Reports of Biochemistry & Molecular Biology
Vol. 4, No. 1, Oct 2015

Original article

www.RBMB.net

In silico Analysis and Modeling of ACP-MIP–PilQ Chimeric Antigen from *Neisseria meningitidis* Serogroup B

Mehrdad Gholami[1], Alireza Salimi Chirani*[2], Mona Moshiri[3], Mansour Sedighi[1], Abazar Pournajaf[1], Masoud Tohidfar[4], Gholamreza Irajian[1]

Abstract

Background: *Neisseria meningitidis*, a life-threatening human pathogen with the potential to cause large epidemics, can be isolated from the nasopharynx of 5–15% of adults. The aim of the current study was to evaluate biophysical and biochemical properties and immunological aspects of chimeric acyl-carrier protein-macrophage infectivity potentiator protein-type IV pilus biogenesis protein antigen (ACP-MIP-PilQ) from *N. meningitidis* serogroup B strain.

Methods: Biochemical properties and multiple alignments were predicted by appropriate web servers. Secondary molecular structures were predicted based on Chou and Fasman, Garnier-Osguthorpe-Robson, and Neural Network methods. Tertiary modeling elucidated conformational properties of the chimeric protein. Proteasome cleavage and transporter associated with antigen processing (TAP) binding sites, and T- and B-cell antigenic epitopes, were predicted using bioinformatic web servers.

Results: Based on our *in silico* and immunoinformatics analyses, the ACP-MIP-PilQ protein (AMP) can induce high-level cross-strain bactericidal activity. In addition, several immune proteasomal cleavage sites were detected. The 22 epitopes associated with MHC class I and class II (DR) alleles were confirmed in the AMP. Thirty linear B-cell epitopes as antigenic regions were predicted from the full-length protein.

Conclusion: All predicted properties of the AMP indicate it could be a good candidate for further immunological *in vitro* and *in vivo* studies.

Keywords: Chimeric protein, *In silico*, *Neisseria meningitides*, Serogroup B, Vaccine

Introduction

Neisseria meningitidis (N. meningitidis) is an airborne, life-threatening pathogen that infects humans with an infectivity rate of approximately 1%. Thirteen serogroups of *N. meningitidis* have been classified based on the capsular immunologic reactivity (1). Five serotypes, including A, B, C, W-135, and Y are responsible for most clinical cases with significant morbidity and mortality (2, 3). Humans are the only host of *N. meningitidis*. Conjugate vaccines using two purified proteins target serogroups A, C, W-135, and Y. No vaccine against serogroup B presently exists, although two vaccines are in developmental stages (4, 5). The recently described 13-kDa adhesion complex protein (ACP) from *N. meningitidis* serogroup B strain MC58 has been proposed as a potential vaccine candidate (6). Based on amino acid sequence analysis from 13 meningococcal strains, three distinct ACP molecule types, dubbed I, II, and III, have been distinguished. The APC molecules in the 13 strains share >98%

1: Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
2: Department of Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3: Department of Pathobiology, Division of Microbiology, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran
4: Agricultural Biotechnology Research Institute of Iran, Tehran, Iran
***Corresponding author: Alireza Salimi Chirani; Tel: +98 2123872556; Fax: +98 2123872556,**
E-mail: Alireza.salimichirani@gmail.com
Received: Jan 10, 2015; Accepted: Mar 17, 2015

similarity, and induce cross-strain bactericidal activity and high levels of serum bactericidal activity. In addition, ACP as an adhesin plays a critical role during host-pathogen interactions, making it a good vaccine candidate (6). Macrophage infectivity potentiator protein (MIP) is a highly-expressed 29-kDa outer membrane protein in *N. meningitidis* serogroup B strain MC58 and is contributes to cross-strain serum bactericidal activity in *N. meningitidis* infections (7, 8). The *N. meningitidis* PilQ as Type IV pilus biogenesis protein is antigenically-conserved and abundant outer membrane protein (9, 10).

The aim of our study was to evaluate biophysical and biochemical properies and immunological aspects of the ACP-MIP-PilQ (AMP) from *N. meningitidis* serogroup B strain using *in silico* and immune informatic studies.

Materials and Methods

Sequence analysis

In this study three protein candidates; ACP with accession number NP_275083.1, MIP with accession number NP_274574.1, and PilQ with accession number NP_274809.1, from *N. meningitidis* serogroup B strain MC58, were selected. The nucleotide and amino acid sequences of the three candidates were retrieved in FASTA format from the gene bank (http://www.ncbi.nlm.nih.gov) for further analyses. Multiple sequences were aligned using ClustalW2 software (www.ebi.ac.uk/Tools/msa) and each sequence was BLASTed on the (http://www.ncbi.nlm.nih.gov/BLAST/) server. In protein drug development, the purpose of creating chimeric proteins is to impart several properties and target multiple platforms together; hence, we integrated three separate functional exposed domains using four helix-forming peptides Glutamic acid- Alanine- Alanine- Alanine- Lysine sequence, (EAAAK)4 as linker (11).

