Characterization of HmuY, a unique heme-binding protein of *Tannerella forsythia* and *Porphyromonas gingivalis* as a nomination for pharmaceutical treatment of periodontitis

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ABSTRACT

Periodontitis, a chronic polymicrobial disease of the gums, affects the soft tissues and bone that support the teeth. *Porphyromonas gingivalis* and *Tannerella forsythia* are the major human bacterial pathogens responsible for periodontitis. They bind to and accumulate on the tooth surface, develop the biofilm matrix and form a periodontal pocket. These pathogens acquire heme from host hemoproteins using the HmuY hemophore for nutrition and growth. This study will mostly concentrate on the characterization of HmuY as a unique heme acquisition system using of bioinformatic tools to introduce it as a candidate novel drug against periodontal pathogens. There results show that HmuY is a stable protein which is a membrane-associated heme-binding lipoprotein. The unique feature and the crystal structure of heme-bound HmuY reveal the presence of His 134 and His 166 residues. Protein interaction network have displayed that TonB-dependent receptor HmuR is in close relationship with HmuY. In conclusion, we revealed molecular properties of HmuY protein that can be as a putative virulence agent during bacterial infection. These features make it an appropriate candidate for biomedical applications against periodontal pathogens.

Key words: HmuY, Heme-binding protein, Periodontitis, *Porphyromonas gingivalis*, *Tannerella forsythia*

INTRODUCTION

Periodontitis, also known as pyorrhea, is a serious inflammation of the periodontium that affects the soft tissue and potentially destroys the bones that support the teeth [1]. Bacteria and the host natural response to infection start to break down the bone and connective tissue that hold teeth in place [2]. Gingival periodontal pocket of human enscones more than 500 bacterial species [3]. Among them, the red-complex bacteria consisting of *Tannerella forsythia* and *Porphyromonas gingivalis* have been strongly implicated in the onset of periodontitis [4, 5]. *T. forsythia* and *P. gingivalis* are Gram-negative anaerobic bacteria which harbor an arsenal of virulence factors including fimbriae, cysteine proteinases, trypsin-like protease, hemagglutinins, heat-shock protein and lipopolysaccharide [5-7]. In addition, these pathogens acquire iron in the form of heme from host hemoproteins for nutrition and growth in a hostile environment by a mechanism composed of HmuY hemophore as a novel heme-binding protein and five additional proteins [8-10]. The goal of periodontitis treatment is to thoroughly clean the pockets around teeth from microbiota and prevents damage to surrounding bone [11]. There are several ways to treat periodontitis such as nonsurgical treatment (i.e. scaling, root planing, and antibiotics) and surgical treatment (i.e. flap surgery, soft tissue grafts, bone grafting, guided tissue regeneration, and enamel matrix derivative application) [12]. However, An easier way to deal with periodontal pathogens is inactivation of HmuY. The aim of this study was to characterize the nature of HmuY by computational approach through *in silico* analysis tools. Finally, we will
be able to design new drugs for nonsurgical treatment of periodontal disease with better understand and correlate the wealth of biochemical data on HmuY protein.

MATERIALS AND METHODS

Retrieval and cataloging of relevant sequence information
Amino acids sequence of HmuY of T. forsythia and P. gingivalis was obtained from the National Center for Biological Information (NCBI) (http://www.ncbi.nlm.nih.gov). A Basic Local Alignment Search Tool (BLAST) was performed against Protein Data Bank (PDB) entries to find similar sequences (hits) that share sequence similarity above a threshold defined by parameters.

Molecular Modeling and functional characterization of HmuY
Analysis tools include compute pI/Mw, a tool for predicting HmuY isoelectric point (pI) and molecular weight (Mw); ProtParam, to calculate various physicochemical parameters; peptide mass, a tool for theoretically cleaving HmuY and calculating the masses of its peptide and any known cellular or artificial posttranslational modifications; peptide cutter, to predict cleavage sites of proteases or chemicals in HmuY sequences; and ProtScale, for amino acid scale representation, such as hydrophobicity plots were used from the tools page, http://www.expasy.org/tools/.

The secondary and three dimensional structures of HmuY protein was obtained using the uniprot server (http://www.uniprot.org) and by SWISS-MODEL Workspace (www.swissmodel.expasy.org), respectively.

Protein interaction network analysis
Protein interaction carried in to find out the most potential metabolic functional associations among all identified choke point proteins through protein interaction database STRING (http://string-db.org).

RESULTS
HmuY was found to be associated with 3H8T_A, a protein from chain A, structure of P. gingivalis heme-binding protein HmuY in complex with heme. HmuY is composed of 4 heme, 12 glycerin, and 8 sulfate ion molecules that is shown in Figure 1.

