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Immunological study on integrated PilQ and disulphide loop region of PilA against acute *Pseudomonas aeruginosa* infection: In silico analysis and *in vitro* production

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ABSTRACT

Objective: Nowadays, *Pseudomonas aeruginosa* (*P. aeruginosa*), the highly regarded opportunistic pathogen, is the leading cause of morbidity and mortality worldwide. The *P. aeruginosa* type IV pili (T4P) as a multiple functional surface organelle in the development of acute *P. aeruginosa* infections have been well documented. Today, in silico analysis is a quick, and cost-effective tool for vaccine development.

Methods: In present study, several turns' motifs along with the chimeric protein were predicted. Based on the hydropathy analysis, numerous antibody-accessible hydrophilic regions were characterized in the chimeric protein. A synthetic chimeric gene, encoding integrated PilQ and disulphide loop region of PilA, was designed. Modeling was done to predict the 3D structure of protein. The model was validated by using Ramachandran plot statistics and by ProSA server. Identification of B-cell and T-cell corresponding epitopes was done by using appropriate servers.

Results: The closer 3D model to the native form of the chimeric protein was achieved. Validation results showed that 95.1% residues were in favor region and 3.6% of amino acid residues were in the allowed region. The B-cell epitope mappings showed that almost all the epitopes had irregular enriched structures. The major histocompatibility complex binding sequence prediction identified several human major histocompatibility complex class I and II restricted T-cell epitopes. The integrated PilQ and PilA disulphide loop encoding regions in the frame of pET28a(+) vector were expressed and purified efficiently.

Conclusions: We expect that the two recognized antigenic determinants from our chimeric protein, "AYHKGNWSGYGKDGNIGIKDEDGMNCGPIAGSCTFPTTGTS-KSPSPFVDLGAKDATSG" and "GPIAGSCTFPTTGTSKSPSP", can be able to evoke strong both humoral and cell-mediated immune responses in mouse models.

1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*), as an important opportunistic human pathogen, can cause various types of infectious diseases such as chronic obstructive pulmonary disease, cystic fibrosis and ventilator-associated pneumonia. Today, *P. aeruginosa* infections in extensive burn and immune-compromised patients have high morbidity and mortality rates[1]. Once *P. aeruginosa* colonizes in human host, the bacterium often spreads rapidly and can cause tissue destruction due to multiple virulence factors[2]. Type IV pili (T4P) and its secretion apparatus in *P. aeruginosa* serves as a key component for twitching motility, colonization and

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adhesion to various receptors[3]. The C-terminal disulphide loop (DSL) region of PilA serves as a functional binding domain of T4P in *P. aeruginosa*. Previous studies confirmed that the LD_{50} of piliated *P. aeruginosa* strains was 10-fold higher than non-piliated mutants[4,5]. Earlier studies showed that specific humoral immunity against the DSL region of pilA can be protective[6,7]. The presence of the protective continuous epitope, two β-turns within the DSL region and its unique curved shape conformation led to production of good-quality, cross-reactive antibodies against this region[8]. In *P. aeruginosa*, the outer membrane PilQ, as a gated channel, facilitates extrusion and retraction of T4P through across the outer membrane. The PilQ molecule is extremely stable T4P biogenesis machine which belongs to the GspD secretin super family[9]. In *P. aeruginosa*, only about 200–300 residues in the C-terminal region of the PilQ monomer make up conserved domain. The PilQ multimeric channel is abundant in *P. aeruginosa* cell membrane[10]. The *P. aeruginosa* PilQ protein was known as an efficient Th17 cell activator and elicited strong interleukin-17 inducer[11]. Up to now, numerous methods were experienced to produce a therapeutic and effective antiserum against *P. aeruginosa*. Despite long period efforts, some of them were not cost-effective and had several disadvantages. However, there is no commercial vaccine against *P. aeruginosa* available on the market[2]. In this study, we decided to exploit bioinformatic tools and modeling approach to better evaluate and characterize the PilQ monomer protein integrated with major pilin DSL region of *P. aeruginosa* PAO1 strain. These immunoinformatics tools help us to choose appropriate antigenic and immunogenic areas of the recombinant integrated PilQ380-706 and DSL region (QD). Our results indicate essential characteristics of QD as an appropriate vaccine candidate. Finally, we expressed and purified QD by bacterial expression system. The results of our study are discussed in details in the following paragraphs.

