Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Microbiology

An Insight on *V.cholerae* GbpA and Toll-Like Receptor 2 Interaction

Sudipto Mandal[1*]

[1]Department of Microbiology, Ramakrishna Mission Vidyamandira, Belur Math, Howrah-711202, India

DOI: https://doi.org/10.36347/sajb.2025.v13i01.003 | **Received:** 18.11.2024 | **Accepted:** 240.12.2024 | **Published:** 06.01.2025

***Corresponding author:** Sudipto Mandal
Department of Microbiology, Ramakrishna Mission Vidyamandira, Belur Math, Howrah-711202, India

Abstract | **Original Research Article**

Vibrio cholerae N-acetylglucosamine binding protein (GbpA), a secretory colonization factor which is essential for adherence to chitin rich surfaces in environment as well as in intestinal lining. It was previously shown to interact with Toll like receptor 2 on host cells and induce inflammatory response. However, the detailed image of the GbpA-TLR2 interaction is yet to elucidate. In this study using confocal imaging we have found that GbpA co-localize with TLR2 on host cell surface. The predicted model of GbpA-TLR2 complex using molecular docking analysis revealed that the concave surface of TLR2 was involved in H-bond interaction with domain 1 of GbpA. With this study we provide insight into *V.cholerae* specific host-microbe interaction involving GbpA and TLR2.

Keywords : *Vibrio cholerae*, GbpA, Toll Like Receptor 2, Molecular Docking.

INTRODUCTION

Attachment of *Vibrio cholerae* to intestinal epithelium is the first important step in pathogenesis of cholera. N-acetylglucosamine binding protein A (GbpA), the gene product of locus VCA0811, plays a key role in this adherence process. It is a chitin-binding protein known to be a common adhesin for chitinous surfaces as well as intestinal epithelium [Kirn T.J *et al*., 2005, Bhowmick R *et al*., 2008]. GbpA is a secretory colonization factor of *V. cholerae* and it mainly remains in the culture supernatant of bacteria [Kirn.T.J *et al*., 2005]. However, it is also found in bacterial surface bound form [Bhowmick R *et al*., 2008, Wong E *et al*., 2012].

The structural analysis of GbpA has indicated that amongst its four domains, the first domain is responsible for binding to chitin and intestinal mucin during colonization whereas domains 2 and 3 were anchoring the protein on the bacterial cell surface; the domain 4 was found structurally similar to chitinase chitin binding domain of *Serratia marcescens* [Wong E *et al*., 2012].GbpA was considered as an important member of *V. cholerae* chitin utilization programme and essential for bacterial attachment to chitinous surface for chitin utilization. This chitin-binding protein was detected to be an important factor of *V. cholerae* for specific attachment to chitin [Montgomery MT *et al*., 1992]. Deletion of this resulted in reduced interaction with chitin leading to diminished chitin utilization [5 Meibom K.L *et al*., 2004]. It was identified as an adhesion factor for the natural surfaces displaying N-acetylglucosamine (GlcNAc) and its oligomers [Meibom K.L *et al*., 2004].

It has been reported that GbpA can induce mucus secretion in intestinal cells and is the factor bridging *V. cholerae* and mucin in the host [Bhowmick R *et al*., 2008]. Furthermore, GbpA was found to interact with intestinal cells and modulate the host cell behavior with certain implications in disease pathogenesis. It has been found to trigger accumulation of reactive oxygen species in host cells which in tum resulted in necrotic cell death [Mandal S *et al*., 2016].

The binding of GbpA with GlcNAc and mucin has been studied and well documented. However, the interaction of GbpA with host cell surface molecule is least explored and still unclear. The host cell surface molecule interacting with it for carrying the early inflammatory signal into the cell interior is still to be identified.

Toll-like receptors (TLRs) superfamily consists of a large group of receptors, which have evolved to recognize a wide range of molecular patterns associated with microbes. TLR signaling involves the up-regulation of pro-inflammatory cytokines, chemokines and the induction of a local-immune response [Harris G *et al*., 2006]. Here, we evaluated the interaction between the Toll-like receptors 2 (TLRs) and GbpA and found that

domain 1 of GbpA is involved in interaction with the concave surface of TLR2.

MATERIALS AND METHODS

Expression and Purification of Recombinant GbpA

Recombinant GbpA was expressed in *E.coli* C43 cells harbouring the pET22b vector encoding the *gbpA* gene (VCA0811) and purified from the bacterial lysate following the previously described method [Wong E *et al*., 2012].

Cell Culture

Human colonic epithelial cell line HT29 was cultured in complete McCoy's 5A (Sigma, St Louis, USA) supplemented with 10% Fetal Calf Serum (Eurobio, Paris, France), Non-essential amino acids and Penicillin-Streptomycin (MP Biomedicals, USA). HEK293-TLR2 cell line was cultured and maintained in DMEM supplemented with 10% Fetal Calf Serum (Eurobio, France), Non-essential amino acids, Penicillin-Streptomycin (MP Biomedicals, USA) and 100 µg ml^{-1} blasticidin (MP Biomedicals, USA). Confluent monolayers were starved in incomplete medium specific for the cell line (with 0.5% FCS) for overnight before treatment with GbpA.

Labeling of Recombinant GbpA Protein with Amine-Reactive Probe

To be used in confocal microscopy the purified GbpA protein was labelled with the amine-reactive fluorescent dye rhodamine-succinimidyl ester following the method described in amine-reactive probe tagging protocol supplied by ThermoFisher Scientific. In brief, the ε-amino groups of lysine residues present in GbpA were targeted for labelling purpose. Firstly, the purified protein solution (1 mg ml^{-1}) was dialysed in PBS solution. The fluorescent dye dissolved in DMSO (10 mg ml^{-1}) was added slowly to the protein solution under mild vortex. The reaction was incubated overnight at 4°C with continuous stirring. To separate the unreacted labelling reagent from the conjugate, extensive dialysis was carried out against PBS solution at 4°C. The conjugate was stored for further usage under the same conditions used for the parent protein.