Primary structure and physico-chemical parameters of AMP

In this study AMP was analyzed using the Expasy ProtParam server (http://web.expasy.org/protparam/) (12). Physico-chemical parameters such as theoretical isoelectric point (pI), molecular weight, and the numbers of positively- (KRH) and negatively- (DE) charged amino acids were determined. Hydrophobic, hydrophilic, aromatic, and hydroxyl amino acids were identified, and the instability and aliphatic indexes, estimated charges at pH 7.00, and grand average of hydropathy (GRAVY) were predicted. All physico-chemical predictions were evaluated using the PBIL-IBCP Lyon-Gerland server (http://pbil.ibcp.fr/htm/index.php) and ExPASy's prediction tools (http://web.expasy.org/). Conserved domains were predicted by NCBI's Conserved Domain Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

Secondary and tertiary molecular structure of AMP

Institute for Genomics Bioinformatics (IGB) (http://www.igb.uci.edu) and Center of Informational Biology (http://cib.cf.ocha.ac.jp/index-e.xml) servers based on Chou and Fasman(13), Garnier-Osguthorpe-Robson (14), and neural network methods (15). The alpha helix, beta sheet, and random coil structure of AMP was predicted by the MINNOU server (16). The solubility of AMP was predicted with 94% accuracy. The alpha, beta, and gamma turns were predicted based on the neural network training on PSI-BLAST (http://www.imtech.res.in/). Transmembrane helices were predicted on the TMHMM data bank (17). Possible presence of transmembrane regions, a signal peptide, and phosphorylation and N-glycosylation sites were predicted using the Protter web server (http://wlab.ethz.ch/protter) (18). The three-dimensional (3D) structure of AMP was predicted by the I-TASSER web server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/). The prediction of the protein's tertiary molecular structure was based on the sequence homology comparison (19, 20).

Proteasome cleavage sites and TAP binding predictions

Proteasome (model 1-SE 0.874, SP 0.534) cleavage of the chimeric protein was predicted by the (http://imed.med.ucm.es) server (21). The transporter associated with antigen processing (TAP) binding affinity was predicted by the TAPPred online server (http://www.imtech.res.in/raghava/tappred/service) (22).

Epitope Mapping

The ProPred-I server (http://www.imtech.res.in/raghava/propred1/) predicted 53 alleles of MHC class I binding sites (23) and the ProPred server (http://www.imtech.res.in/raghava/propred/) predicted 51 alleles of MHC class II HLA-DR binding sites associated with the AMP (24). The AMP dependent B-cell epitopes were predicted by the IEDB-AR server (http://tools.immuneepitope.org).

Results

The sequences retrieved from the BLASTp program were associated with *N. meningitidis*. Tables 1, 2, and 3 summarize BLASTp results of APC, MIP, and PilQ, respectively. The NCBI BLASTp software indicated that the retrieved MIP sequence was related to peptidyl prolyl isomerase, a fraction of the high-abundance *N. meningitidis* MIP. The MIP amino acid sequences were 99 to 100% identical between *N. meningitidis* strains.

Table 1. Summary of BLASTp of ACP from *N. meningitidis*

Sequence ID	Length	Max score	Total score	Query cover	E value	Identities	Positive	Gap
NP_275083.1	124	254	254	100%	2e-87	100%	100%	0%
WP_002215074.1	124	252	252	100%	1e-86	99%	100%	0%
WP_012222154.1	186	253	253	100%	5e-86	100%	100%	0%
WP_011799040.1	186	251	251	100%	2e-85	99%	100%	0%

Table 2. Summary of BLASTp of MIP from *N. meningitidis*

Sequence ID	Length	Max score	Total score	Query cover	E value	Identities	Positive	Gap
CBA04183.1	302	549	549	100%	0.0	100%	100%	0%
WP_002212827.1	272	548	548	100%	0.0	100%	100%	0%
WP_002239187.1	272	547	547	100%	0.0	99%	100%	0%
WP_002236000.1	272	546	546	100%	0.0	99%	100%	0%

Table 3. Summary of BLASTp of PilQ from *N. meningitidis*

Sequence ID	Length	Max score	Total score	Query cover	E value	Identities	Positive	Gap
WP_002249070.1	769	709	709	100%	0.0	100%	100%	0%
WP_009348000.1	777	709	709	100%	0.0	100%	100%	0%
WP_002225652.1	769	710	710	100%	0.0	100%	100%	0%
EOC56528.1	223	709	709	100%	0.0	100%	100%	0%
WP_002249070.1	769	709	709	100%	0.0	100%	100%	0%

