



Proteome Screening, Identification and Designing of Potent Antigenic Peptides of Neisseria Meningitides: A Cause of Meningitis

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Received: March 5th 2014, Accepted: March 14th 2014, Published: May 1st 2014

Abstract:

Meningitis is an acute inflammation of the protective membranes covering the brain and spinal cord, known collectively as the meninges. Meningitis can be life-threatening because of the inflammation's proximity to the brain and spinal cord; therefore, the condition is classified as a medical emergency. The scope of the current work is to predict and design a potent antigenic peptide of the bacteria Neisseria Meningitides so as to develop a potential vaccine. The bacterial proteome is collected from NCBI database followed by the screening for the foreign proteins. The proteins were further screened for the identification of most antigenic sites within them. The selected antigenic peptides were designed using Argus Lab software followed by the geometry optimization and energy evaluation. Based on the energy values the best antigenic peptides were selected and can be further processed for the production of potential vaccine to eradicate the disease.

Keywords: PVS Server, Antigenic emboss, peptide designing.

Introduction:

Meningitis is an acute inflammation of the protective membranes covering the brain and spinal cord, known collectively as the meninges. The inflammation may be caused by infection with viruses, bacteria, or other microorganisms, and less commonly by certain drugs. Meningitis can be life-threatening because of the inflammation's proximity to the brain and spinal cord; therefore, the condition is classified as a medical emergency. The most common symptoms of meningitis are headache and neck stiffness associated with fever, confusion or altered consciousness, vomiting, and an inability to tolerate light (photophobia) or loud noises (phonophobia). Children often exhibit only nonspecific symptoms, such as irritability and drowsiness. If a rash is present, it may indicate a particular cause of meningitis; for instance, meningitis caused by meningococcal bacteria may be accompanied by a characteristic rash.

A lumbar puncture diagnoses or excludes meningitis. A needle is inserted into the spinal canal to extract a sample of cerebrospinal fluid (CSF) that envelops the brain and spinal cord. The

CSF is examined in a medical laboratory. The first treatment in acute meningitis consists of promptly administered antibiotics and sometimes antiviral drugs. Corticosteroids can also be used to prevent complications from excessive inflammation. Meningitis can lead to serious long-term consequences such as deafness, epilepsy, hydrocephalus and cognitive deficits, especially if not treated quickly. Some forms of meningitis (such as those associated with meningococci, *Haemophilus influenzae* type B, pneumococci or mumps virus infections) may be prevented by immunization.

Materials and methods:

Protein sequence analysis:

The Protein sequences of N.Meningitides were retrieved from NCBI database. It is a readily available website which is used to display publicly available information on Prokaryotic protein sequences in FASTA format.

Primary screening of proteome: In primary screening all the non required protein sequences are removed (i.e., hypothetical, putative, predicted). These sequences are removed because the role of these sequences in pathogenesis is not known clearly.

Secondary screening: In secondary screening P BLAST ⁽⁷⁾ analysis is done against the *Homo sapiens* to identify the sequences that have no significant or less identical matches (<33%) with the protein sequences of the *Homo sapiens*.

Tertiary screening: In tertiary screening TFASTY ⁽⁸⁾ is done to check the similarity with *Homo sapiens*, coding DNA sequences (CDS). TFASTY compares a protein sequence to a DNA sequence database, calculating similarities with frame shifts to the forward and reverse orientations. The sequences which showed no similarity or <33% similarity with human CDS were taken and then the further analysis was done.

Antigenic Site Determination:

To find out the antigenic sites within the proteins which we got after the tertiary screening Antigenic EMBOSS ⁽⁹⁾ was performed with those sequences.

Antigenic peptide prediction (Protein variability server) ⁽¹⁰⁾ was used to verify the antigenic sites which give result in forms of peaks. Both finds out the potential antigenic regions of a protein sequence.

Calculation of Surface accessibility area:

It is the surface area of a biomolecule that is accessible to a solvent .Surface accessibility area of antigenic peptides which we got from the antigenic site determination was calculated to find out the protein having highest pathogenesis with specific sites.

Designing and Optimization of the vaccine:

The potential candidate vaccine was designed and optimized by using ArgusLab 4.0.1.

Result and discussion:

BLAST Analysis:

Fasta sequences are selected after the BLAST results based on low sequence similarity.

Antigenic Site Determination:

Results from Antigenic Emboss gave the idea of the antigenic sites presents within these proteins along with antigenic score. Sites having low SAA were considered (Table No.1) and further confirmation for the selection of antigenic sites was done with the Antigenic peptide prediction (Protein variability server) method {Table No. 2(i, ii, iii)}.Highlighted areas shows the same sequence as in Antigenic EMBOSS, which were taken for further analysis.

