**Bioinformatics prediction and experimental validation of VH antibody fragment interacting with Neisseria meningitidis factor H binding protein**

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**Abstract**

Objectives: We previously conducted an *in silico* research on the interactions between the ribosome display-selected single chain variable fragment (scFv) and factor H binding protein (fHbp) of *Neisseria meningitidis*. We found that heavy chain variable (VH) fragment of this scFv had considerable affinity to fHbp. These results led us to evaluate the ability of this small antibody fragment in binding and detection of fHbp antigen.

Materials and Methods: In this study, at first, the three-dimensional structure of VH fragment was simulated by Kotai Antibody Builder web server. By using ClusPro 2.0 web server, the 3D structure of the soluble form of fHbp (PDB: 2KCO) was docked to the modeled VH fragment to extract the structure of the complex's binding. Molecular dynamics (MD) simulation was carried out using GROMACS 4.5.5 package for 65 ns. Secondly, coding sequence of VH fragment was cloned separately and expressed in *Escherichia coli*. After purification of the VH fragment, its binding activity to fHbp protein was analyzed by enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) method.

Results: Important amino acids involved in antigen-antibody interaction were identified by analyzing the fHbp-VH complex. The ability of the VH antibody fragment to bind and detect fHbp antigen has been confirmed by the results of *in silico* analysis, ELISA and SPR methods.

Conclusion: These results showed that this small fragment of antibody could be used for designing diagnostic kits.

**Introduction**

Advances in bioinformatics and computational methods caused structure prediction of antibody and *in silico* study of the antigen-antibody interactions (1, 2). The most important web servers for antibody modeling are Kotai antibody Builder (https://bio.tools/kotai_antibody_builder) and PIGSpro (https://cassandra.med.uniroma1.it/pigspromo/) that can build models of immunoglobulins by homology methods. In order to modeling of antibody structure, the antibodies’ crystal format was employed. This crystal structures are utilized as a template (3). In lack of the presence of experimental structures, protein- protein docking program is a good option to predict the conformation of protein complexes and binding interactions of antigens with antibodies (4).

Recently, a wide variety of antibody fragments provided possibilities for the production of new therapeutic and diagnostic agents. In comparison with the whole antibodies, these fragments have significant advantages including smaller size, simpler manufacturing processes, more efficient tissue penetration, and ability to generate multi-specific fragments. Genetic manipulations of these fragments are also feasible because of their small size (5). A single-chain fragment variable (scFv) is a polypeptide chain (~28 kDa). This polypeptide consisted of both heavy (VH) and light (VL) chain variable regions of an immunoglobulin. There is also a short polypeptide (10-25 amino acids) linker, which has made a covalently link. Because of glycine and serine residues in linker structure, scFv would be remained flexible and resistant to proteases (6).

In our previous studies, we identified and characterized a scFv antibody against *Neisseria meningitidis* factor H binding protein (fHbp) (7). Bioinformatics evaluation of the interactions between the anti-fHbp scFv and fHbp showed that VH fragment of this scFv had considerable affinity to fHbp. Docking led to identifying the essential interacting residues in both VH and VL chains regions of scFv (8).
In this study, VH fragment of the selected anti-fHbp scFv was separately cloned and expressed in Escherichia coli. After purification of the VH fragment coding sequence, its binding activity to fHbp protein was analyzed utilizing enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) methods.

**Materials and Methods**

**Modeling of VH antibody fragment and fHbp protein**

In order to generate the three-dimensional (3D) structure of the VH fragment, Koval Antibody Builder (PDB: 1jhl) was employed (9). This web server builds 3D structures of variable domains of antibodies using their sequence. Modeller 9.16 has been utilized to construct the model of fHbp antigen. The 3D structure of the soluble form of fHbp (PDB: 2KC0) was applied as a scheme for fHbp protein homology modeling. After modeling of VH fragment and fHbp protein, the energy minimization process was made using steepest descent algorithm of GROMACS 4.5.3 package.