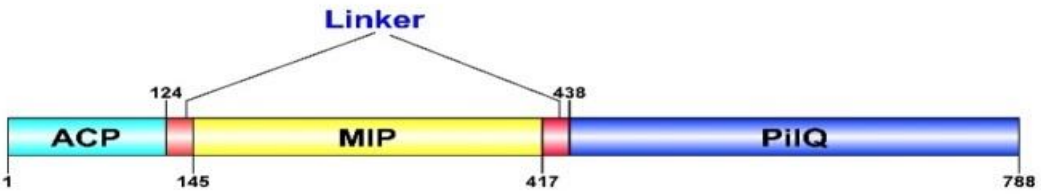

Fig. 1. Schematic of antigenic chimera of AMP genes with $(EAAAK)_4$ linkers.

The multiple alignments using ClustalW2 software revealed many conserved regions within the selected regions of ACP, MIP, and PilQ among most *N. meningitidis* serogroups. The multiple alignments of each protein are shown in supplementary Figs. S1, S2, and S3.

Primary structure analysis

The AMP contained 786 amino acids. Of these, 100 are positively-charged, and 89 are negatively charged. Its molecular weight is 83558.3 Daltons and the theoretical pI is 8.94. The *in vitro* the estimated half-life in mammalian reticulocytes is about 30 hours, whereas the *in vivo* estimated half-life in *Escherichia coli* was predicted as less than 10 hours. The AMP also contains 277 hydrophobic (ALIVMW), 327 hydrophilic (DEKNQRST), 41 aromatic (FYW), and 126 hydroxyl (STY) residues. The amino acid composition is summarized in Table 4.

Table 4. The amino acid composition of AMP

Amino acid	No. of residues	% of residues	Amino acid	No. of residues	% of residues
Ala (A)	100	12.7%	Lys (K)	78	9.9%
Arg (R)	22	2.8%	Met (M)	17	2.2%
Asn (N)	39	5.0%	Phe (F)	21	2.7%
Asp (D)	38	4.8%	Pro (P)	27	3.4%
Cys (C)	5	0.6%	Ser (S)	50	6.4%
Gln (Q)	35	4.5%	Thr (T)	59	7.5%
Glu (E)	51	6.5%	Trp (W)	3	0.4%
Gly (G)	66	8.4%	Tyr (Y)	17	2.2%
His (H)	1	0.1%	Val (V)	48	6.1%
Ile (I)	49	6.2%	Pyll (O)	0	0.0%
Leu (L)	60	7.6%	Sec (U)	0	0.0%

The conserved domains of the AMP were predicted using PRS-BLAST. The protein contains six distinct functional regions. The first and forth domains were identical to bacterial type II and III secretion systems. The second, third, and fifth domains belonged to the isomerase family. The predicted domains are given in Table 5.

Table 5. Predicted domain of the recombinant protein by PRS-PLAST

Name	Accession number	Description	Interval	E-value
Secretin	pfam00263	Bacterial type II and III secretion system protein	617-778	2.11e-53
FKBP_N	pfam01346	FKBP-type peptidyl-prolylisomerase	187-299	1.34e-37
FKBP_C	pfam00254	FKBP-type peptidyl-prolylcis-trans isomerase	306-394	2.15e-32
Secretin_N	pfam03958	Bacterial type II/III secretion system short domain	460-529	1.01e-11
FkpA	COG0545	FKBP-type peptidyl-prolylcis-trans isomerases	186-396	8.09e-76
pilus_MshL	TIGR02519	Pilus (MSHA type) biogenesis protein MshL	498-777	3.47e-34

Secondary structure prediction

The secondary structure prediction showed that the AMP contains 15 alpha helixes, 33 beta strands, and 48 random coils. The secondary structure was predicted using the MINNNOU server (Fig. 3). The joint results from both the IGB server (Fig. 2) and the MINNNOU web site were identical.

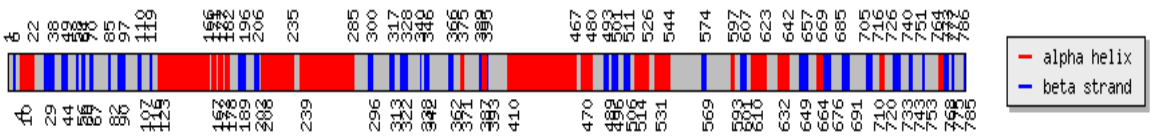

Fig. 2. The Joint result of secondary structure prediction based on Chou and Fasman, Garnier-Osguthorpe-Robson, and neural network methods

