OPEN ACCESS Freely available online

Immunome Research

Research Article

Immunoinformatics Prediction of Epitope Based Peptide Vaccine against Listeria Monocytogenes Fructose Bisphosphate Aldolase Protein

Mustafa Elhag*, Mustafa Abubaker, Nagla Mohamed Ahmed

Department of Bioinformatics, Africa City of Technology, Sudan

ABSTRACT

Listeria monocytogenes represents an important food-borne pathogen worldwide that can cause life-threatening listeriosis disease especially in pregnant women, fetuses, elderly people, and immuno-compromised individuals with high mortality rates. Moreover, no vaccine against it exists. This study predicts an effective epitope-based vaccine against Fructose 1,6 Bisphosphate Aldolase (FBA) enzyme of *Listeria monocytogenes* using immunoinformatics approaches. The sequences were retrieved from NCBI and several prediction tests were conducted to analyze possible epitopes for B-cell, T-cell MHC class I and II. 3D structure of the most promising epitopes was obtained. Two epitopes showed high binding affinity for B-cells, while four epitopes showed high binding affinity for MHC-I and MHC-II. The results were promising to formulate a vaccine with more than 98% population coverage. We hope that these promising epitopes serves as a preventive measure for the disease in the future and recommend *in vivo* and *in vitro* studies.

Keywords: Epitope; Fructose-1,6-bisphosphatealdolase; Immunoinformatics; Enzyme

INTRODUCTION

Bacteria of the genus *Listeria* that are widely distributed in the environment comprise a group of gram-positive, facultative anaerobe, non-sporulating rods. It consists of ten different species with *Listeria monocytogenes* commonly found in humans [1]. *L. monocytogenes* represents an important food-borne pathogen worldwide, that can cause life-threatening listeriosis disease in the susceptible groups including pregnant women, fetuses, elderly people, and immuno-compromised individuals, with a considerable mortality rate (20%–30%). The disease includes, but not limited to sepsis, meningitis, encephalitis, spontaneous abortion, or fever and self-limiting gastroenteritis in a healthy adult. Food-borne listeriosis has a global economic and health burden due to the wide spread of *L. monocytogenes* in food and food processing environments [2]. It capable of proliferating in different stressful environmental conditions, including high salinity, low temperature, and a wide range of pH values. Although the incidence of listeriosis is relatively rare compared to other food-borne illnesses, listeriosis accounts for approximately 19% of deaths among all food-borne illness [3]. Studies have shown that *L. monocytogenes* is the third leading cause of death from food-borne illness in the United States, with approximately 260 deaths annually. Mortality rates with confirmed *L. monocytogenes* infections are around 15% but can be higher depending on patient status and comorbidities. *L. monocytogenes* has been responsible for several fatal outbreaks involving ready-to-eat meat, dairy products, and fish products, and most recently fruits and vegetables. It has 13 different serotypes based on a variety of flagella and surface antigens [4].

They reported FBA as a novel immunogenic surface target useful for the detection of the genus *Listeria*. 30-kDa protein is a fructose-1,6-bisphosphate aldolase, an enzyme of the glycolytic pathway that catalyzes the cleavage of its substrate fructose-1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate [5]. There are two main classes of FBA: class I is known to form tetramers, and is present mainly in higher eukaryotes, such as animals, plants, and algae; while class II can form many different multimers, and is present mainly in bacteria. Class II FBA has been studied as a potential target of new antibiotics and as vaccine antigen. Besides, many studies have shown that FBA may play a role in pathogenesis by interacting with host's plasminogen or promoting adhesion to

Corresponding Author: Mustafa Elhag, Department of Bioinformatics, Africa City of Technology, Sudan; E-mail:mustafa.alfakih@gmail.com

Received: May 20, 2021; **Accepted:** June 3, 2021; **Published:** June 10, 2021

Citation: Elhag M, Abubaker M, Ahmed NM (2021) Immunoinformatics Prediction of Epitope Based Peptide Vaccine Against Listeria Monocytogenes Fructose Bisphosphate Aldolase Protein. Immunome Res. 17:191.

host's cells. Thus, FBA is considered a moonlighting protein in many species and may have significant role in both physiology and pathogenesis. Understanding of epitope/antibody interaction is the key to construct potent vaccines and effective diagnostics [6]. These epitopes are capable of inducing B cell and T cell mediated immunity. Most of immunoinformatics researches are stressed on the design and study of algorithms for mapping potential B- and T-cell epitopes that speed up the time and lower the costs needed for laboratory analysis of pathogen products. Using such tools and information to analyse the sequence areas with potential binding sites can lead to the development of new vaccines [7]. The aim of this study is to predict an effective epitope-based vaccine against FBA enzymes of *L. monocytogenes* using immunoinformatics approaches. No previous reports were found in *L. monocytogenes* epitope based vaccine so this may be considered the first study to use insilico approach to design an epitope-based vaccine.

