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Structural and functional studies of a 50 kDa antigenic protein from *Salmonella enterica* serovar Typhi

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ABSTRACT

The high typhoid incidence rate in developing and under-developed countries emphasizes the need for a rapid, affordable and accessible diagnostic test for effective therapy and disease management. TYPHIDOT®, a rapid dot enzyme immunoassay test for typhoid, was developed from the discovery of a ~50 kDa protein specific for *Salmonella enterica* serovar Typhi. However, the structure of this antigen remains unknown till today. Studies on the structure of this antigen are important to elucidate its function, which will in turn increase the efficiency of the development and improvement of the typhoid detection test. This paper described the predictive structure and function of the antigenically specific protein. The homology modeling approach was employed to construct the three-dimensional structure of the antigen. The built structure possesses the features of TolC-like outer membrane protein. Molecular docking simulation was also performed to further probe the functionality of the antigen. Docking results showed that hexamminecobalt, $Co(NH_3)_6^{3+}$, as an inhibitor of TolC protein, formed favorable hydrogen bonds with D368 and D371 of the antigen. The single point (D368A, D371A) and double point (D368A and D371A) mutations of the antigen showed a decrease (single point mutation) and loss (double point mutations) of binding affinity towards hexamminecobalt. The architecture features of the built model and the docking simulation reinforced and supported that this antigen is indeed the variant of outer membrane protein, TolC. As channel proteins are important for the virulence and survival of bacteria, therefore this ~50 kDa channel protein is a good specific target for typhoid detection test.

1. Introduction

Salmonella enterica serovar Typhi is the causative agent for typhoid fever, a multi-systemic enteric infection in humans [1,2]. The common antibiotics for typhoid treatment are fluoroquinolones but multi drug-resistance strains have evolved rapidly [3]. Vaccines for typhoid fever are available but cheaper and safer vaccines with higher efficacy via a single dosage is yet to be developed [4]. A total of 21.6 million new typhoid cases and 216,500 deaths per year [5,6] have prompted the research on new typhoid detection methods to replace conventional culture methods and biochemical test which are time consuming and lacked sensitivity and specificity [7–10]. The staggering typhoid incidence rate of 1100 cases per population of 100,000 in developing and under-developed countries [1,6] also emphasizes the need to develop a more affordable and accessible typhoid diagnostic test with high sensitivity and specificity. However, today's diagnostic methods which emphasized on the RNA-/DNA-based are complex, costly and unavailable where needed most. Thus, a rapid typhoid diagnostic test is necessary for early detection to prevent delay in treatment and management of possible outbreak.

Ismail et al. [11] reported a ~50 kDa antigenic protein specific for *S. typhi* which was later developed into TYPHIDOT®, a rapid dot enzyme immunoassay (EIA) diagnostic test with 87–90% sensitivity and specificity up to 95% [12]. However, the structure of the antigen has yet to be elucidated. In order to understand the function of the protein at molecular level, it is necessary to determine the three-dimensional structure of the protein since the biological function of a protein is dependent upon the structure that it adopts. The protein structures solved experimentally mainly by X-ray crystallography or NMR [13–19], are able to provide the detailed information about a protein structure but these methods are time-consuming and expensive. Unfortunately, the rate of solving the structure of new proteins by experimental means is still far behind the rate of new protein sequences being generated. Thus, there is considerable interest in using theoretical methods to predict the three-dimensional structure of a protein from its amino acid sequences.

Elucidating the structure of the 50 kDa antigen would be valuable in the quest to understand the biochemical functions and

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Table 1
Top 10 hits of different Enterobacteriaceae organisms from BLAST search against non-redundant protein database with the antigen from *Salmonella enterica* serovar Typhi.

Organism	Accession no.	Title	Sequence identity (%)/similarity (%)	*E*-Value
Citrobacter koseri ATCC BAA-895	YP_001455919.1	Outer membrane channel protein	91/96	<1.0 *e*−180
Escherichia fergusonii ATCC 35469	YP_002384075.1	Transport channel	89/94	<1.0 *e*−180
Escherichia albertii TWO7627	ZP_02900740.1	Outer membrane protein TolC	89/94	<1.0 *e*−180
Escherichia coli E22	ZP_03043238.1	Outer membrane protein TolC	89/94	<1.0 *e*−180
Citrobacter youngae ATCC 29200	ZP_03838598.1	Outer membrane channel protein	90/96	<1.0 *e*−180
Enterobacter cloacae	CAL18627.1	TolC protein	86/93	<1.0 *e*−180
Shigella boydii Sb227	ABB67411.1	TolC	89/94	<1.0 *e*−180
Shigella dysentariae Sd197	ABB63214.1	Outer membrane channel	89/94	<1.0 *e*−180
Shigella flexneri 2a str. 2457T	NP_838556.1	Outer membrane channel protein	89/94	<1.0 *e*−180
Klebsiella pneumonia 342	YP 002236547.1	Outer membrane protein TolC	85/92	<1.0 *e*−180

mechanisms of this antigen at the cellular level. The built model for this antigen could aid in optimizing typhoid diagnostic tests, therapeutic agents or vaccine development in the future via epitope(s) determination. Understanding the epitopes would help to increase the sensitivity and specificity for a recombinant antigen-based diagnostic test. Besides that, the built model can also aid in the design of specific complementary determining regions of the antibody in order to increase the antibody–antigen binding affinity for possible antibody-based diagnostic test. The structural and functional elucidation the antigen would also allow for the search for new inhibitor(s).