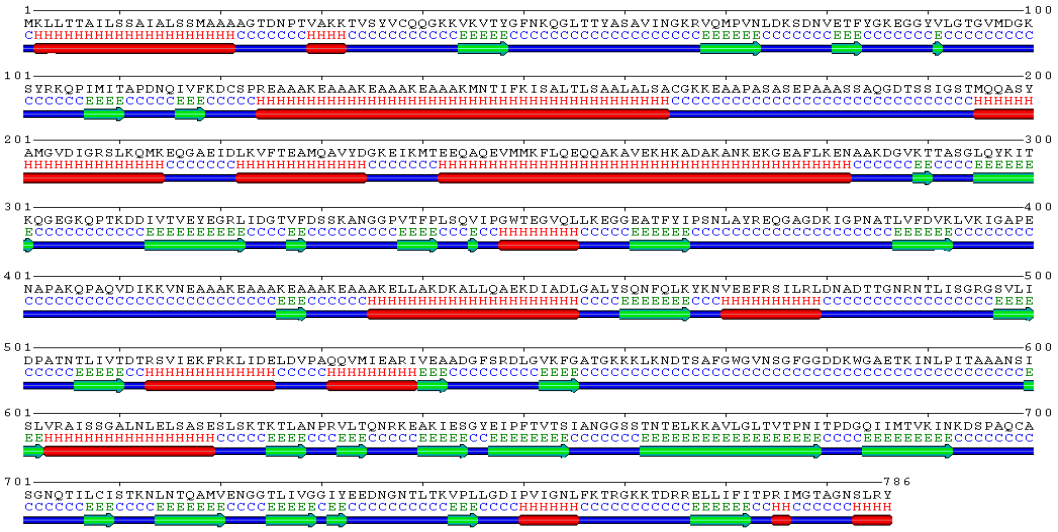

Fig. 3. The secondary structure prediction of chimeric AMP based on relative solvent accessibility

The secondary structure profile of AMP, according to the position and number of each structure, is shown in Table 6. No transmembrane helix topology was identified in the sequence using the TMHMM data bank. The sequence of AMP also was analyzed for putative signal peptide, N-glycosylation, and phosphorylation sites using the Protter web server. The Protter analysis predicted an N-terminal signal peptide region (red) from amino acids 1 to 21, and five N-linked glycosylation sites (green) at positions 26, 384, 564, 682, and 703. No phosphorylation sites or transmembrane regions were identified. The results are illustrated on supplement S4.

Table 6. The secondary structure profile of AMP according to the position of each conformation

Secondary structure	Position
Alpha helix (Hh)	2-21,29-32,124-164,195-214,222-234,242-282,348-355,435-455,470-479,513-525,531-539,603-619,750-755,775-776,783-786
Extended strand (Ee)	44-48,68-73,81-83,91,107-110,116-118,289-290,295-301,313=322,327-328,338-341,345,361-366,387-392,426-428,460-466,497-500,506-510,540-542,552-555,600-602,625-628,632-634,640-644,647-654,662-679,684-692,707-709,714-720,725-729,731-732,743-745,767-772
Random coil (Cc)	22-28,33-43,49-67,74-80,84-90.92-106,111-115,119-123,165-194,215-221,235-241,283-288,291-294,302-312,323-326,329-337,342-344,346-347,356-360,367-386,393-425,429-434,456-459,467-469,480-496,501-505,511-512,526-530,543-551,556-599,620-624,629-631,635-639,645-646,655-661,680-683,693-706,710-713,721-724,730,733-742,746-749,756-766,773-774, 777-782
Alpha turn (Aa)	21-30,76-80,96-105,171-190,306-310,331-335,356-360,371-385,421-430,481-495,561-565,581-600,656-660,736-745,761-765,781-785
Beta turn (Tt)	1-2,17-33,36-42,46-56,63-67,73-90,94-107,111-116,129-141,168-192,210-219,232-240,265-270,283-294,300-312,322-326,330-345,356-360,368-385,396-406,415-436,448-454,464-468,477-496,501-505,525-529,547-550,556-600,606-611,619-629,635-639,643-651,655-660,679-683,691-704,707-716,720-724,733-750,755-765,771-786
Gamma turn (Gg)	1-3,15-17,19-33,39-42,51-57,64-66,74-81,85-89,94-106,111-115,120-127,131-134,136-141,167-193,212-221,234-241,266-271,282-288,290-294,301-311,323-326,330-337,341-346,356-360,371-385,397-407,417-436,448-454,465-469,479-496,502-505,527-529,557-568,572-574,576-600,607-609,621-624,636-638,656-660,680-683,692-696,698-704,721-724,734-750,757-765,778-786

Hydropathy prediction

Hydropathy was predicted by two Kyte-Doolittle and Hopp-Woods hydropathy plots, which can indicate potential transmembrane and surface regions.

The positive values in the Kyte-Doolittle plot indicate hydrophobic amino acids, which may be parts of alpha helixes spanning lipid bilayers, whereas the Hopp-Woods scale (25) indicates hydrophilic, residues and use to identify potential antigenic sites in protein sequences.

