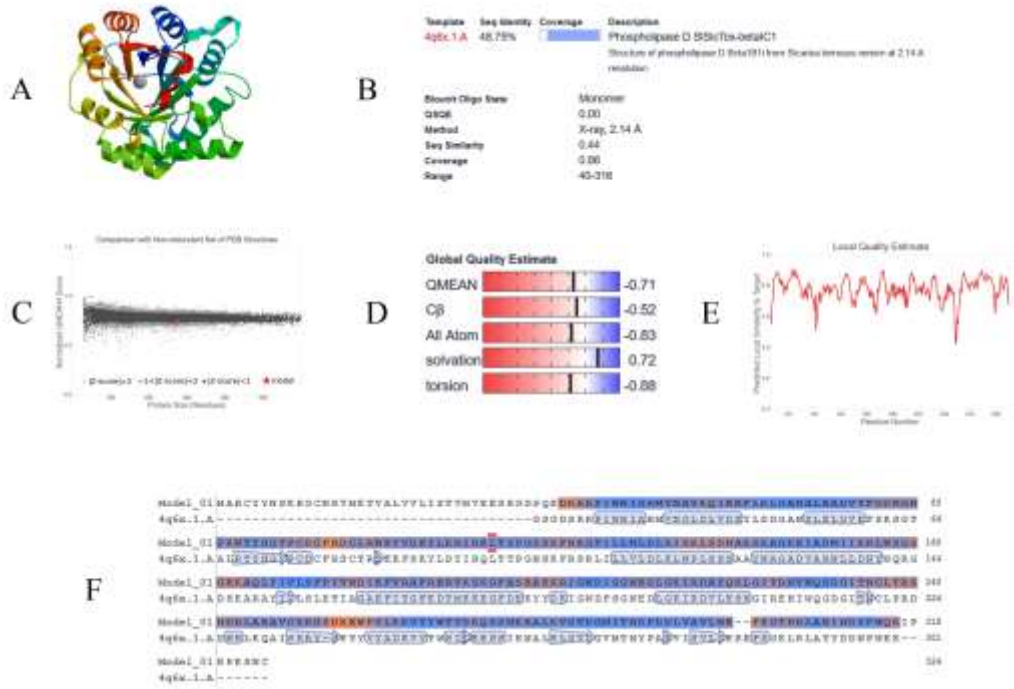

Figure 3. SWISS MODEL-based analysis of the constructed 3D structure for HL-PLD1. A: Predicting the HL-PLD1 protein's tertiary structure. B: Coverage and identity of sequence. C:

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Estimation of global Quality; the composite estimator QMEAN with 4 individual terms torsion, solvation, all atoms, and Cβ atoms. The positive values indicate that the average scores of the model are greater than experimental structures. Negative values, however, imply that the scores of the model are, on average, below the experimental structures. The QMEAN Z score is given on top. D: Comparison with PDB structures' non-redundant set: The protein length (the number of residues) is given on the x axis, and the normalized QMEAN score is represented on the y axis. Every dot shows the experimental structure of one protein. Experimental structures with a |Z-score| from 1 to 2 are shown in grey, whereas the black dots signify the experimental structures with a normalized QMEAN score within 1 standard deviation of the mean (|Z-score| from 0 to 1. The experimental structures even farther from the mean value are shown in light grey. A red star represents the actual model. E: Local quality: for each model residue (on the x axis), the expected similarity to the native structure (the y axis). It has been expected that residues with a score lower than 0.6 should usually have a low quality. F: Alignment of Model-Template. (The reader can refer to the online version of the paper for an interpretation of the color references).

Physical and chemical properties

There were 324 amino acids in HLPLD' sequence of protein, and the molecule weight (MW) of HL-PLD1 was calculated to be 37kDa. Moreover, the aliphatic index of HL-PLD1 (79.72) was calculated, which indicated the occupation of relative volume via aliphatic side chains (alanine, isoleucine, valine and leucine). It was resulted that at different temperatures the HL-PLD1 protein showed more stability. Moreover, the HL-PLD1's indices of GRAVY (−0.525) and instability (34.57) were counted (Table 1).

The GRAVY's negative value indicates the hydrophilicity of the protein, hence giving an estimate of the protein stability within a test tube. The HLPLD1 sequence was considered to be a stable protein given that the stability of a value below forty was predicted. The prediction of the protein structures leads to a better comprehension of the functioning of proteins and gives us knowledge on how proteins might be controlled, influenced, and modified.

