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In Silico Determination and Validation of PorA Structure and Ligand Binding Site as a vaccine candidate in *Neisseria meningitidis*

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Abstract:

*Neisseria meningitidis*is a major cause of bacterialmeningitis and septicemia worldwide. In theabsence of a comprehensive vaccine against this organism,the characterization of its variable surface antigens isimportant for epidemiologic monitoring and vaccine development. The serologic characterization schemefor meningococci comprises the following: groups, basedon variants in the capsular polysaccharide; types, based onvariants of the PorB outer membrane protein (OMP); subtypes,based on variants of the PorA OMP; and immunotypes,based on variants in the lipooligosaccharide.The development of an effective vaccine against serogroup B strains, which have been responsible for the majority of infections in temperate countries, is therefore likely to require alternative approaches.The two major components of the OMV vaccine are the meningococcal porin proteins encoded by the *porA*and *porB*genes.In order to study the vaccine potential of individual outer membrane proteins, we analyze the PorB structure to identified functional residues wichinvolved in ligand bindig site.

Key Words: *Neisseria meningitides,* PorA, ligand bindig site, Bioinformatic

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1. Introduction

The development of an effective vaccine against life-threatening episodes of meningitis and septicaemia, caused by serogroup B strains of *Neisseria meningitidis*, is a continuing healthcare priority. The lack of an effective vaccine against serogroup B is further emphasized by the development of conjugate polysaccharide vaccines against serogroup C strains and the recent demonstration of their efficacy (http://www.phls.org.uk). Similar strategies to produce conjugate vaccines incorporating serogroup A, W135, and Y capsular polysaccharides are likely to succeed. However, the serogroup B polysaccharide mimics human neural cell adhesion molecules and is nonimmunogenic in purified form (1). The development of an effective vaccine against serogroup B strains, which have been responsible for the majority of infections in temperate countries, is therefore likely to require alternative approaches. The nonimmunogenicity of the serogroup B capsular polysaccharide has led to the development of outer membrane vesicle (OMV) vaccines, based on outer membranes that have been depleted of toxic lipopolysaccharide. Experimental OMV vaccines have undergone phase III trials in humans (2-5) and have been shown to produce limited protection in adults (2). However, the immune responses were of limited duration (6), and no protection was shown in children under 2 years of age, the group at greatest risk of infection (4, 7). In addition, because of the multicomponent nature of the OMVs, the relative responses varied between individuals and only a proportion of the antibodies were protective (6).

The two major components of the OMV vaccine are the meningococcal porin proteins encoded by the *porA*and *porB*genes. The product of the *porA*gene, the class 1 or PorA protein, varies between strains and is responsible for meningococcal serosubtype specificity. The *porB*gene

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exists as one of two alleles encoding the proteins responsible for serotype specificity, the class 2 (PorB2) and class 3 (PorB3) proteins, the expression of which is mutually exclusive. The PorA and PorB proteins are members of the family of *Neisseria* porins: cloning of the encoding genes has permitted structural and immunological studies of the proteins and has led to a structural model for the organization of the porins, in which a series of _-sheets traverse the membrane to form eight surface-exposed hydrophilic loops (8). Major variations between the proteins are largely restricted to the loops, which vary in length and amino acid sequence, generating differences in immunological specificity both within and between the porin classes (9, 10).

The cloning of genes encoding the outer membrane proteins has facilitated the production of pure proteins free from other *Neisseria* antigens for investigation as potential vaccine candidates. The generally accepted correlate of protection against meningococcal infection is the presence of antibodies with the ability to activate complement-mediated killing of meningococci (11). Several studies with recombinant meningococcal PorA and Opc proteins, as well as with purified PI porin from *Neisseria gonorrhoeae*, have clearly demonstrated that the production of bactericidal antibodies was dependent on refolding of the protein to induce native conformation, which could be achieved by incorporation either into artificial membranes (liposomes)(12-18) or detergent micelles (19- 20).