**Molecular docking of VH antibody fragments and fHbp protein and molecular dynamics simulation**

Antibody-antigen molecular docking was performed by ClusPro 2.0 (https://cluspro.bu.edu/publications.php) while working in antibody mode. Identifying the antibody and antigen was carried out by considering antibody and antigen as receptor and ligand, respectively. All default parameters were accepted during the analysis. Automatic masking of non-Complementarity-determining regions (CDRs) regions of the antibody chains was utilized to obtain better docking results. In order to analyze, the models that have the lowest energy level were chosen as the best models (10). Furthermore, for molecular dynamics (MD) simulation, the finest docked model constructed by ClusPro was employed. Then, the energy of the whole molecular system was minimized, while the number of iterations was 5000 and the steepest descent algorithm was applied to implement GROMACS 96 43a1 force field.

GROMACS 4.5.3 package was employed to simulate the complexes of antibody-antigen. In order to adjust the inside box temperature, we used Berendsen temperature coupling method. The procedure that was used for computing electrostatic interactions was Particle Mesh Ewald technique. The pressure was preserved at 1 atm and the permissible compressibility was 4.5×10⁻⁵ atm. The linear constraint solver was worked to constrain bond lengths involving hydrogen atoms, permitting a time step of 2 ps (11). For each concentration of fHbp protein, different ranges of 0.25, 0.5, 1, and 2 μg/ml were used separately. The Beatty’s equation was used as the secondary antibody. The immunoreaction was started by the addition of 100 μl of anti-His tag antibody (2 μg/l in PBS+0.05% Tween 20) (Abcam, UK) was used as the secondary antibody. The affinity constant 

Expression and purification of VH antibody fragment

E. coli BL21 (DE3) was transformed with pET28a-VH antibody fragment. It was grown in 50 ml of LB medium containing 50 μg/ml Kanamycin at 37 °C with shaking at 180 RPM to a density of OD₆₀₀=0.6. Then, Isopropyl-D-1-thiogalactopyranoside (IPTG) (Merck, Germany) was added to a final concentration of 1 mM. Samples were collected at 3, 5 and 8 hr after induction and treated with lysis buffer (Tris 50 mM, 10% glycerol, 0.1% Triton X-100) (Merck, Germany). The cell lysate was evaluated by SDS-PAGE (12).

**ELISA-based evaluation of the binding activity of VH antibody fragment**

Microtiter plate was coated with recombinant fHbp protein (2 μg/ml in phosphate buffer (PBS), pH=7.5) and incubated 16 hr at room temperature. PBS containing 3% (w/v) bovine serum albumin (BSA) was used for blocking for 2 hr. After three washing steps, 100 μl of extracted VH fragment diluted with PBST (5 μg/ml) was added to the wells as the primary antibody and incubated for 7 hr at 37 °C. Horseradish peroxidase (HRP)-conjugated anti-His tag antibody (2 μg/l in PBS+0.05% Tween 20) was used as the secondary antibody. The immunoreaction was started by the addition of 100 μl of TMB (tetramethylbenzidine) (13). After 15 min, the peroxidase reaction was stopped by adding 100 μl of 2N H₂SO₄. Absorbance was measured at 450 nm. Finally, the affinity of VH fragment against recombinant fHbp antigen was determined by ELISA.

Surface Plasmon Resonance analysis of VH fragment antibody and fHbp protein interactions

A double channel cuvette-based Surface Plasmon Resonance (SPR) (Autolab ESPRIT, Ecochemie B.V.,
Netherlands) was used for analysis of interaction between VH fragment and fHbp protein and calculation of the affinity of interaction. One channel was used for the test, and the other was used for reference sample measurements. The result of the SPR measurement was automatically monitored using data acquisition software version 4.3.1. All kinetic data were achieved using the SPR kinetic evaluation software version 5 (Ecochemie B.V.).

11-Mercaptoundecanoic acid (11-MUA), 1-ethyldimethyiaminopropyl carbodiimide hydrochloride (EDC), N-hydroxysuccinimic (NHS), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulmonic acid) and Ethanolamine were purchased from Sigma-Aldrich (Steinheim, Germany).