The Kyte-Doolittle plot of the AMP (Fig. 4) showed six short significant hydrophobic regions (gray) around amino acids 150, 390, 500, 600, 670, and 760. In the Hopp-Woods scale, the negative values assigned to apolar residues; thus, antigenic sites are likely to be in the positive region. In Fig. 5, the Hopp-Woods plot illustrates nine potentially antigenic areas in the chimer.

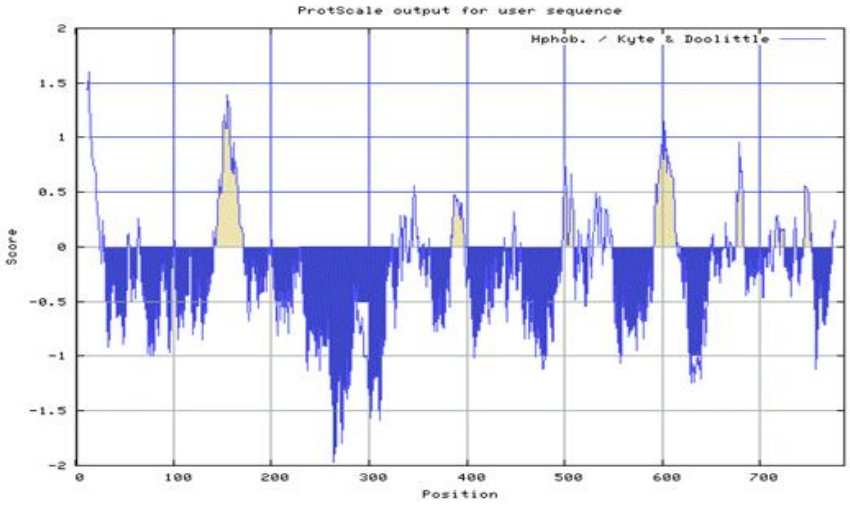

Fig. 4. The Kyte-Doolittle plot of the AMP

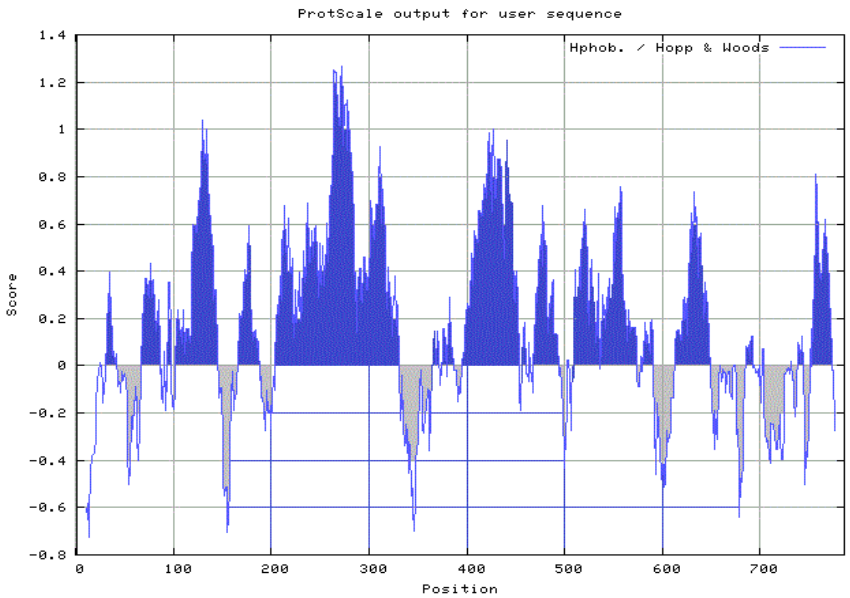

Fig. 5. The Hopp-Woods plot of AMP

In proteomics studies, tertiary structure refers to geometric shape, and is symbolic of single-, double-, or triple-bonded protein molecules. The I-TASSER server is an on-line platform for protein structure predictions. The 3D model is based on multiple-threading alignments and iterative template fragment assembly simulations with the goal to provide the most accurate structural and functional predictions using state-of-the-art algorithms. The 3D structure of the AMP is shown in Fig. 6.