2. Materials and methods

2.1. Primary sequence analysis and construct design

The sequence 380-706 of PilQ and DSL region of PilA molecule from *P. aeruginosa* PAO1 strain were retrieved from gene Bank (NCBI, http://www.ncbi.nlm.nih.gov) in FASTA format with accession numbers NP_253727.1 and NP_253215.1 respectively. Selected sequence of PilQ was subjected to blastp against non-redundant protein sequence database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The resulted sequences with a similarity > 90%, coverage > 90%, and E-value < 10^{-4} were selected and aligned with multiple sequence alignment tools from European Bioinformatics Institute server (http://www.ebi.ac.uk)[12]. In the next step, we used the helix-forming sequence (Glu-Ala-Ala-Ala-Lys) [$(EAAAK)_4$] as an integrating linker between PilQ and PilA DSL region[13]. In actual fact, in the study, the longest, conserved and well solvent-exposed sequence of PilQ from *P. aeruginosa* was selected which attached to the DSL by the linker sequence. Schematic representation of chimeric QD domains was designed by Domain Graph 2.0[14]. The primary structure and the several basic physicochemical properties of the QD sequences were evaluated by using ProtParam tool (http://web.expasy.org/protparam/). The protein sub-cellular localization of QD among Gram-negative bacteria was predicted by PSLpred server (http://www.imtech.res.in/raghava/pslpred/). PSLpred server uses a hybrid approach-based method within overall high accuracy of 91.2%[15]. Presence of signal sequence in our chimeric sequence was checked by SignalP (http://www.cbs.dtu.dk/services/SignalP-3.0/)[16], and Signal-3L software (http://www.csbio.sjtu.edu.cn/bioinf/Signal-3L/)[17].

The solubility of QD was analyzed by Recombinant Protein Solubility Prediction (http://biotech.ou.edu/#rt) with accuracy of 94%[18]. The QD surface accessibility was calculated by Immune Epitope Database (IEDB) surface accessibility prediction (http://tools.immuneepitope.org) at default threshold. The QD encoding sequence was optimized by ExPASy tools (http://www.expasy.org/) based on 15% cut-off for codon efficiency and except for positions with strong secondary structures. The chimeric QD encoding sequence in frame of expression vector pET28a(+) plasmid was constructed by Biomatik Corporation, Canada. The *Escherichia coli* (*E. coli*) BL21 (DE3) was used as host expression strain.

2.2. Antigenicity prediction

The potential hydrophilic regions and antigenicity value prediction were performed based on Kyte-Doolittle technique [19], Hopp/Woods hydrophilicity scale[20], Kolaskar and Tongaonkar method by IEDB B-cell antigenic prediction site [21]. We used VaxiJen v2.0 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) to ensure accuracy of antigenic areas[22]. VaxiJen is the first server for alignment-independent prediction of protective antigens solely based on the physicochemical properties of proteins for vaccine development.

2.3. Prediction of protein secondary and 3D structures

The secondary structure of QD was studied by the MINNOU server (http://minnou.cchmc.org/) by using default parameters. The α, β, and γ turn prediction was analyzed by the AlphaPred, BetaTPred2, and GammaPred servers, respectively[23–25]. The IMTECH server also predicted weakly polar interactions between the side-chain aromatic rings and hydrogen's of backbone amides (Ar-HN interactions)[26].

The 3D structure of QD was predicted by online iterative threading assembly refinement (I-TASSER) software at http://zhanglab.ccmb.med.umich.edu/I-TASSER/[27]. The predicted result was validated by ProSA server (https://www.came.sbg.ac.at/prosa.php)[28]. In proteomics studies, structure refinement model is a closer form of molecule to the native structure.

The refinement process was done in terms of H-hydrogen bonding networks, backbone topology, chain residue standing, and atomic-level energy minimization. The selected 3D structure of QD was corrected by minimization of atomic-level energy by using ModReiner online server (http://zhanglab.ccmb.med.umich.edu/ModRefiner/)[29]. We used the refined model at predictions of discontinuous B-cell epitopes. After refinement process, the 3D modeling of QD was visualized by ChemBio office software. The 3D hydrophobicity modeling of QD was predicted by Discovery Studio (Accelrys Co. Ltd) software.