Analysis of Important \textit{H. pylori} Outer Membrane Proteins by Detection of Common Sequences in Exposed Areas; in Silico Study

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\textit{H. pylori} Outer membrane proteins (OMPs) play a main role in the pathogenesis. Our study aimed to study on computational investigation for determination of \textit{H. pylori} OMPs structures, accessible surface area and common amino acid sequences in exposed regions. BabA/BabB, AlpA/AlpB and SabA/SabB were analyzed. NCBI and PDB were used as databases. PHD, GOR4, SignalP3.0, CPH Models, PHYRE and VADAR servers and two software including Swiss PDB viewer and Discovery studio were used for all analysis. Seven, six, five, five, four and two sequences of exposed amino acid were identified for SabB, SabA, AlpA, AlpB, BabA and BabB, respectively. Sequence similarities between BabA/BabB, AlpA/AlpB, BabA and BabB were 7.6%. Similarity between BabA/BabB and AlpA/AlpB were approximately 41%. Similarity between SabA and SabB were 71.19%. The common areas in expose amino acid region were 86-89/56-58 in SabA/SabB and 170-171, 34-37/186-187, 54-57 in AlpA/AlpB, respectively. Detection of exposed amino acid sequences of OMPs is very helpful for characterization of their corresponding receptor. A number of common regions, between SabA/SabB and AlpA/AlpB, located on the exposed amino acid areas but none of the similar areas in BabA/B is located on the exposed amino acid regions. Exposed amino acid of OMPs is the main sequences which are related to interaction with stomach epithelial cells.

Key words: AlpA, AlpB, BabA, SabA, Exposed amino acid sequences.

After discovery of \textit{Helicobacter pylori} pathogenesis approximately 30 years ago many scientists have shown that \textit{H. pylori} strains cause various diseases including chronic gastritis, peptic ulcer, gastric adenocarcinoma and primary gastric B-cell lymphoma (J. V. Solnick and D. B. Schauer, 2001).

It has been proposed that \textit{H. pylori} adhesins play important role for binding to the gastric mucosa and outcome of infection (D. Ilver \textit{et al.}, 1998, J. Mahdavi \textit{et al.}, 2002, N. Murata-Kamiya, 2011). Adherence of \textit{Helicobacter pylori} and colonization to the gastric mucosa induces host immune and inflammatory responses. However, \textit{H. pylori} escape from host immune response with different mechanisms (E. P. Reeves \textit{et al.}, 2008).

There are several members of proteins known as outer membrane proteins (OMP) on the


Indeed, Lewis b available on the surface of MUC5AC in gastric mucus (C. De Bolos et al., 1995). Prior study had shown that the presence of babA gene with cagA (cytotoxin-associated gene A) and vacA (vacuolating cytotoxin gene A), together increase the risk of gastritis, ulcer disease, gastric cancer and MALT lymphoma (M. Gerhard et al., 1999, F. O. Olfat et al., 2005, C. F. Zambon et al., 2003).

SabA is one of the major H. pylori adhesins and the sialyl-Lewis x antigen is a binding site for SabA. The interaction of H. pylori with MUC1 in gastric mucus mediates by both SabA and BabA (S. K. Linden et al., 2009). Induction of interleukin-8 (IL-8) secretion by two highly homologous OMPs, AlpA and AlpB, facilitate gastric injury and inflammation (H. Lu et al., 2007).

These two genes are capable of binding to the host cell laminin probably as adhesins. Additionally, other host related receptors have not been recognized yet (O. A. Senkovich et al., 2011). AlpA and AlpB mutants in C57BL/6 mice showed low colonization in gastric epithelial cells (H. Lu, J. Y. Wu, E. J. Beswick, T. Ohno, S. Odenbreit, R. Haas, V. E. Reyes, M. Kita, D. Y. Graham and Y. Yamaoka, 2007).

Some of OMPs are associated with multiple intracellular signaling pathways and consecutive production of pro-inflammatory cytokines, such as interleukin-8 (IL-8), in epithelial cells and increased peptic ulcer disease (S. Censini et al., 1996, Y. Yamaoka et al., 2002).

In the present study we have focused on OMPs that play important role in the gastric disease.

The aim of this research is an extensive computational analysis for investigate OMPs in view of Secondary structures, tertiary structure, signal sequence, internal and external loops. Finding of homology modeling, prediction of accessible surface amino acids among sequences of external loops and similarity of exposed amino acid area in these groups of proteins. The result of this study will be useful for detection adhesion motifs, reorganization of cell surface receptors and to get a better understanding of OMPs interaction with gastric epithelial cells and host inflammatory response.

MATERIALS AND METHODS

Databases

Amino acids sequences of BabA, BabB, AlpA, AlpB, SabA and SabB were analyzed via recovering the genes from National Center for Biotechnology Institute and Uni-prot KB/Swiss-Prot.