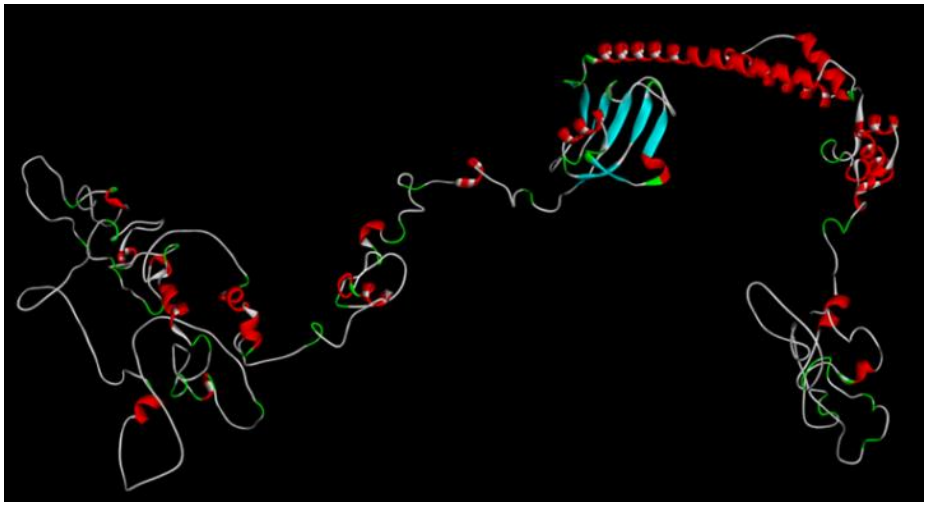

Fig. 6. The 3D structure of the AMP

Proteasome cleavage sites and TAP binding predictions

Proteasome degradation is critical to the function of the adaptive immune system. Antigenic peptides are displayed by the major histocompatibility complex (MHC) class I proteins on the surface of antigen-presenting cells. The analysis predicted 203 proteasome and 367 immunoproteasome cleavage sites in the AMP; of these, 192 sites were identified as both proteasome and immunoproteasome cleavage sites. The TAP transporter is found in the endoplasmic reticulum (ER) organelle, associated with the peptide-loading complex (PLC). The ten high affinity TAP-binding sequences associated with the AMP are shown in Table 7.

Table 7. TAP transporter binding affinity of AMP

Rank	Sequence	Position	Score	Predicted Affinity
1	AKMNTIFKI	143-152	8.395	High
2	EEFRSILRL	471-479	8.130	High
3	AQEVMMKFL	245-253	7.585	High
4	AAAKMNTIF	141-149	7.430	High
5	TVAKKTVSY	28-36	7.398	High
6	KRVQMPVNL	66-74	7.327	High
7	GTAGNSLRY	778-786	7.135	High
8	KEAKIESGY	638-646	7.102	High
9	AEIDLKVFT	218-226	7.035	High
10	ATFYIPSNL	362-370	7.002	High

Prediction of T-cell, B-cell, and antigenic epitopes

The ProPred-I server predicted 22 epitopes in the full length of AMP which recognized by MHC I alleles with a cutoff of 0.05. The 53 unique human MHC I alleles were involved in the prediction. The prediction was based on the level of binding affinity for the most frequent human MHC I alleles. Among the 53 MHC I alleles, SALTLSAAL, VTVEYEGRL, WGAETKINL, NTLTKVPLL, SVIEKFRKL were the most important epitopes recognized by the most number of human MHC I alleles.

In addition analysis of binding affinity of the AMP for most frequent human DR MHC II alleles reveal that 22 AMP epitopes bind to some of human DR MHC II alleles with a cutoff of 0.04. Table 8 shows conformational high affinity epitopes in the AMP sorted by matching position.

Table 8. Prediction of conformational high affinity epitopes in the AMP recognized by human MHC alleles

Predicted epitopes 51 human DR MHC II alleles (Cutoff 0.04)	Number of MHC class II binding alleles	Matching Position	Predicted epitopes 53 unique MHC I alleles (Cutoff 0.05)	Number of MHC class I binding alleles	Matching Position
MKLLTTAIL	34	1-9	AILSSAIAL	13	7-15
ILSSAIALS	19	8-16	YGKEGGYVL	13	84-92
VNLDKSDNV	12	72-80	KEAAAKEAA	7	421-429
YVLGTGVMD	11	90-98	SALTLSAAL	26	152-160
YRKQPIMIT	18	102-110	APASASEPA	12	171-179
IVFKDCSPR	16	116-124	KEQGAEIDL	11	214-222
MNTIFKISAL	15	145-154	AQEVMMKFL	10	245-252
IFKISALTLS	33	148-157	EAFLKENAA	14	276-284
VMMKFLQEQ	21	248-256	VTVEYEGRL	16	314-322
LQYKITKQG	17	295-303	ELLAKDKAL	14	437-445
LVFDVKLVK	27	387-395	LISGRGSVL	8	491-499
LAKDKALLQ	21	439-447	SVIEKFRKL	15	514-522
FQLKYKNVEE	18	463-472	AADGFSRDL	12	542-550
FRSILRLDN	35	473-481	FSRDLGVKF	9	546-554
VLIDPATNT	11	498-506	FGATGKKKL	12	554-562
IVTDTRSVI	20	508-516	WGAETKINL	16	583-592
LKNDTSAFG	11	562-570	AANSISLVR	14	596-604
LVRAISSGAL	30	602-611	ESLSKTKTL	13	619-627
YEIPFTVTS	14	646-654	KTLANPRVL	13	625-633
IIMTVKINK	24	685-693	GGSSTNTEL	9	658-666
ILCISTKNL	15	706-714	ILCISTKNL	8	706-714
LLIFITPRIMG	45	768-778	NTLTKVPLL	16	738-746