Table 1. Different physicochemical features of HL-PLD1 protein.

Number of amino acids (Nos.)	**324**
Molecular weight (Da)	**36979.18**
Theoretical pI	**8.29**
Total number of negatively charged residues	**47**
(Asp+Glu) Total number of positively charged	**50**
Residues (Arg+Lys) instability index	**34.57**
Aliphatic index	**79.72**
Grand average of hydropathicity (GRAVY)	**-0.525**

The B-cell epitopes prediction

As an online server for predicting the linear epitopes of B cells, Bcepred utilized the physicochemical characteristics of a sequence of protein like polarity, mobility/flexibility, hydrophilicity, accessibility, surface exposed, and turns with an accuracy of 58.7% at a 2.38 threshold. The segment of amino acid residue having a score higher than the threshold was assumed to be the predicted B-cell epitope. Table 2 shows the Bcepred-anticipated epitopes

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concerning the chemo-physical parameters such as turns, accessibility, flexibility, exposed surface, antigenic propensity, and hydrophilicity.

Table 2. The predicted B-cell epitopes on HLPLD1 using Bcepred concerning various parameters.

Parameter	Epitope sequence
Hydrophilicity	CKRDSDK, KRDSDKE, EESRGDS, ESRGDSQ, SRGDSQE, RGDSQED, GDSQEDK, DSQEDKK, SQEDKKR, DDNGNPK
Antigenicity	TVALVVL, VALVVLI, ALVVLIS, LVVLIST, KTVALVV, AQLYIVL, LYIVLSF, VLSFPYV, VMKTVAL, MKTVALV
Flexibility	SRGDSQE, RGDSQED, GDSQEDK, DDNGNPK, LTSPGSS, TSPGSSK ,SPGSSKF, WSGSGEK, SGSGEKA , PGSSKFR
Accessibility	DSQEDK, SQEDKK, QEDKKR, EDKKRP, SEAEKR, RDAYQK, TRSHDR,KRDSDK, PRPKSN
Turn	NSKRGCN, TFDDNGN, FDDNGNP, DDNGNPK, DNGNPKW, NGNPKWT, TSPGSSK, SPGSSKF, TNDPDDL, PRPKSNC

Using ABCpred, in an antigenic sequence, the predicting of B-cell epitopes was carried out, based on the Artificial Neural Network, ANN, with an accuracy of 65.93%. In this software, the forecasted B-cell epitopes were ordered in accordance with their obtained scores under a trained recurrent neural network. The ABCpred software outputs are given in both tabular and graphical formats. Graphically, the B-cell epitopes are shown in blue for clarification. In Table 3, 10 epitopes with the highest scores are shown. Table 4 ranks the peptides having a score over the threshold. On the basis of the server, the peptides that have a higher score are more probable to be used as epitope. This study has used the default parameters of the program. B-cell epitopes of HLPLD1 were predicted using ABCpred server, which placed the predicted epitopes above threshold regarding their scores. For a peptide, having a higher score demonstrated that the peptide was more likely to be an epitope, and thus 16 epitopes were anticipated, on our sequence, by the suggested server. The most elevated score (0.80) was associated with the linear epitope GHMVNAVKQI.

Table 3. The predicted B-Cell epitopes on HLPLD1 using ABCpred (ANN, artificial neural network).

Rank	Sequence	Start position	Score
1	GHMVNAVKQI	49	0.80
2	FASEAEKRIG	193	0.79
3	WEYVDEYLKR	99	0.78
4	SRGDSQEDKK	33	0.78
5	IIKRLWSGSG	152	0.78
6	LDLKISKLSD	129	0.76
7	DVTFDDNGNP	72	0.74
8	GTPCDCFRDC	87	0.72
9	HCYYNSKRGC	3	0.72
10	NDIEFVRAFR	176	0.71

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Table 4. The predicted linear B-cell epitopes on HLPLD1 using BCPREDS.