Table No-1: Antigenic sites obtained from Antigenic EMBOSS

SL.NO.	PROTEIN NAME	ANTIGENIC SITES/ EPITOPES	PEPTIDE	SAA (SURFACE ACCECIBILITY AREA)	START POSITION	END POSITION
1.	Structure Of A N. Meningitides Protein	(7)	GGSYALRVQGE	2/11= 0.18	54-G	64-E
2.	catabolite repression control protein	(12)	EYIYCGSLYVAHQKM	2/15= 0.13	142-E	156-M
3.	exonuclease III	(12)	ARLHQLQAVIDK	3/12= 0.25	13-A	24-K
4.	Exodeoxyribonuclease III	(12)	DHAPVVVD	3/8= 0.38	262-D	269-D

Table No-2: Antigenic sites obtained from Protein Variability Server

There are 6 antigenic determinants in your sequence:

n	Start Position	Sequence	End Position
1	13	FVDMASLPAEMPLIPVNGADTLIVDGEAVSLT	44
2	57	YRYLTYGA	64
3	68	PGGSYALRVQG	78
4	87	AGTAVYNGEVLHF	99
5	113	FAAKVDFGS	121
6	174	VAGKYSY	180

There are 11 antigenic determinants in your sequence:

n	Start Position	Sequence	End Position
1	7	RIISVNVNGIQA	18
2	20	AERGLLSWLQAQNADVICLQ	39
3	44	SAFDLDDPSFQLDGFLYACDA	65
4	69	EQGGVALYSRLQPKAVISG	87
5	96	YGRYLQADFDKVSIALLLPS	116
6	146	REYIYCGSLYVAHQK	160
7	169	CQQMPGFLAPE	179
8	182	WLDEVFGN	189
9	191	GYADALRE	198
10	224	FDYQVLTTP	231
11	247	RFSQHAPLIVDYDW	260

There are 13 antigenic determinants in your sequence:

n	Start Position	Sequence	End Position	n	Start Position	Sequence	End Position
1	4	IIIGSLMK	12	1	4	IIMPYR	10
2	22	RARLHQLQAVID	33	2	22	NSIRARVDRVTD	33
3	35	HQPDVIG	41	3	36	ERADVVDLA	44
4	47	VHDDQFPLKD	56	4	56	PTMPFAALGYDVVHCGF	72
5	58	EAMGYHVVIYHGQ	69	5	74	QWNGVAIASRVGIDAVEV	91
6	71	GHYGVAMLSK	80	6	109	AEARALGATCGGVRVWSLYVFN	130
7	104	GDYPLADGGHVRILNG	119	7	134	IDSPHYTYKWKWLAALH	150
8	139	FYQDLMS	145	8	161	PSTPVALVGD	170
9	152	SPEQPVIVMG	161	9	172	NIAPVDD	178
10	164	NISRIDLDI	172	10	180	VWDISLSESTHVTA	194
11	184	KTGKCSF	190	11	199	AFNAIVDAGFTDVVRP	214
12	237	LRIDLILATKALAKQ	251	12	240	IDFILGSPTLAELVTHA	256
13	268	SDHAPVWS	275	13	274	SDHAPVWV	281

Table No-2: Peptide with Surface accessibility area (SAA)

Protein	Method used to predict antigenic site	Sequence	SAA
1.	Antigenic Emboss	GGSYALRVQGE	2/11= 0.18
	Protein Variability Server	PGGYALRVQG	2/11=0.18
2.	Antigenic Emboss	EYIYCGSLYVAHQKM	2/15= 0.13
	Protein Variability Server	REYIYCGSLYVAHQK	3/15=0.2
3.	Antigenic Emboss	ARLHQLQAVIDK	3/12= 0.25
	Protein Variability Server	RARLHQLQAVID	3/12=0.25
4.	Antigenic Embo	DHAPVVVD	3/8= 0.38
	Protein Variability Server	SDHAPVVV	3/8=0.37

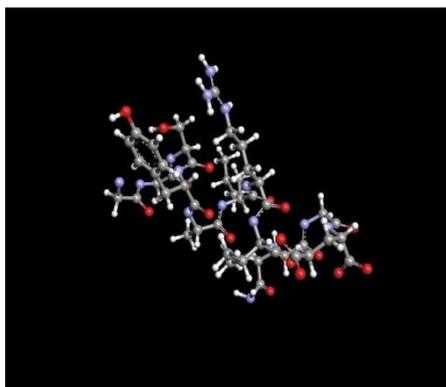
Designing and Energy Optimization:

The peptides i.e.1st GGSYALRVQGE, 2nd EYIYCGSLYVAHQKM, 3rd ARLHQLQAVIDK and 4th DHAPVVVD were designed using ArgusLab4.0.1. After designing the peptides, their geometry was optimized and energy minimization was performed using Swiss pdb Viewer for the peptide with low energy to make the structures stable.

Table No-3: Geometry Optimization using Argus Lab (4.0.1) and Energy Minimisation done by Swiss pdb Viewer table showing the details:

PEPTIDE NO.	PEPTIDE SEQUENCE	GEOMETRY OPTIMIZATION	ENERGY MINIMISATION
1	GGSYALRVQGE	78.78kcal/mol	-235.435
2	EYIYCGSLYVAHQKM	161.26kcal/mol	-207.054
3	DHAPVVVD	96.72kcal/mol	-455.926
4	ARLHQLQAVIDK	129.83kcal/mol	627.301

Figure No. 1: Designed peptide (1st Protein's antigenic site) after energy minimization using ArgusLab4.0.1



Conclusion:

In the above work the complete proteins of the Neisseria Meningitides were identified and the sequences were collected from NCBI Database. The sequences were further screened to identify the foreign proteins to the human proteome. These foreign proteins were further tested for the presence of antigenic sites using EMBOSS Antigenic. From among the selected epitopes some of them having maximum antigenic propensity were screened based on PVS server. The final selected peptides were designed in Argus Lab. The geometry of the peptides was optimized and the energy evaluation was performed. Based on the energy values the best peptide selected for the Vaccine development was Structure of A.N Meningitides. The peptide can be synthesized and attenuated so as to use it for vaccination against the Meningitis.

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