The meningococcal porin proteins induce an antibody response during infection, which correlates with the development of bactericidal and opsonic activity (21- 23), and the PorA protein has been identified as making a major contribution to the development of bactericidal activity after immunization of volunteers with the experimental OMV vaccine (6). However, it has been suggested that the reduced size of the surface-exposed loops of PorB, particularly PorB3, compared with PorA make it less accessible to antibodies and hence less susceptible to

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immunological attack after immunization with OMV (24). Nevertheless, some monoclonal antibodies directed against PorB3 demonstrate bactericidal activity (25), suggesting that it remains a potential vaccine candidate. In order to study the vaccine potential of individual outer membrane proteins, we analyze the PorB structure to identified functional residues wichinvolved in ligand bindig site.

2. Methods

2.1. Sequence availability and homology search

The PorA protein sequence with AccessionAAF70297.1 and GI 7839495 acquired from NCBI at http://www.ncbi.nlm.nih.gov/protein was saved in FASTA format for further analyses. The sequences served as a query for protein BLASTat http://blast.ncbi.nlm.nih.gov/Blast.cgi against non redundant protein database. Probable putative conserved domains of the query protein were also searched for, at the above address.

2.2. Primary sequence analysis

Protparamonline software at http://expasy.org/tools/protparam.html was employed for estimation and determination of properties such as molecular weight, theoretical pI, amino acid composition, total number of negatively and positively charged residues, instability index and aliphatic index.

2.3. 3D structure prediction

The SWISS-MODEL Workspace at http://swissmodel.expasy.org/ is a web-based integrated service dedicated to protein structure homologymodelling. It assists and guides the user in building protein homology models at different levels of complexity. Building a homology model comprises four main steps: identification of structural template(s), alignment of target sequence and template structure(s), model building, and model quality evaluation. These steps

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can be repeated until a satisfying modelling result is achieved. Each of the four steps requires specialized software and access to up-to-date protein sequence and structure databases.

2.4. Ligand binding site predictions

Cofactor athttp://zhanglab.ccmb.med.umich.edu/COFACTOR/ is a structure-based method for biological function annotation of protein molecules. Important amino acid involved in ligand binding site is predicted by this server.

2.5. Pocket detection

DogSiteScorer at http://dogsite.zbh.uni-hamburg.de/is an automated pocket detection and analysis tool which can be used for protein drugability assessment. Predictions with DoGSiteScorer are based on calculated size, shape and chemical features of automatically predicted pockets, incorporated into a support vector machine for druggability estimation.

2.6. Identification of functionally and structurally important residues

InterProSurfat http://curie.utmb.edu/pattest9.htmlpredicting functional sites on protein surface using patch analysis was employed.PorA 3D structure, served as an input file for this server.

3. Result and Discussion

3.1. Sequence availability and homology search

The protein sequence with 386 residues obtained from NCBI and saved in FASTA format. Protein sequence serving as query for BLAST produced a set of sequences as the highest similar sequence. BLAST search revealed numerous hits to the PorA subunit sequence. All hits were ofNeisseria meningitidis. Putative conserved domains were detected within this sequence and are shown in Figure 1. Porin superfamily. These outer membrane channels share a beta-barrel structure that differ in strand and shear number. Classical (gram-negative)porins are non-specific channels for small hydrophillic

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molecules and form 16 beta-stranded barrels, which associate as trimers. Maltoporin-like channels have specificities for various sugars and form 18 beta-stranded barrels, which associate as trimers. Ligand-gated protein channels cooperate with a TonB associated inner membrane complex to actively transport ligands via the proton motive force and they form monomeric, barrels. The 150-200 N-terminal residues form a plug that blocks the channel from the periplasmic end.