Position	Epitope	Score
73	VTFDDNGNPKWTYHGTPCDC	0.963
199	KRIGWDISGNEDLGKIRDAY	0.937
136	LSDNAKSKAGKEIADMIIKR	0.934
100	EYVDEYLKRIRELTSPGSSK	0.906
252	DSDKEWPSLKKVYYWTVDKQ	0.896
31	EESRGDSQEDKKRPIWNIGH	0.833
221	LGITDNVWQSDGITNCLTRS	0.808
305	RLANINDSPWQKIPRPKSNC	0.767

Using BCPRED, a combination of a support vector machine (SVM) and a subsequence kernel (SSK) were recruited so that continuous B-cell epitopes could be predicted (El-Manzalawy et al., 2008). The input of BCPRED was a single sequence of amino acid in a plain format, and a score was given to each of the epitopes. Table 4 shows the top-scored epitopes. By default, the running features of the program were as follows: length of epitope: 20 amino acids, and classifier specificity: 75% when an overlap filter was applied. Using the information from epitope immunogenic peptides can well be predicted and novel potential vaccines can be designed. For prediction of the linear B-cell epitopes of HLPLD1, BCPREDS server was employed, and hence a segment having a score over the threshold was selected as the predicted epitope of B cell. The higher score indicates that the binding affinity is higher. Table 4 represents 8 top-scored epitopes, with the highest score pertaining to VTFDDNGNPKWTYHGTPCDC epitope.
Besides, the IEDB was used to forecast continuous antibody epitopes from the HLPLD1 sequences, using methods such as hydrophilicity, surficial access, beta-turn, flexibility, antigenicity, and predicting the Bepipred linear epitope to predict B cell epitopes. The IEDB outputs were presented in the forms of tables and graphs. Graphically, the Y axis depicted the corresponding score for each residue, whereas the X axis represented the position of a residue in the sequence. In the tables, the calculated scores for different residues are represented. In Fig. 4, the IEDB prediction results are given. The minimum, maximum and threshold scores for the considered parameters were as follows: beta-turn (0.544, 1.447, 0.997), surface accessibility (0.051, 7.469, 1.000), flexibility (0.898, 1.128, 1.002), antigenicity (0.879, 1.266, 1.016), and hydrophilicity (-6.186, 7.386, 1.714), Bepipred linear epitope (-0.010, 2.068, 0.350) prediction. Based on the aforementioned results, a number of potential B-cell epitopes existed on HLPLD1 sequence, which could be used as appropriate targets for further antivenom and diagnostic studies.

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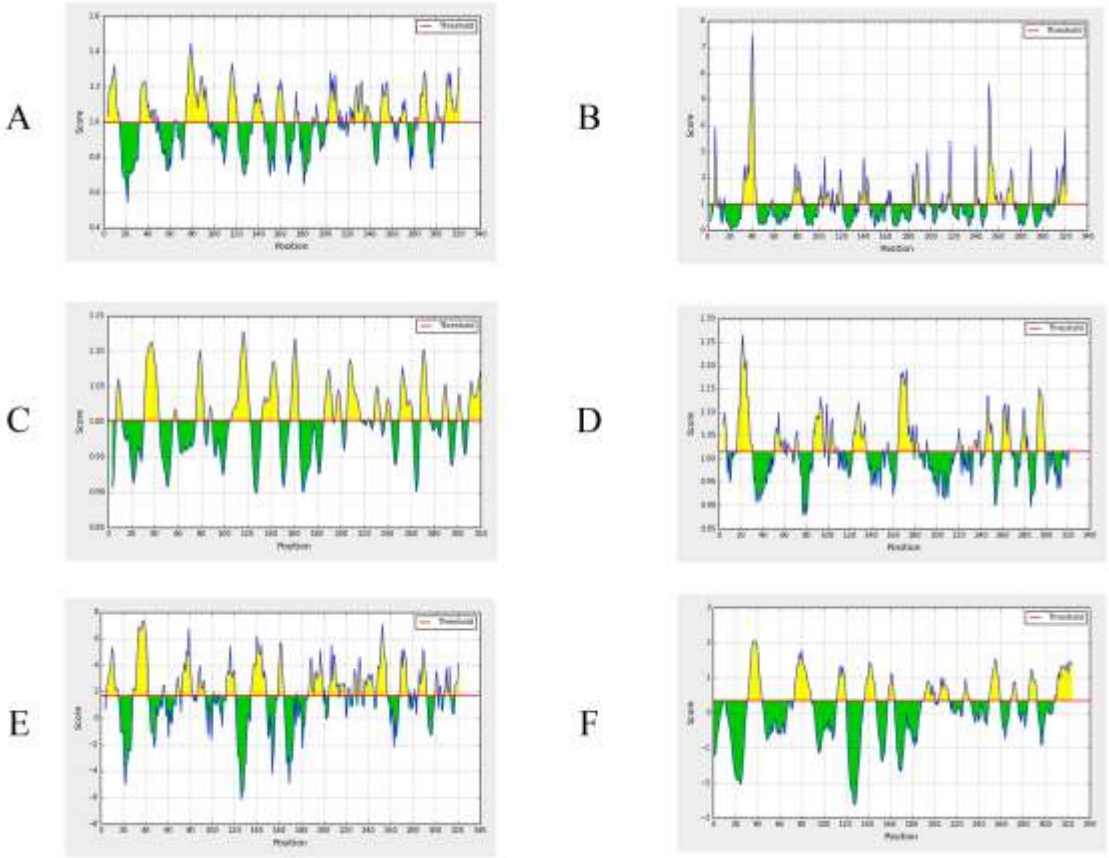