Antibody immobilization on gold disc

In this study, VH fragment antibody was covalently immobilized on 11-MUA linker via amine coupling. Amine coupling is the most generally applicable covalent coupling chemistry used to immobilize protein ligands (15). For immobilization of VH fragment, at first, the surface of gold disks was cleaned using piranha solution (3:1 v/v mixture of 98% H2SO4:30% H2O2). In order to form self-assembled monolayer on gold disk, the gold disc was immersed in 11-MUA solution (0.001 mol l−1) overnight. Second, the surface of MUA/gold disk was washed with acetate buffer (0.01 mol l−1) for immobilization of antibody on the gold disk. At this point, resonance angle was recorded as baseline. Then, MUA/gold disk was gained by injecting 100 μl of freshly prepared 1:1 mixture of EDC (0.4 mol l−1) and NHS (0.1 mol l−1) in distilled water (DW) over the MUA/gold disk for 300 sec. Afterwards, 50 μl of VH antibody fragment (60 μg ml−1) was immobilized on MUA/gold disk surface. After 800 sec, it was washed by coupling buffer (NaAc.3H2O). After immobilization of VH antibody fragment on MUA/gold disk, neutralizing unreacted activated ester groups on the formed 11-MUA with 1 mol l−1 ethanolamine (pH = 8.5) is crucial.

Channel 1 was utilized to immobilize the VH antibody fragment, whereas channel 2 was employed as negative control without VH.

VH fragment was coated on MUA/gold disk surface. Then, different concentrations of fHBP (5, 2.5, 1.25, 0.625, 0.3125, and 0.156 μg ml−1) were passed over the disk, and the sensograms in optimized pH of coupling buffer (NaAc.3H2O) were achieved (16).

Results

Homology modeling, molecular docking and MD simulation

Using homology modeling method by Modeller 9.16, three-dimensional model of fHbp antigen was built (Figure 1a). The 3D models of the scFv antibody and VH fragment antibody were created using Kotai Antibody Builder (Figure 1a). The energy minimization process of both models was made using steepest descent algorithm of GROMACS package (Figure 1b).

Molecular docking between fHbp and the VH fragment antibody was considered using ClusPro 2.0 to study the binding mechanism and interaction modes of VH fragment antibody (Figure 2a). Among the 10 models built by the ClusPro 2.0 server, the greatest model with the lowest energy was selected. The docked complex was then simulated using GROMACS 4.5.3 package (11). Protein system was solvated in a triclinic box with simple point charge water model. The structure was found to be positively charged at pH=7.4. Therefore, Chloride ion (Cl−) was added to the simulation box to create the system electrically neutral. Electrostatic interactions were calculated using Particle Mesh Ewald method (PME) (17). The pressure was maintained at 1.0 atm. Then, the system was subjected to MD simulation for 65 nsec. Figure 2b shows the number of hydrogen bonds between VH antibody fragment and fHbp antigen during simulation time. Root Mean Square Deviation (RMSD) analysis is presented in Figure 2c. After 3 nano second, structure of the protein reaches a certain distance from the reference structure and then keeps that distance more or less, until it reached plateau on 54 nsec and maintain its value.
Cloning, expression, and purification of VH fragment antibody

The sequences of VH fragment was amplified with VH/for-NcoI and VH/back NotI primers. The PCR product was ligated into pET28a (+) expression vector. The His-tagged VH fragment antibody was expressed in E. coli BL21 (DE3) and purified by Ni-NTA agarose resin. The expressed protein (12 kDa) was investigated by SDS-PAGE and western blotting (Figure 3).

Functional assay of the purified scFv by ELISA

Affinity constant ($K_{\text{aff}}$) of the purified VH fragment was determined to be $2.25 \times 10^{10}$ M$^{-1}$ ($K_{\text{aff}}$ is the antigen-antibody affinity constant in 1/mol (M$^{-1}$)), (Figure 4).