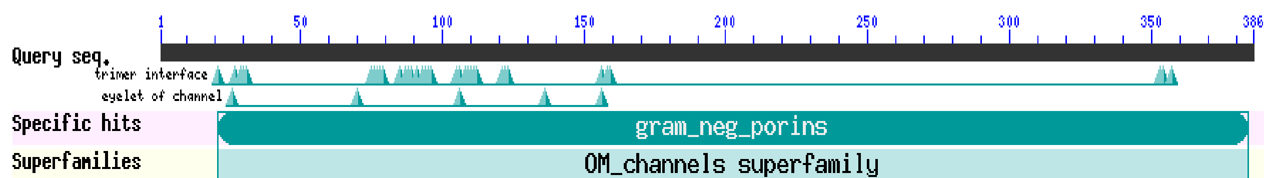

Figure 1.Putative conserved domains have been detected.

3.2. Primary sequence analysis

The protein sequence served as input for the computation of various physical and chemical parameters. The computed parameters included the molecular weight, theoretical pI, instability index, aliphatic index and grand average of hydropathicity (indicates the solubility of the proteins: positive GRAVY (hydrophobic), negative GRAVY (hydrophilic)) are summarized below.

Number of amino acids: 386

Molecular weight: 41731.4

Theoretical pI: 8.71

Amino acid composition:

Ala (A) 40 10.4%

Arg (R) 20 5.2%

Asn (N) 23 6.0%

Asp (D) 25 6.5%

Cys (C) 0 0.0%

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Gln (Q) 16 4.1%

Glu (E) 16 4.1%

Gly (G) 42 10.9%

His (H) 5 1.3%

Ile (I) 15 3.9%

Leu (L) 29 7.5%

Lys (K) 24 6.2%

Met (M) 2 0.5%

Phe (F) 18 4.7%

Pro (P) 13 3.4%

Ser (S) 31 8.0%

Thr (T) 20 5.2%

Trp (W) 4 1.0%

Tyr (Y) 17 4.4%

Val (V) 26 6.7%

Total number of negatively charged residues (Asp + Glu): 41

Total number of positively charged residues (Arg + Lys): 44

Atomic composition:

Carbon C 1851

Hydrogen H 2871

Nitrogen N 523

Oxygen O 576

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Sulfur S 2

Formula: $C_{1851}H_{2871}N_{523}O_{576}S_2$

Total number of atoms: 5823

Extinction coefficients:

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 47330

Abs 0.1% (=1 g/l) 1.134

Estimated half-life:

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).

>20 hours (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 28.88

This classifies the protein as stable.

Aliphatic index: 74.35

Grand average of hydropathicity (GRAVY): -0.437

3.3. 3D structure prediction

Building a homology model comprises four main steps: identification of structural template(s), alignment of target sequence and template structure(s), model building, and model quality evaluation. These steps can be repeated until a satisfying modelling result is achieved. Each of the four steps requires specialized software and access to up-to-date protein sequence and

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structure databases.Swiss model software recruited for homology modeling introduced 1 model. Predicted model is shown in figureFigure2.

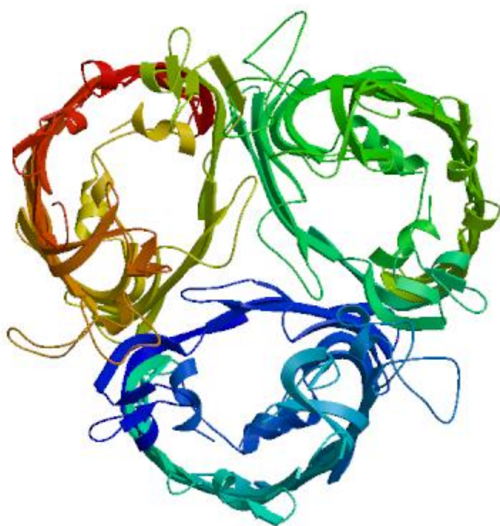

Figure2.3D structure of PorA

3.4. Ligand binding site predictions

Ligand binding sites determined using COFACTOR software, indicate involvement of conserved residues include3,5,47,114,116,363in binding site with the highest $Cscore^{LB}$ (the confidence score of predicted binding site) (Figure 3).