Table 9. Predicted linear epitopes in the AMP

No	Position	Peptide	Length	No.	Position	Peptide	Length
1	20-31	AAAGTDNPTVAK	12	16	423-441	DIKKVNEAAAEAAAKE	19
2	74-92	VNLDKSDNVETFYGKEGGY	19	17	489-497	SILRLD N	9
3	97-105	GVMDGKSYR	9	18	552-556	EARIV	5
4	123-145	CSPREAAAKEAAAKEAAAKEA	23	19	564-579	FSRDLGVKFGATGKKK	16
5	168-200	ACGKKEAAPASASEPAAASSAQG DTSSIGST	33	20	583-595	DTSAFGWGVNSGF	13
6	216-222	LKQMKEQ	7	21	628-633	SGALNL	6
7	241-249	GKEIKM T	9	22	635-638	LSAS	4
8	261-282	LQEQQAKAVEKHKADAKANKEK	22	23	642-655	SKTKTLANPRVLTQ	14
9	286-298	FLKENAAKDGVKT	13	24	665-676	GYEIPFTVTSIA	12
10	304-318	QYKIT KQGEGKQP	15	25	687-693	ELKKAVL	7
11	333-345	IDGTVFDSSKANG	13	26	703-713	PDGQIIMTVKI	11
12	352-356	LSQVI	5	27	730-733	CIST	4
13	363-368	VQLLKE	6	28	744-752	ENGGTLIV	9
14	377-390	YIPSNLAYREQGAG	14	29	771-776	GDIPVI	6
15	403-417	VKLVKIGAPENAPAK	15	30	790- 795	RELLIF	6

B-cell epitopes are antigenic regions that can induce B-cell responses. They generally consist of groups of amino acids that lie close together on the protein surface and determine antigenicity. B-cell receptors (antibodies) exclusively recognize these regions. Predicted linear B-cell epitopes are presented in Table 9.

Discussion

Drug discovery is an expensive process involving high research and development costs and extensive clinical testing. Typical development time is estimated to be 10-15 years. Modern powerful computers and appropriate software can perform *in silico* analyses faster and more economically and predict the relative success of drug development more reliably than traditional methods (26). Currently no meningococcal vaccines against *N. meningitidis* serogroup B are available; therefore, on-hand vaccines are generally used during serogroup B outbreaks (30). Presently, most of *in silico* studies have focused on reverse vaccinology and genomic *in silico* analysis (27, 28), or concentrated on other components of bacteria such as the factor H binding protein (29) or detergent-derived outer membrane vesicles (30). The three dimensional structure shown that two helix-forming peptide linkers (EAAAK)4 well separated the three segment of the AMP. According to our results conformational turns, which represent likely antigenic sites, were found throughout the entire sequence of the AMP which can be used as a good stimulator of the immune system. The absence of a transmembrane lipophilic alpha helix introduces the AMP as a water-soluble protein. The presence of several high-affinity TAP-binding regions within the AMP may make it a good inducer of both the humoral and the cellular immune response. For this reason it is thought the AMP can be a good candidate for immunological *in vitro* and *in vivo* studies.

Acknowledgment

The current research was supported by Iran University of Medical Sciences, Tehran, Iran. The authors declare no competing interest.

References

1. Pace D, Pollard AJ, Messonier NE. Quadrivalent meningococcal conjugate vaccines. Vaccine. 2009; 27 Suppl 2:B30-41.
2. Brigham KS, Sandora TJ. *Neisseria meningitidis:* epidemiology, treatment and prevention in adolescents. Current opinion in pediatrics. 2009; 21(4):437-43.
3. Woodard JL, Berman DM. Prevention of meningococcal disease. Fetal and pediatric pathology. 2006; 25(6):311-9.
4. Panatto D, Amicizia D, Lai PL, Gasparini R. *Neisseria meningitidis* B vaccines. Expert review of vaccines. 2011; 10(9):1337-51.
5. Safadi MA, Barros AP. Meningococcal conjugate vaccines: efficacy and new combinations. Jornal de pediatria. 2006; 82(3 Suppl):S35-44.
6. Hung M-C, Heckels JE, Christodoulides M. The adhesin complex protein (ACP) of *Neisseria meningitidis* is a new adhesin with vaccine potential. mBio. 2013; 2013;4(2).
7. Hung MC, Salim O, Williams JN, Heckels JE, Christodoulides M. The *Neisseria meningitidis* macrophage infectivity potentiator protein induces cross-strain serum bactericidal activity and is a potential serogroup B vaccine candidate. Infection and immunity. 2011; 79(9):3784-91.
8. Christodoulides M, McGuinness BT, Heckels JE. Immunization with synthetic peptides containing epitopes of the class 1 outer-membrane protein of *Neisseria meningitidis*: production of bactericidal antibodies on immunization with a cyclic peptide. Journal of General Microbiology. 1993; 139(8):1729-38.
9. Collins RF, Frye SA, Kitmitto A, Ford RC, Tønjum T, Derrick JP. Structure of the *Neisseria meningitidis* outer membrane PilQ secretin complex at 12 Å resolution. Journal of Biological Chemistry. 2004; 279(38):39750-6.
10. Frye SA, Assalkhou R, Collins RF, Ford RC, Petersson C, Derrick JP, et al. Topology of the outer-membrane secretin PilQ from *Neisseria meningitidis*. Microbiology (Reading, England). 2006; 152(Pt 12):3751-64.
11. Wu YJ, Fan CY, Li YK. Protein purification involving a unique auto-cleavage feature of a repeated EAAAK peptide. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2009; 877(31):4015-21.

12. Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch A. Protein identification and analysis tools on the ExPASy server. In: Walker JM, editor. The Proteomics Protocols Handbook. Springer; 2005. pp. 571–607.
13. Chou PY, Fasman GD. Prediction of the secondary structure of proteins from their amino acid sequence. Advances in enzymology and related areas of molecular biology. 1978; 47:45-148.
14. Garnier J, Osguthorpe DJ, Robson B. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. Journal of molecular biology. 1978; 120(1):97-120.
15. Qian N, Sejnowski TJ. Predicting the secondary structure of globular proteins using neural network models. Journal of molecular biology. 1988; 202(4):865-84.
16. Cao B, Porollo A, Adamczak R, Jarrell M, Meller J. Enhanced recognition of protein transmembrane domains with prediction-based structural profiles. Bioinformatics (Oxford, England). 2006; 22(3):303-9.
17. Moller S, Croning MD, Apweiler R. Evaluation of methods for the prediction of membrane spanning regions. Bioinformatics (Oxford, England). 2001; 17(7):646-53.
18. Omasits U, Ahrens CH, Muller S, Wollscheid B. Protter: interactive protein feature visualization and integration with experimental proteomic data. Bioinformatics (Oxford, England). 2014; 30(6):884-6.
19. Roy A, Yang J, Zhang Y. COFACTOR: an accurate comparative algorithm for structure-based protein function annotation. Nucleic acids research. 2012; 40 (Web Server issue):W471-7.
20. Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. Nature protocols. 2010; 5(4):725-38.
21. Diez-Rivero CM, Lafuente EM, Reche PA. Computational analysis and modeling of cleavage by the immunoproteasome and the constitutive proteasome. BMC bioinformatics. 2010; 11:479.
22. Bhasin M, Raghava GP. Analysis and prediction of affinity of TAP binding peptides using cascade SVM. Protein science : a publication of the Protein Society. 2004; 13(3):596-607.
23. Singh H, Raghava GPS. ProPred1: prediction of promiscuous MHC Class-I binding sites. Bioinformatics (Oxford, England). 2003; 19(8):1009-14.
24. Singh H, Raghava GP. ProPred: prediction of HLA-DR binding sites. Bioinformatics (Oxford, England). 2001; 17(12):1236-7.
25. Hopp TP, Woods KR. Prediction of protein antigenic determinants from amino acid sequences. Proceedings of the National Academy of Sciences of the United States of America. 1981; 78(6):3824-8.
26. Ekins S, Mestres J, Testa B. *In silico* pharmacology for drug discovery: methods for virtual ligand screening and profiling. British journal of pharmacology. 2007; 152(1):9-20.
27. Kelly DF, Rappuoli R. Reverse vaccinology and vaccines for serogroup B *Neisseria meningitidis*. Advances in experimental medicine and biology. 2005; 568:217-23.
28. Pizza M, Scarlato V, Masignani V, Giuliani MM, Arico B, Comanducci M, et al. Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. Science (New York, NY). 2000; 287(5459):1816-20.
29. McNeil LK, Zagursky RJ, Lin SL, Murphy E, Zlotnick GW, Hoiseth SK, et al. Role of Factor H Binding Protein in *Neisseria meningitidis* Virulence and Its Potential as a Vaccine Candidate To Broadly Protect against Meningococcal Disease. Microbiology and Molecular Biology Reviews. 2013; 77(2):234-52.
30. Ferrari G, Garaguso I, Adu-Bobie J, Doro F, Taddei AR, Biolchi A, et al. Outer membrane vesicles from group B *Neisseria meningitidis* delta gna33 mutant: proteomic and immunological comparison with detergent-derived outer membrane vesicles. Proteomics. 2006; 6(6):1856-66.