MATERIALS AND METHODS

Protein sequence retrieval

A total of 1750 *Listeria monocytogenes* FBA strains were retrieved from National Center for Biotechnology Information (NCBI) database on March 2019 in FASTA format. These strains were collected from different parts of the world for immunoinformatics analysis. The retrieved protein strains had length of 284 with name fructose-1,6-bisphosphate aldolase [8].

Determination of conserved regions

The retrieved sequences of *L. monocytogenes* FBA were subjected to Multiple Sequence Alignment (MSA) using ClustalW tool of Sequence Alignment Editor Software version 7.2.5 to determine the conserved regions. Molecular weight and amino acid composition of the protein were also obtained.

Sequenced-based method

The reference sequence of *L. monocytogenes* FBA was submitted to different prediction tools at the Immune Epitope Database (IEDB) analysis resource to predict various B and T cell epitopes. Conserved epitopes would be considered as candidate epitopes for B and T cells [9].

B cell epitope prediction

B cell epitopes is the portion of the vaccine that interacts with B lymphocytes. Candidate epitopes were analysed using several B cell prediction methods from IEDB, to identify the surface accessibility, antigenicity and hydrophilicity with the aid of random forest algorithm, a form of unsupervised learning. The bepipred linear epitope prediction 2 was used to predict linear B cell epitope with default threshold value 0.533. The emini surface accessibility prediction tool was used to detect the surface accessibility with default threshold value 1.000. The kolaskar and tongaonker antigenicity method was used to identify the antigenicity sites of candidate epitope with default threshold value 1.032. The parker hydrophilicity prediction tool was used to identify the hydrophilic, accessible, or mobile regions with default threshold value 1.695.

T cell epitope prediction MHC class I binding

T cell epitopes is the portion of the vaccine that interacts with T lymphocytes. Analysis of peptide binding to the Major Histocompatibility Complex (MHC) class I molecule was assessed by the IEDB MHC-I prediction tool to predict cytotoxic T cell epitopes. The presentation of peptide complex to T lymphocyte undergoes several steps. Artificial Neural Network (ANN) 4.0 prediction method was used to predict the binding affinity. Before the prediction, all human allele lengths were selected and set to 9 amino acids. The half-maximal inhibitory concentration (IC50) value required for all conserved epitopes to bind at score less than 100 were selected [10].

T cell epitope prediction MHC class II binding

Prediction of T cell epitopes interacting with MHC Class II was assessed by the IEDB MHC-II prediction tool for helper T cells. Human allele references set were used to determine the interaction potentials of T cell epitopes and MHC Class II allele. NN-align method was used to predict the binding affinity. IC50 values at score less than 500 were selected.

Population coverage

In IEDB, the population coverage link was selected to analyse the epitopes. This tool calculates the fraction of individuals predicted to respond to a given set of epitopes with known MHC restrictions. The appropriate checkbox for calculation was checked based on MHC-I, MHC-II separately and combination of both.

Docking analysis and homology modelling

The predicted peptides were docked with MHC alleles with the least IC50 score and the highest population coverage. To dock the predicted peptides, MDockPeP and HPEPDOCK servers were used. The peptide sequences and MHC were submitted in FASTA and pdb formats. The 3D structure was obtained using raptorX. a protein structure prediction server developed by Xu group, excelling at predicting 3D structures for protein sequences without close homologs in the Protein Data Bank (PDB). USCF chimera was the program used for visualization and analysis of molecular structure of the promising epitopes.

RESULTS

Multiple sequence alignment

The conserved regions and amino acid composition for the reference sequence of *L. monocytogenes* FBA are illustrated in Figure 1 and 2 respectively. Glycine and alanine were the most frequent amino acids (Table 1).

Amino Acid	Number	Mol%
Ala	30	10.56

Cys	3	1.06
Asp	17	5.99
Glu	22	7.75
Phe	6	2.11
Gly	30	10.56
His	10	3.52
Ile	23	8.1
Lys	23	8.1
Leu	17	5.99
Met	8	2.82
Asn	10	3.52
Pro	11	3.87
Gln	7	2.46
Arg	3	1.06
Ser	14	4.93
Thr	14	4.93
Val	28	9.86
Trp	2	0.7
Tyr	6	2.11

Table 1: List of conserved peptides with their antigenicity, emini surface accessibility and parker hydrophilicity scores.

B-cell epitope prediction

The reference sequence of *L. monocytogenes* FBA was subjected to bepipred linear epitope 2, emini surface accessibility, kolaskar and tongaonkar antigenicity and parker hydrophilicity prediction methods to test for various immunogenicity parameters. Two epitopes have successfully passed the three tests. 3D structure of the proposed B cell epitopes is shown (Figure 1).