Affinity investigation of antigen on VH/ MUA/gold disk by SPR

For the assessment of VH affinity, firstly, various concentrations of the antigen (fHbp) from $9 \times 10^{-9}$ to $3 \times 10^{-7}$ mol l$^{-1}$ was passed over the VH fragment antibody/MUA/gold disk surface. Antibody-antigen interaction graph is completely evident. Thus, VH/MUA/gold disk has a suitable affinity against fHbp. Affinity constant was determined to be $9.74 \times 10^{9}$ M$^{-1}$ (Figure 5).

Discussion

In the treatment of infectious diseases, rapid, accurate, and precise detection of pathogens is very important. Detection of pathogenic bacteria can be performed by using antibodies.
There are different methods to produce new generation of diagnostic and therapeutic agents, thanks to innovative technologies such as antibody fragments technology and recombinant DNA advances. Recombinant antibody fragments (rAbFs) are minimal antigen-binding proteins with the full antigen-binding capacity of whole antibodies. It has been shown that scFv, Fab (fragment antigen binding), and Nanobodies (Nbs) have the advantages of economical production, superior biodistribution, easy genetic manipulation, and chemical modification. These advantages enable us to generate fragments with desired properties. The development of selection technologies (e.g. phage- and ribosome-display) and the advent of various production systems have simplified rAbF production.

Meningococcal meningitis is a serious bacterial infection caused by the gram-negative bacterium *N. meningitidis*. This infection causes inflammation and swelling of the membranes that cover brain and spinal cord and can be fatal if it is not treated quickly and efficiently. FHp, a surface-exposed lipoprotein of *N. meningitidis*, is the main virulence factor; FHp is used in vaccines since it is expressed by all strains of serogroup B meningococcus (18). This antigen binds to factor H (FH), a regulatory protein of the complement system, and recruits FH to the surface of the bacterium. Interaction and binding of FH with FHp down-regulates alternative complement pathway. Thus, the bacterium evades host innate immunity and survives in human serum (19, 20).

Although, crystal experiment is one of the best methods for studying protein-ligand binding mechanism, it is time-consuming. Computational biology can solve the problem. It is a conceivable method to study the protein-ligand interaction on *in silico* at a suitable speed.

Payandeh et al., designed new antibody using computational biology and *in silico* approach by rational engineering methods (21).

In this research, according to our previous *in silico* study (22), a new bioinformatics study was performed on the VH fragment and FHp antigen. In our selected scFv, the number of amino acids involved in antibody’s heavy chain and antigen interaction was more than the scFv, the number of amino acids involved in antibody’s complement pathway. Interaction and binding of FH to FHb antigen is more flexible than the VH fragment in ELISA is more flexible than the VH fragment in SPR. This is 2.5 times more than the affinity of selected antibodies can be predicted, and the results can be used to design and select antibodies with the highest affinity. On the other hand, the affinity of selected antibodies can be evaluated by both ELISA and SPR methods.

**Conclusion**

In the present study, the results of bioinformatics studies, ELISA, and SPR confirmed the ability of the VH antibody fragment to bind and detect FHp antigen. These results showed that this small fragment of antibody could be used in designing diagnostic kits.

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**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