Accession number of BabA, BabB, AlpA, AlpB, SabA and SabB in NCBI were 7010027, 12356019, 12355264, 12355263, 9349659 and 7009867, respectively. Accession number of these genes in Uni-prot KB/Swiss-Prot were B6JMB5, E6NLN7, E6NNU8, E6NNU7, D7FE80 and B6JLV4, respectively. Proteins tertiary structure were taken in Protein Data Bank (H. M. Berman et al., 2000).

Servers

The method has been used to predict secondary structure of the proteins was based on PHD and GOR 4 servers (H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne, 2000, Aleksey A Porollo et al., 2004, B. Rost et al., 1994). The presence and location of signal peptide cleavage sites in amino acids sequences was predicted by SignalP 4.1 Server (H. Nielsen et al., 1997).
There are many freely accessible Web servers for protein structures prediction on the Internet. Our model can be built by Phyre servers, namely, structure prediction systems using such algorithm to provide models in PDB format and homology modeling (L. A. Kelley and M. J. Sternberg, 2009).

It is well known that a tertiary structure is mainly responsible for protein's function. Role of loops and turns in protein structure, folding, stability, and function is determined (J. S. Fetrow, 1995, P. T. Jones et al., 1986). Every OMP has a particular pattern of exposed amino acid sequences in external loops, which can be predicted by GETAREA and VADAR servers. Swiss PDB viewer and Discovery studio server were used for processing of data. Uniprot alignment was performed for sequence similarity OMPs detection.

RESULT

Sequences of the outer membrane proteins were retrieved from the data bases and analysis for detection of surface amino acids were

![Graphs](image)

Fig. 1. SignalP3.0 server for prediction of the location of signal peptide cleavage sites in amino acid sequences. A (BabA), B (BabB), C (AIPa), D (AlpB), E (SabA), F (SabB).
performed via above-mentioned servers. Our findings showed that BabAhad four exposed sequences of amino acid;30-55,140-155,245-260,309-320, BabBwith two exposed sequences of amino acid;36-41,100-115, AlpAwith five exposed sequences of amino acid;12-37,80-90,130-135,170-175,183-190, AlpBwith five exposed sequences of amino acid;50-55,75-82,100-105,172-180,180-187, SabAwith six exposed sequences of amino acid;78-80,86-122,140-160,195-200,240-260,300-310, SabBwith seven exposed sequences of amino acid;52-58,82-115,135-155,230-240,270-295,290-298.

SignalP 4.1 Server allowed us to recognize signal sequences of the outer membrane proteins and incision sites which are shown in fig. 1. Moreover, secondary structures (beta sheet, alpha helices, random coil) for the OMPs are shown through PHD and GOR 4 servers (figure 2). The tertiary structures were predicted by Phyre servers (figure 3) following via GETAREA and VADAR servers for detecting accessible surface area amino acids (figure 4).

Uniprot alignment for measuring similarity between the OMPs showed that although there is a low similarity (7.6%) between BabA, BabB, AlpA, AlpB, SabA and SabB, the server has found high similarities among BabA/ BabB (41.16%), AlpA/ AlpB (41.95%) and SabA/SabB (71.19%).

Although the most parts of similarities between proteins were not in the exposed regions, a number of conserved sequences were found in exposed external loops. The common regions were

![Fig. 2. Secondary structure prediction of OMPs by GOR4 server; A(BabA), B(BabB), C(AlpA), D(AlpB), E(SabA), F(SabB).](image-url)
Despite high sequence similarity between BabA and BabB, none of the similar areas were located on the exposed regions. The similarity between BabA, BabB, AlpA, AlpB, SabA and SabB were not in exposed amino acid sequences.

**DISCUSSION**

The gastric pathogen, *H. pylori*, is one of the bacteria that lead to development of chronic gastritis, gastric or duodenal ulcer, gastric cancer and MALT-lymphoma (M. J. Blaser, 1997). *H. pylori* induce strong immune responses in host cells. Moreover, the infection was not eradicated in most cases and patients may subject to severe gastroduodenal disease and gastric malignancy. Adaptation of *H. pylori* to their host immune system cause persistence of the bacteria for years and decades in their niches (N. Murata-Kamiya, 2011). Despite multiple treatments in *H. pylori* associated disease, there is a high incidence in developing countries (M. Oleastro and A. Menard, 2013).