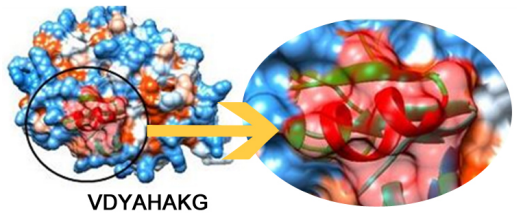

Figure 1: Proposed B cell epitope. The arrow shows position of (VDYAHAKG) with red colour at structural level using chimera software.

Prediction of cytotoxic T-lymphocyte epitopes and modelling

The reference sequence was analyzed using MHC-I binding prediction tool to predict T cell epitopes interacting with different types of MHC-I alleles, based on Artificial Neural Network (ANN) with half-maximal inhibitory concentration. 61 peptides were predicted to interact with different MHC-I alleles. The most promising epitopes and their corresponding MHC-I alleles are shown in followed by the 3D structure of the proposed T cell epitope (Figure 2).

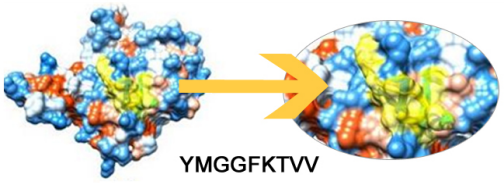

Figure 2: Proposed T cell epitopes that interact with MHC-I. The arrow shows position of (YMGGFKTVV) with yellow colour at structural level using chimera software.

Prediction of the helper T-lymphocyte epitopes and modelling

Reference sequence was analyzed using MHC-II binding prediction tool based on NN-align with half-maximal inhibitory concentration there were 388 predicted epitopes found to interact with MHC-II alleles. The most promising epitopes and their corresponding alleles are shown in along with the 3D structure of the proposed epitope (Figure 3).

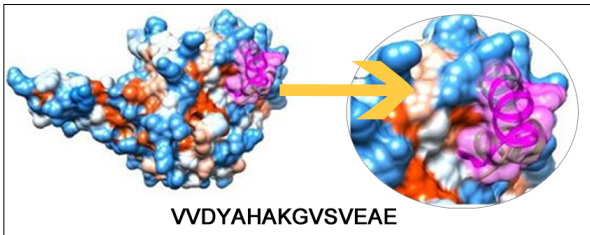

Figure 3: Proposed T cell epitopes that interact with MHC-II. The arrow shows position of (VVDYAHAKGVSVEAE) with magenta colour at structural level using chimera software.

Population coverage analysis

All MHC-I and MHC-II epitopes were assessed for population coverage against the whole world using IEDB population coverage tool. For MHC-I, epitopes with highest population coverage were YMGGFKTVV (45.36) and ALAAALGSV (40.6). For MHC class II, the epitopes that showed highest population coverage were VVDYAHAKGVSVEAE (69.26) and GLVEDLKITVPVAIH & LVEDLKITVPVAIHL (66.79) (Table 3). When combined together, the epitopes that showed highest population coverage were VVDYAHAKGVSVEAE (69.26) and GLVEDLKITVPVAIH & LVEDLKITVPVAIHL (66.79).

Epitope	Coverage Class II (%)	Total HLA hits
AAKYMGGFKTVVKMT	39.48	6
ADPQECLRVVKEANI	28.88	5
AGKYAVGQFNINNLE	22.54	6

AHAKGVSVEAEIGTV	0	4
AIELGHSKINVNTEC	3.02	2
AIHLDHGSSFDSCKA	23.9	3
AKGVSVEAEIGTVGG	0	1
AKYMGGFKTVVKMTE	35.55	8
ALAAALGSVHGPYHG	46.79	7
ALAGKYAVGQFNINN	18.23	4

Table 2: Population coverage of proposed peptides interaction with MHC class II.

DISCUSSION

In the present study, we proposed different peptides that can be recognized by B cell and T cell to produce antibodies against FBA of *Listeria monocytogenes* for the first time. Peptide vaccines overcome the side effects of conventional vaccines. Peptide vaccines are characterized by easy production, stimulating effective immune response and no potential infection possibilities. The reference sequence of *L. monocytogenes* FBA was subjected to Bepipred linear epitope prediction 2 test, Emini surface accessibility test and Kolaskar and Tongaonkar antigenicity test and Parker hydrophilicity test in IEDB, to determine the binding to B cell and to test the immunogenicity and hydrophilicity respectively. The reference sequence was analyzed using IEDB MHC-I and II binding prediction tools to predict T cell epitopes. 26 epitopes were predicted to interact with MHC-I alleles with IC50<100. Four of them were most promising and had the affinity to bind the highest number of MHC-I alleles (ITVPVAIHL, MPIVNMTDM, MLKKALAGK, YMGGFKTVV). Eleven epitopes (YAHAKGVSV, ALAAALGSV, TVVKMTEGL, MLKKALAGK, ITVPVAIHL, HAKGVSVEA, IVNMTDMLK, ALGSVHGPY, KALAGKYAV, NIDALAAAL, LAAALGSVH) appeared in both MHC-I and II results.