**References**

1. Maier JK, Labute P. Assessment of fully automated antibody homology modeling protocols in molecular operating environment. Proteins 2014;82:1599-1610.
2. Pipiani S, Saini V, Niraj RR, Pushp A, Kumar A. Homology modelling and molecular docking studies of human placental cadherin protein for its role in teratogenic effects of anti-epileptic drugs. Comput Biol Chem 2016;60:1-8.
3. Sevy AM, Meiler J. Antibodies: Computer-Aided Prediction of Structure and Design of Function. Microbiol spect 2014;2-1:14.
4. Hosseini SA, Tahmoorespur M, Seidhavat MH, Monhemi H, Nassiri M. Designing of a functional chimeric protein for production of nanobodies against human CD20: Molecular dynamics simulation and *in vitro* verification. Int J Pept Res Ther 2018;1:7-7.
5. Xenaki KT, Oliveira S, van Bergen En Hemegouwen PMP. Antibody or Antibody Fragments: Implications for Molecular Imaging and Targeted Therapy of Solid Tumors. Front Immunol 2017;8:1287-1292.
6. Alibakhshi A, Kahaki FA, Ahangarzadeh S, Yaghobi H, Yarian F, Azemard M, et al. Targeted cancer therapy through antibody fragments-decorated nanomedicines. J Control Release 2017;268:323-334.
7. Yarian F, Kazemi B, Bandehpour M. Identification and characterization of a novel single-chain variable fragment (scFv) antibody against Neisseria meningitidis factor H-binding protein (FHp). J Med Microbiol 2018;67:820-827.
8. Bandehpour M, Yarian F, Ahangarzadeh S. Bioinformatics evaluation of novel ribosome display-selected single chain variable fragment (scFv) structure with factor H binding protein through docking. J Theor Comput Chem 2017;16:1-10.
9. Yamashita K, Ikeda K, Amada K, Liang S, Tsuchiya Y, Nakamura H, et al. Kotai antibody builder: automated high-resolution structural modeling of antibodies. Bioinformatics 2014;30:3279-3280.
10. Bandehpour M, Ahangarzadeh S, Yarian F, Lari A, Farnia P. *In silico* evaluation of the interactions among two selected single chain variable fragments (scFvS) and ESAT-6 antigen of *Mycobacterium tuberculosis*. J Theor Comput Chem 2018;16:12-18.
11. Hess B, Kutzner C, van der Spoo D, Lindahl E. GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable
molecular simulation. J Chem Theory Comput 2008;4:435-447.
12. Yarian F, Bandehpour M, Seyed N, Kazemi B. Cloning, expression and purification of the factor H binding protein and its interaction with factor H. Iran J Microbiol 2016;8:29-35.
13. Ahangarzadeh S, Bandehpour M, Kazemi B. Selection of single-chain variable fragments specific for Mycobacterium tuberculosis ESAT-6 antigen using ribosome display. Iran J Basic Med Sci 2017;20:327-333.
14. Beatty JD, Beatty BG, Vlahos WG. Measurement of monoclonal antibody affinity by non-competitive enzyme immunoassay. J Immunol Methods 1987;100:173-179.
15. Taheri RA, Rezayan AH. Evaluating the potential of an antibody against recombinant OmpW antigen in detection of Vibrio cholerae by surface plasmon resonance (SPR) biosensor. Plasmonics 2017;12:1493-1504.
16. Taheri RA, Rezayan AH, Rahimi F, Mohammadnejad J, Kamali M. Comparison of antibody immobilization strategies in detection of Vibrio cholerae by surface plasmon resonance. Biointerphases 2016;11:1493-1504.
17. Cheatham TE, Miller, JL, Fox T. Molecular dynamics simulations on solvated biomolecular systems: the particle mesh Ewald method leads to stable trajectories of DNA RNA and proteins. J Am Chem Soc 1995;117:4193-4194.
18. Shirley M, Taha MK. MenB-FHbp meningococcal group B vaccine (TRUMENBA®): A review in active immunization in individuals aged >/= 10 years. Drugs 2018;78:257-268.
19. McNeil LK, Zagursky RJ, Lin SL, Murphy E, Zlotnick GW, Heiseth 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. Microbiol Mol Biol Rev 2013;77:234-252.
20. Donald RG, Hawkins JC, Hao L, Liberator P, Jones TR, Harris SL, et al. Meningococcal serogroup B vaccines: Estimating breadth of coverage. Hum Vaccin Immunother 2017;13:255-265.
21. Payandeh Z, Rajabibazl M, Mortazavi Y, Rahimpour A, Taromchi AH, Dastmalchi S. Affinity maturation and characterization of the ofatumumab monoclonal antibody. J Cell Biochem 2019;120:940-950.
22. Bandehpour M, Yarian, F, Ahangarzadeh S. Bioinformatics evaluation of novel ribosome display-selected single chain variable fragment (scFv) structure with factor H binding protein through docking. J Theor Comput Chem 2017;16:1750021-1750031.