![Figure 1. Molecules in HmuY protein](http://example.com/figure1.png)

The score for protein alignment between HmuY and 3H8T_A was 397, with a 100% identity. analysis at the NCBI GenBank database showed that HmuY has 216 amino acids that its estimated structure weight was 23879.0 and 24496.4 dalton in P. gingivalis and T. forsythia, respectively. Amino acids compositions of HmuY in these pathogens are displayed in Table 1.
Table 1. The amino acid composition of HmuY in P. gingivalis and T. forsythia

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>P. gingivalis (%)</th>
<th>T. forsythia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>6.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>2.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>3.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>6.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Gln (Q)</td>
<td>3.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>6.0</td>
<td>6.9</td>
</tr>
<tr>
<td>Gly (G)</td>
<td>10.2</td>
<td>5.6</td>
</tr>
<tr>
<td>His (H)</td>
<td>1.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Ile (I)</td>
<td>3.7</td>
<td>5.6</td>
</tr>
<tr>
<td>Leu (L)</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Lys (K)</td>
<td>9.7</td>
<td>14.8</td>
</tr>
<tr>
<td>Met (M)</td>
<td>2.3</td>
<td>5.1</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Pro (P)</td>
<td>4.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Ser (S)</td>
<td>6.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Thr (T)</td>
<td>8.8</td>
<td>7.9</td>
</tr>
<tr>
<td>Trp (W)</td>
<td>1.4</td>
<td>1.9</td>
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<tr>
<td>Tyr (Y)</td>
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<td>2.3</td>
</tr>
<tr>
<td>Val (V)</td>
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<td>8.3</td>
</tr>
<tr>
<td>Pyl (O)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sec (U)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Initial structure prediction showed that HmuY had 26 positively charged residues (Arg + Lys) and 26 negatively charged residues (Asp + Glu) in P. gingivalis and 36 positively and 29 negatively charged residues in T. forsythia. The very high aliphatic index was 64.49 and 73.10 and instability index (II) was computed to be 16.65 and 16.61 in P. gingivalis and T. forsythia, respectively. In modelled secondary structure (Fig. 2), alpha helices residues has dominance over other secondary structure i.e. beta-turn, gamma-turn, beta-hairpin, and coil.

Figure 2. Secondary structure prediction of HmuY protein

Three dimensional structures of HmuY have been generated by SWISS-MODEL Workspace (Fig. 3) and its quality was confirmed with ProsaWeb server (Fig.4).

Figure 4. A). Overall model quality with ProsaWeb server. B) Local model quality with ProsaWeb server.

Its theoretical isoelectric point (pI), extinction coefficient, and grand average of hydropathicity value were 6.83, 33015, and -0.448 respectively in P. gingivalis and were 9.23, 29450, and -0.427 respectively in T. forsythia. These results show that HmuY is a stable protein. HmuY is an all β-protein constituted by 15 β-strands. N- and C-terminus are located on the protein surface corresponding to the palm and they point toward the wrist.
The unique feature of the HmuY heme pocket is the presence of His 134 that is surrounded by Tyr127, Met129, Met136, and Pro168 and His 166 is encompassed by Phe156, Phe164, Pro171 (Fig. 5).

Protein interaction network have displayed proteins, their length and type of relationship with HmuY protein (Fig. 6). It has several predicted functional partners such as hemR (TonB- dependent receptor HmuR), PG_1553 (CobN/ magnesium chelatase), PG_1556 (hypothetical protein), PG_1237 (LuxR family transcriptional regulator), PG_1555, PG_1554, PG_1685, PG_1302, and mfa2 (hypothetical protein), and PG_1019 (putative lipoprotein). The highest and the lowest scores belonged to hemR (0.980) and PG_1302 (0.596), respectively.
DISCUSSION

There are numerous microorganisms in oral cavity that are differ among individuals, reflecting diet, sampling times of day, and geographical locations [5, 13]. Human Oral Microbiome Database (HOMD) (http://www.homd.org/) is one of the most important databases of taxa present in the oral cavity [14]. Among other microbiota that cause multiple diseases such as periodontal disease, pathobiome pathogens such as P. gingivalis and T. forsythia appear in periodontitis [5]. As a keystone pathogens, they impair host immune responses and destroy tooth-supporting tissues [2]. In infective process, these pathogens must gain nutrients to survive and replicate at the infection site, and among such nutrients is heme [8].

Use of systemic antibiotics immediately after nonsurgical treatment can increase the degree of clinical attachment gain and probing depth reduction [15]. Recently, the use of conventional antibiotics is limited due to the emergence of antibiotic resistance [16]. So studies have led to design of new drugs.

According to information obtained from previous studies, the heme can be considered as a drug target [17, 18]. This is an iron-dependent cofactor of several essential enzymes and proteins. Heme is release from host heme-binding proteins of P. gingivalis and T. forsythia through the action of proteases and transport to the bacterial cell [19]. HmuY has been identified in other bacteroidetes (Microscilla marina, Prevotella intermedia, and Bacteroides from the species vulgatus, fragilis, ovatus, thetaiotaomicron, caccuei, stercori, and coprocola), proteobacteria (Plesioctis pacifica, Stigmatella aurantica, and Myxococcus xanthus), spirochaetes (Leptospira biflexa), and chlorobi (Chloroherpeton thalassium) [20]. HmuY is an outer-membrane associated lipoprotein which encodes a 23-kDa protein that consists of 216 amino acids. Comparative analysis of the predicted secondary structure of HmuY protein displayed that it is present outside of the cell. Subsequent inhibition of HmuY protein, bacteria will not be able to gain nutrients such as heme and the reduction of bacterial survival may occur following the loss of HmuY protein. So, it is noteworthy that HmuY is a putative virulence factor that can be a good potential drug target against periodontal pathogens.

CONCLUSION

These data on structure and function of the HmuY, heme-binding protein, which may have also a role in the host immune responses and in interaction with host cells, may lead to the development of novel pharmaceutical treatment. The high stability of HmuY protein have been acquired by its inimitable structure makes it an appropriate candidate for biotechnological and biomedical applications against periodontal pathogens.

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REFERENCES