The best epitope with the highest population coverage for MHC-I was YMGGFKTVV with 45.36% in two HLA hits, and the coverage of population set for whole MHC-I epitopes was 94.15%. Excluding certain alleles for MHC-II, the best epitope was VVDYAHAKGVSVEAE scoring 69.26% with thirteen HLA hits and the coverage of population set was 80.8% for the whole MHC-II epitopes. These epitopes could potentially induce T-cell immune response when interacting strongly with MHC-I and MHC-II alleles effectively generating cellular immune response against the invading pathogen. The peptide VVDYAHAKGVSVEAE had the highest population coverage percent 69.26 in thirteen HLA hits for both MHC-I and MHC-II. Recent outbreaks of *L. monocytogenes* demands the development of vaccine especially for immuno-compromised, elderly people and pregnant women. Many studies had predicted peptide vaccines for different microorganisms such as COVID-19, Pseudomonas, Rubella, Ebola, Dengue, Zika, HPV, Lagos rabies virus and Treponema pallidum using immunoinformatics tools. Limitations of the study include the exclusion of certain HLA alleles for the MHC-II.

CONCLUSION

Vaccination is a method to protect and minimize the possibility of infection. Design of vaccines using *insilico* prediction methods is highly appreciated due to the significant reduction in cost, time and effort. Peptide vaccines overcome the side effects of conventional vaccines. We presented different peptides that can produce antibodies against FBA of *Listeria monocytogenes* for the first time. Two B cell epitopes passed the antigenicity, accessibility and hydrophilicity tests. Four MHC-I epitopes were the most promising ones, while four for MHC-II. Eleven epitopes were shared between MHC I and II. For the population coverage, the epitopes covered 98.94% of the alleles worldwide excluding certain alleles.

ACKNOWLEDGMENT

Many thanks go to National Center for Biotechnology Information (NCBI), BioEdit, Immune Epitope Database Analysis Resource (IEDB), RaptorX server, and UCSF chimera team. We are grateful to Editor-in-Chief and all the unknown reviewers for their valuable comments on the previous version of this manuscript.

REFERENCES

1. Hain T, Chatterjee SS, Ghai R, Kuenne CT, Billion A, Steinweg C, et al. Pathogenomics of Listeria spp. Int J Med Microb.2007;297(7-8): 541-557.
2. Hunt K, Blanc M, Alvarez-Ordonez A, Jordan K, et al. Challenge Studies to Determine the Ability of Foods to Support the Growth of Listeria monocytogenes. Pathogens. 2018;7(4):80.
3. Lomonaco S, Decastelli L, Nucera D, Gallina S, Bianchi DM, Civera T, et al. Listeria monocytogenes in Gorgonzola: Subtypes, diversity and persistence over time. Int J Food Microb. 2009;128(3):516-520.
4. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, et al. Foodborne Illness Acquired in the United States—Major Pathogens. Emerg Infect Dis. 2011;17(1):7-15.
5. Jackson KA, Gould LH, Hunter JC, Kucerova Z, Jackson B. Listeriosis outbreaks associated with soft cheeses, United States, 1998–2014. Emerg Infect Dis. 2018;24(6):1116-1118.
6. Silk BJ, Date KA, Jackson KA, Pouillot R, Holt KG, Graves LM, et al. Invasive listeriosis in the Foodborne Diseases Active Surveillance Network (FoodNet), 2004–2009: Further targeted prevention needed for higher-risk groups. Clin Infect Dis. 2012. 54(5):S396-S404.
7. Katebi AR, Jernigan RL. Aldolases utilize different oligomeric states to preserve their functional dynamics. Biochem. 2015;54(22):3543-3554.
8. Daher R, Coincon M, Fonvielle M, Gest PM, Guerin ME, Jackson M, et al. Rational design, synthesis, and evaluation of new selective inhibitors of microbial class II (zinc dependent) fructose bis-phosphate aldolases. J Med Chem. 2010;53(21):7836-7842.

9. Chaves EGA, Weber SS, Bao SN, Pereira LA, Bailao AM, Borges CL, et al. Analysis of Paracoccidioides secreted proteins reveals fructose 1,6-bisphosphate aldolase as a plasminogen-binding protein. BMC Microbiol. 2015;15(1): 53.

10. Blau K, Portnoi M, Shagan M, Kaganovich A, Rom S, Kafka D, et al. Flamingo cadherin: A putative host receptor for Streptococcus pneumoniae. J Infect Dis. 2007;195(12):1828-1837.