Figure 4. The IEDB server output using the parameters of A: beta-turn, B: surficial accessibility, C: flexibility, D: antigenicity, E: hydrophilicity, and F: Prediction of the Bepipred linear epitope. The residues with higher scores, which are shown in yellow on the graphs, are more likely to be part of the epitope.

DISCUSSION

Heminecrolysin is the first hemolytic toxic fraction of *Hemiscorpius lepturus* and the main protein is venom, the function of which is mediated by the activity of phospholipase D (5-7). Heminecrolysin is able to induce an immune response to *Hemiscorpius lepturus* venom. Nevertheless, only the dermonecrotic impact of Hemiscorpius lepturus venom can be neutralized by the antibodies generated against Heminecrolysin, while the inflammatory impacts are neutralized by antibody against the whole range of Hemiscorpius lepturus venom (6, 7). Bioinformatics exerts an essential role in the development of vaccines by forecasting the protein functions, structures, and other biological properties and determining the chosen vaccine epitope's specific immune response. By predicting B-cell epitopes, it is aimed to arrive at a simpler identification of these epitopes, which practically aims to replace epitopes with antigens under the antibody generation or structure function research (29). The prediction of linear B-cell epitope can be attained using different bioinformatic tools, e.g., Lasergene, ABCpred, and Bcepred (5, 19, 22).

The sequence and structure of the Hl-PLD1 and those of the PLDs of brown spiders were remarkably homologous. As the deadly dermonecrotic component of *H. lepturus*, Hl-PLD1 was

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very similar to Loxoscelidae PLD, showing a high level of phospholipase D activity (11). It has also been recognized that by using monospecific PLD1antibodies, the *H. lepturus* PLD1 activity is inhibited, which leads to the prevention of lethality or complications following the envenomation. As a highly important fatal toxin, the HL-PLD1 molecule can, therefore, be used in immunization and removing the serious envenomation complications. Epitopes are remarkably affected by a protein's secondary structure (30). It was observed that random coil had nearly half of protein's structure followed by alpha helix and extended strand. The random coil on the protein surface seems to be loose and tends to twist; therefore, it can be a potential epitope (31). Being normally located inside the protein, the beta-turn and alpha-helix have a high chemical-bond energy, which reserves the structure of the protein, and hence they do not seem to act as epitopes (21). The hydrophilicity of the protein is signified by GRAVY's negative value, and the instability index gives an estimate of the protein stability in the test tube. Given that the value below 40 was estimated to be stable, the HLPLD1 sequence was categorized as a stable protein. The prediction of protein structures leads to a better knowledge on the proteins' functioning and on how proteins might be controlled, influenced, and modified. The GOR IV approaches were thus utilized to predict the structure elements of HL-PLD1 protein. Based on the HL-PLD1 secondary structure outcomes, the said protein comprised 37.96% alpha-helix, 22.22% extended strand, and 39.81% random coil. It was found that the alpha-helix and beta-turn inside the protein, with a great energy of hydrogen bond, were responsible for keeping the protein structure, hence producing a powerful interaction with antibodies (30). Moreover, based on DiNNNA server, 4 bonds of disulfide (also called disulfide bridges) were predicted in the HL-PLD1 sequence. Disulfide bonds exert an important influence on the structure and activity of a protein (32). Disulfide bonds sequences equal to or smaller than 5 are easier to forecast than those with over 5 bonds. Besides, when disulfide bonds are precisely predicted, the conformational space are reduced and the modeling of the 3D structure and folding of proteins are boosted (33). The present study has made use of some reliable bioinformatic tools for the prediction of the linear B-cell epitopes on HL-PLD1, hence stating potential objectives for future research on the *Hemiscorpius lepturus* antivenom. In fact, a more precise prediction of the protein structures and epitopes has been achieved by using numerous predicting indices and tools (34). In this context, Bcepred, BCPREDS, ABCpred and IEDB were used as 4 viable bioinformatics mechanisms to predict linear B-cell epitopes on the HL-PLD1 protein. Linear epitope prediction is not limited to the application of informatics, and additional properties (e.g., prediction of Emini surface accessibility, Koloaskar-Tongankar antigenicity, prediction of Chou-Fasman beta-turn, floppy prediction, and Parker hydrophilicity prediction) must also be taken into account (26). Based on our obtained results, the existence of potential epitopes in this protein demonstrates that this protein would be an interesting antivenom candidate after its test in animal models. Besides, Bcepred was employed to forecast the linear epitopes of B-cell considering such chemical and physical features as hydrophilicity, accessibility, flexibility/mobility, turns, and exposed surface. A key property of B-cell epitopes is its surface accessibility, which must be determined before selecting the potent B-cell epitope because it shows the most exposed part of a protein's surficial area and possibly induce an immune response mediated by B cells. In this work, the hydrophilicity/hydrophobicity prediction represented over 10 domains of hydrophilicity on the HL-PLD1 sequence (35). The hydrophilic side chains, due to their water affinity, move towards the protein's outside. By predicting a protein's hydrophilicity and hydrophobicity, the protein's folding and stability can better be understood. Moreover, a protein's hydrophilic regions that are often located on the protein's