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**Fig. 3.** Tertiary structure prediction of OMPs by CPH model server. Data are shown with Discovery StudioVisualizer; A(BabA), B(BabB), C(AlpA), D(AlpB), E(SabA), F(SabB).
The remarkable progress in recent years has resulted from taking advantage of bioinformatics tools with the structure and function of proteins and application of this information for protein engineering, modeling and manufacturing of vaccines. Outer membrane proteins could possibly be one of the most important proteins in *H. pylori*. Furthermore, OMPs act as adhesins and involve in gastric colonization (B. Kalali *et al*., 2014). It was found that OMPs of bacteria are appropriate candidates for vaccine production (Ravinder Singh *et al*., 2014). In the current study, we have systematically analyzed exposed amino acid sequences for detecting adhesion motifs and possible receptors as well. Structural similarities between proteins are a suitable prediction for finding a functional similarity among the proteins. Our study has focused on similarity of exposed amino acid area in several *H. pylori* OMPs. Our results have shown that there was high similarity between AlpA and AlpB (41.95%), SabA/SabB (71.1%) and BabA/BabB (41.16%). Although the mentioned similarities are previously reported by a number of research groups, our study has concentrated on the common exposed amino acid sequences. Furthermore,
finding a common amino acid sequence of the proteins which share a high similarity in their exposed amino acid sequences will likely provide us a possible target site for new drugs or vaccines. Moreover, detection of these sequences is very useful for finding complementary receptors on surface of gastric epithelial cell and specific targeting of cell surface receptors. BabA play a role in CagA translocation by the H. pylori TFS and lead to inflammation and intestinal metaplasia (N. Ishijima et al., 2011, S. M. Sheu et al., 2012). Although, BabA interacts with a Lewis b on the human gastric MUC5AC, the biding role of the other proteins in HOP (helicobacter outer membrane proteins) group (BabB, BabC, and HP0227) has not been identified so far (D. Ilver, A. Arnqvist, J. Ogren, I. M. Frick, D. Kersulyte, E. T. Incecik, D. E. Berg, A. Covacci, L. Engstrand and T. Boren, 1998). Sequence similarity between BabA/B genes was 41.16% and the proteins are highly similar in their N- and C-terminal regions but none of the similar areas has located on the exposed amino acid region.

Two greatly homologous OMPs, AlpA and AlpB located in the alpAB operon. AlpA/B causes interleukin-8 (IL-8) secretion and expansion of the inflammatory responses (S. Odenbreit et al., 2002, S. Odenbreit et al., 1996, S. Odenbreit et al., 1999). Even though, the pair proteins will bind to host laminins, their corresponding receptors for these proteins remains unknown up to now (O. A. Senkovich, J. Yin, V. Eksheyyan, C. Conant, J. Traylor, P. Adegboyeaga, D. J. McGee, R. E. Rhoads, S. Slepenkov and T. L. Testerman, 2011). Various types of bioinformatic tools were used to analyses sequence similarity and detection of exposed amino acid sequences. As a result, a few regions of exposed amino acid sequences were detected as common regions which were 170-171, 34-37 in AlpA and 186-187, 54-57 in AlpB, respectively. The biological importance of AlpA and AlpB in adherence to host cells and tissues was reported in several studies and Exposed amino acid sequences of OMPs family play important role in binding to gastric tissue (S. Odenbreit, G. Faller and R. Haas, 2002, S. Odenbreit, M. Till and R. Haas, 1996, S. Odenbreit, M. Till, D. Hofreuter, G. Faller and R. Haas, 1999). Moreover, these two proteins are expressed in all H. pylori strains at steady rates whereas other H. pylori OMPs produce at variable rates which emphasis a critical function of AlpA and AlpB in the bacteria (S. C. Baik et al., 2004).


Our finding has shown that the similarity rate between two proteins SabA/SabB was 71.19% but there was a short amino acid sequence in the exposed area which was common between two proteins (86-89/56-58).

Detection of Exposed amino acid sequences in OMPs is very helpful for characterization of the receptors. Adherence to the gastric epithelium with specific receptors is a crucial step in beginning of infection. In addition, it can be considered for better understanding of the pathways of H. pylori colonization. In this way, we can recognize possible receptor on gastric epithelial cells. In fact, detection of these receptors probably helps us for control of H. pylori infection and to understand fundamental mechanisms of H. pylori colonization in chronic infection. Additionally, Adhesion motifs of the OMPs are a stimulator for host cell immune response and induce pro-inflammatory intracellular signaling. Therefore, these proteins can be used as a purpose for vaccine expansion and protein docking study. Indeed, further studies should be performed in vaccine design and potential targets in future.

**CONCLUSION**

Exposed amino acid of OMPs is the main sequences which are related to interaction with stomach epithelial cells. Despite extensive studies over several H. pylori adhesions, some of their receptors are still unknown.
A number of common regions between SabA/SabB and AlpA/AlpB, located on the exposed aminoacidic areas. In spite of high similarity in BabA/B Homologous, none of the similar areas is located on the exposed amino acid regions.

This study has provided accessible surface amino acid sequences, which will be helpful for detection of possible receptors on gastricepithelial cells and design novel targets to prohibit progress of disease that leads to severe pathologic outcomes.

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