In this study, we aimed to construct the three dimension predictive model of the antigen. We also elucidated its functionality via molecular docking simulation and validated the docking simulation by performing mutations on the built structure. Results obtained show that the architecture of the built model resembles the variant of outer membrane protein TolC.

2. Methodology

The sequence of the antigen was obtained from GenBank (id: CAD07712.1). Signal peptide prediction was performed by SignalP 3.0 [20], PrediSi [21], SIG-Pred [22], SOSUIsignal [23] and SPEPLip [24] servers. The secondary structure prediction was made by Jpred [25], Phyre [26], and PSIpred [27] and SSpro [28] servers. The sequence was used to search against Basic Local Alignment Search Tool (BLAST) [29] for non-redundant (nr) protein sequences database. The sequence was further searched against BLAST for suitable templates in RCSB Protein Data Bank (PDB). Multiple sequence alignment with members of Enterobacteriaceae family was performed by ClusterW2 [30]. From the results obtained from BLAST and ClusterW2, outer membrane protein, TolC of *Escherichia coli* (PDB id: 1EK9 [31], 1TQQ [32] and 2VDD [33]) were selected as the templates for the construction of residue 25 to 448 of the antigen. Proteasome from *Saccharomyces cerevisiae* was selected as the template for the C-terminal 43 residues (residue 449–491) of the antigen.

MODELLER 9v7 [34] was employed to build the three dimensional model for the antigen. A total of 100 models were randomly generated. A model with the best discrete optimized potential energy (DOPE) [35] was chosen. The built model was subsequently energy minimized with 45 cycles of the steepest descent followed by 5 cycles of conjugate gradient from the basic minimize- Sander module of Amber version 8 [36] to reduce unfavorable contacts and stesric clashes. Ramachandran plot [37] from PROCHECK [38] was employed to verify the constructed model. Secondary structure calculation of the constructed model was done using STRIDE [39].

Docking of hexamminecobalt, $Co(NH_3)_6^{3+}$, to the binding site of the antigen was performed by AutoDock version 3.0.5 [40]. PM6 charges for $Co(NH_3)_6^{3+}$ were calculated by MOPAC2009 [41] as recommended by AutoDock 3.0.5 [42]. The antigen was added with polar hydrogen atoms using the program *protonate* and the partial charges were loaded using *kollua.amber* option of AutoDock. The grid maps with $60 \times 60 \times 60$ points and a spacing of 0.375 Å were generated under AutoGrid of AutoDock. The molecular docking was performed employing Lamarckian genetic algorithm (LGA) with pseudo-Solis and Wets local search and with the following parameters: population size of 50; energy evaluations of 1,500,000; maximum generations of 27,000; translational step of 0.2 Å; orientational and torsional step of 5.0°; crossover rate of 0.80; mutation rate of 0.02; elitism of 1; local search rate of 0.06; 300 iterations per local search with termination value of 0.01; consecutive successes or failures before doubling or reducing local search step size of 4 and a total of 100 docking runs. Same docking simulation approach was performed towards a single point mutation on the antigen of D392A, D395A and, double mutations of both D392A and D395A. Mutant type antigen was mutated from wild type antigen using HyperChem 7.0 (Hypercube Inc., FL).

3. Results

Data from the GenBank showed that the antigen consists of 491 residues (amino acids). Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.gov/) against non-redundant protein database showed that this antigen has at least 85% and 92% for sequence identity and similarity, respectively, with the outer membrane channel protein of Enterobacteriaceae family, namely *Citrobacter*, *Escherichia*, *Enterobacter*, *Shigella* and *Klebsiella* (with

Table 2
Templates obtained from BLAST search against RCSB protein data bank (PDB) with the antigen from *Salmonella enterica* serovar Typhi.

Organism	PDB id.	Title	Sequence identity (%)/similarity (%)	*E*-Value
Escherichia coli	1TQQ	Chain A, structure Of TolC in complex with hexamminobalt	89/94	<1.0 *e*−180
Escherichia coli	2VDD	Chain A, crystal structure of the open state of TolC outer membrane component Of multidrug efflux pumps	91/96	<1.0 *e*−180
Escherichia coli	1EK9	Chain A, 2.1a X-ray structure Of TolC: an integral outer membrane protein and efflux pump compnent from *Escherichia coli*	91/95	<1.0 *e*−180
Saccharomyces cerevisiae	1G0U	Chain I, a gated channel into the proteasome core particle	33/43	7.5
Saccharomyces cerevisiae	1RYP	Chain J, crystal structure of the 20S proteasome from yeast at 2.4 Å resolution	33/43	7.5