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surface are potentially antigenic (36). In order to obtain data on the HL-PLD1protein's surficial accessibility and hydrophilicity, the prediction tools of Emini's surficial accessibility and Parker's hydrophilicity were applied (37, 38). Using Kolaskar and Tongaonkar antigenicity predicting tools, the values of the protein antigenic propensity for B-cell epitope was calculated (39). The beta turns of the B-cell epitope were often hydrophilic and functionally available at the surface. Based on the Chou-Fasman Beta turn prediction, the protein potential antigenic region was also identified, given that a positive correlation existed between the two factors (40). Furthermore, a protein's flexible region causes an antibody to be successfully bound to an epitope. Consequently, Karplus Schulz flexibility prediction tool was used to predict the HL-PLD1 flexibility (41). This study has predicted a number of B-cell epitopes on HL-PLD1 based on different features. Parameters like hydrophilic property, flexibility, surface attainability, turns, exposed surface, and antigenic tendency were in correlation with the continuous epitopes' location. Using BCPREDS, 8 potential antigenic epitopes were predicted on the HL-PLD1. Likewise, according to the ABCpred results, 10 out of 16 epitopes predicted on the HL-PLD1 could be used as vaccine targets. The IEDB results demonstrated several potential epitopes on the HL-PLD1. Jahangiri et al. (2019) adopted the ABCpred, BCPREDS, IEDB, and Bcepred bioinformatics to predict linear B-cell epitopes on *Plasmodium vivax* Apical Membrane Antigen (*Pv*AMA-1) considering the existence of a number of potential epitopes on AMA-1 that are a good choice for diagnostic and vaccine studies (21). Bueno et al. (2011) employed a BepiPred server to define a highly antigenic B-cell epitope within PvAMA-1 vaccine choices and showed that it had serological reactivity as the natural infection occurred (42). Using ABCpred, BCPREDS, Bcepred and BepiPred, Zobayer et al. (2018) predicted some linear B-cell epitopes. In another study, two 9-mer peptides (i.e., GDRIPDEKN and PHVPEYSSS) were reported to be the best B-cell epitopes of Api m3 allergen of *Apis mellifera* (43). Cao et al. (2016) identified six highly antigenic B-cell epitopes of three major toxin components of *Deinagkistrodon acutus* by using the bioinformatics analysis (44). In their study, Batista Oliveira et al. (2016) introduced FDDNANPEY-TYHGIP and DKVGHDFSGNDDISDVGK peptides as good choices for inducing a protective antibody response to sphingomyelinase D from the venom of Loxosceles intermedia spider (17).

CONCLUSION

In this study, using reliable bioinformatics tools, important aspects of Hl-PLD1protein such as its chemical and physical properties, hydrophilicity, structures, antigenicity, and B-cell epitopes were identified. To the best of our knowledge, scant attention has been paid to predicting and analyzing chemical, physical, and biological properties of Hl-PLD1 comprehensively. In this study, the Hl-PLD1 protein sequence was analyzed using online software including Bcepred, ABCpred, and BCPREDS. The findings showed that GHMVNAVKQI and VTFDDNGNPKWTYHGTPCDC peptides, having the highest safety scores of 0.80 and 0.963 respectively, could be useful for conducting in vivo studies with the aim of designing a highly effective antivenom against Hl-PLD1.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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