The calculated BS-score for this predicted binding site was 1.64.BS-score is a measure of local similarity (sequence & structure) between template binding site and predicted binding site in the query structure. Based on large scale benchmarking analysis, observed that a BS-score >1 reflects a significant local match between the predicted and template binding site. Template proteins with similar binding site are listed in Table1.

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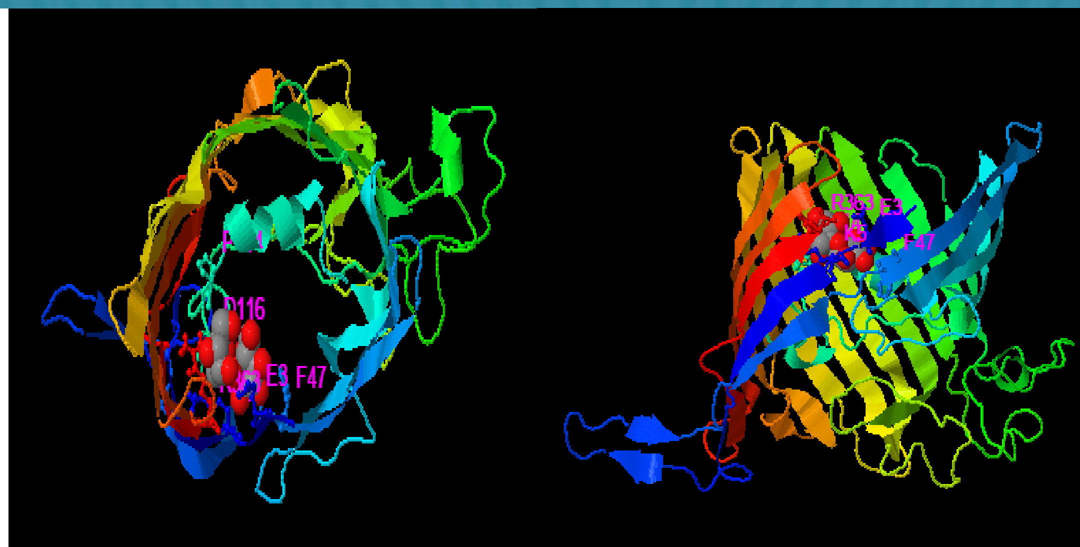

Figure3.PorA ligand binding site predictions.

Rank	Cscore^{LB}	PDB Hit	TM-score	RMSD[a]	IDEN[a]	Cov.	BS-score	Lig. Name	Predicted binding site residues
1	0.13	3a2sX	0.890	1.01	0.480	0.906	1.64	SUC	3,5,47,114,116,363
2	0.12	2j1nC	0.699	3.44	0.163	0.804	1.30	D12	252,253,286,287,300,301
3	0.05	2zldA	0.684	3.64	0.177	0.799	0.88	PEPTIDE	106,113,114,115,125,126
4	0.05	2fgrA	0.730	2.61	0.227	0.802	0.96	PEPTIDE	60,61,62,63,89
5	0.04	2xe5C	0.701	3.32	0.163	0.802	1.14	D10	142,149,150,195
6	0.04	3o0eD	0.690	3.62	0.173	0.802	0.83	PEPTIDE	63,90,95,102,106,113,114,115,142,144,148,198

(a) Cscore^{LB} is the confidence score of predicted binding site. Cscore^{LB} values range in between

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[0-1]; where a higher score indicates a more reliable ligand-binding site prediction.

(b) BS-score is a measure of local similarity (sequence & structure) between template binding site and predicted binding site in the query structure. Based on large scale benchmarking analysis, we have observed that a BS-score >1 reflects a significant local match between the predicted and template binding site.

(c) TM-score is a measure of global structural similarity between query and template protein.

(d) RMSD[a] the RMSD between residues that are structurally aligned by TM-align.

(e) IDEN[a] is the percentage sequence identity in the structurally aligned region.

(f) Cov. represents the coverage of global structural alignment and is equal to the number of structurally aligned residues divided by length of the query protein.

Table1.Template proteins with similar binding site.

3.5. Pocket detection

Pockets and descriptors have been calculated for PorA structure with DoGSiteScorer: Active Site Prediction and Analysis Serverissumerized in Table 2.

Pocket descriptor table

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0	895.94	1034.23	589.45	28.74	0.84
P1	519.55	791.58	419.41	24.30	0.86
P2	426.43	530.73	352.47	15.94	0.72
P3	422.85	582.62	396.18	11.54	0.59
P4	215.49	253.67	127.10	11.09	0.39

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Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P5	192.96	334.30	111.79	10.38	0.35
P6	174.40	383.46	272.20	13.54	0.45
P7	157.89	221.63	88.59	11.38	0.40
P8	153.92	334.53	155.30	7.17	0.20
P9	130.82	240.64	208.78	7.94	0.25
P10	123.14	172.41	91.11	7.28	0.22

Subpocket descriptor table

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0SP0	282.05	406.00	202.32	12.63	0.10
P0SP1	202.24	210.67	137.09	0.40	0.36
P0SP2	156.42	269.84	149.04	7.92	0.20
P0SP3	134.98	268.80	200.45	8.66	0.16
P0SP4	77.18	185.57	107.96	6.46	0.19
P0SP5	43.07	83.69	68.01	2.04	0.18
P1SP0	327.36	444.28	233.03	15.37	0.37
P1SP1	122.62	227.02	63.54	7.68	0.12
P1SP2	69.57	200.32	134.85	7.39	0.07
P2SP0	365.57	473.44	317.87	15.94	0.32
P2SP1	60.86	120.63	74.65	4.93	0.14

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Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P5SP0	108.03	245.26	93.02	8.09	0.08
P5SP1	84.93	163.51	45.05	8.64	0.14
P6SP0	110.40	305.01	232.59	6.55	0.28
P6SP1	64.00	163.02	121.57	6.50	0.27

legend: undruggable =>druggable

Table2. Pockets and descriptors calculated for PorA

3.6. Identification of functionally and structurally important residues

Interprosurf annotated functional residues on the 3D structure of PorA.Residues Predicted by Auto Patch Analysis are:**168,169 169,170,171**.Results are shown in Figure 4.

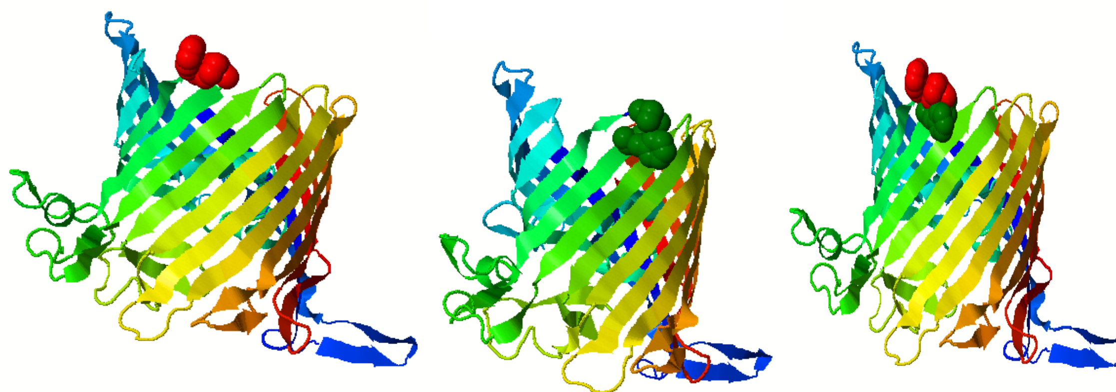

Figure 4.Functional residues on PorA 3D structure.

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