Co-Localization Study by Confocal Microscopy

HT29 cells were seeded and grown overnight on coverslips and then incubated with 250 ng $µl^{-1}$ of GbpA-rhodamine in DMEM medium for 1 h at 37°C. After two washes with PBS cells were incubated with anti-TLR2 Alexafluor 488-conjugated mAb for 1 h at ambient temperature. The cells were washed and mounted with antifade reagent containing DAPI (Life technologies). Confocal microscopy was performed for studying the co-localization of GbpA and TLR2 using confocal microscope. The co-localization correlation co-efficient was determined by analysing the images using the Nikon NIS-Element software.

Ligand- Receptor (GbpA- TLR2/1) Interaction by *in Silico* Approach

To check the interaction pattern of GbpA with TLR2, we retrieved the crystal structures of GbpA and TLR2/1 heterodimer from Protein Data Bank. The PDB ID for TLR2/1heterodimer [PubMed: 17889651] and GbpA [PubMed: 22253590] are 2Z80 and 2XWX respectively. Protein-protein docking was performed with these two crystal structures using Clus Pro 2.0 protein-protein docking server (http://cluspro.bu.edu) under default parameters.

RESULTS

GbpA Binds to TLR2 on Host Cell Surface

The molecular interaction between GbpA and its cognate receptor TLR2, a confocal study was performed in HT29 cells. The fluorescence microscopy analysis revealed that the rhodamine-tagged GbpA finely co-localized with TLR-2 (Fig.1). From the co-localization pattern of GbpA and TLR2 it was evident that the protein interacts with the host cell surface receptor TLR2. The yellow coloured region in the merged image prominently indicated the interaction of GbpA with its cognate receptor TLR2. The Pearson's R-value was found to be 0.95 which denoted the fine co-localization of the two fluorescent probes.

TLR2 is involved in Interaction with GbpA

The interaction of GbpA with TLR2/1 heterodimer on cell surface was predicted by protein-protein docking analysis using ClusPro 2.0 protein-protein docking server for our prediction. The predicted model suggested that GbpA most likely to interact with the central domain of the concave surface of TLR2/1 heterodimer (Fig.3). Here we found out the most favourable site of GbpA binding to TLR2. The resulted 93 complexes from four different predictions (Balanced, Electrostatic-favoured, Hydrophobic-favoured and Vander Waals interaction values) were analysed to get the most favourable binding pose of GbpA with TLR2/1 heterodimer. In case of most of the complexes (78 out of 93 conformations), GbpA bound to the concave surface of TLR2 central domain (LRR). Further analysing these 78 conformations, we observed that the N-terminal domain of GbpA is critical for interaction with TLR2. To select the most favourable binding pose out of all these interactions we have used three parameters; H-bond count, lowest energy value and electrostatic charge complementarity. The final selection of GbpA complex consists of 33 H-bond interactions involving the residues from concave surface of TLR2 central domain and with a lowest binding free energy value of (-)770.7 kcal/mol.

The hydrophobic region of GbpA comprising residues Ile82-Leu93 (IASAESALAAAL) positioned at the middle of the TLR2 concave surface which is mostly negatively charged due to the presence of three consecutive Glutamic acid residues (residue number: Glu177, Glu178 and Glu180. It is a highly electrostatic favourable interaction between GbpA and TLR2 as

evident by the H-bond interaction patterns. The negatively charged residues of GbpA: Glu51, Glu62, Glu67, Glu86 and Asp188 interacted with positively charged residues Lys37, Lys253, Arg257, Arg286, Lys305 and Lys339 of TLR2, while positively charged residues from GbpA: Arg78 and Lys81 were found to interact with Glu177, Glu225 and Glu281 of TLR2.

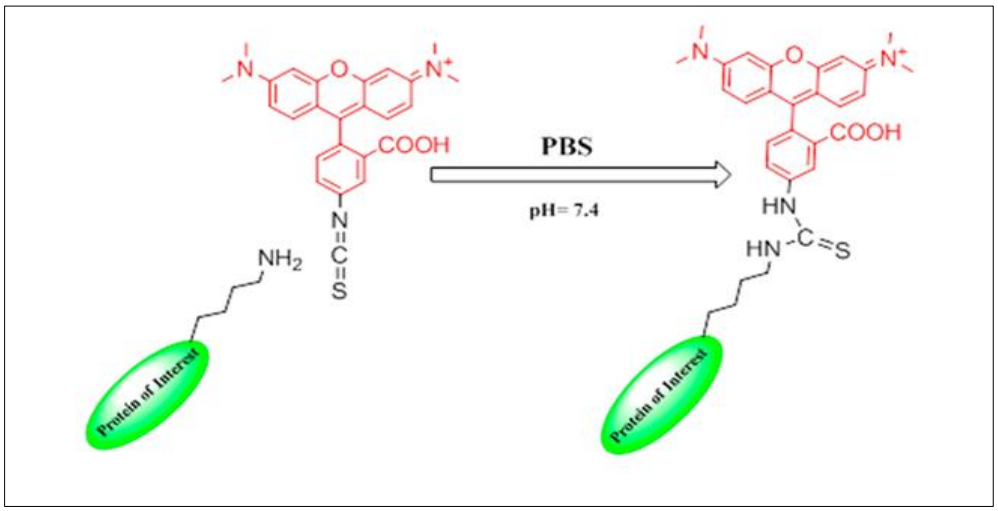

Fig. 1: Schematic diagram showing the chemical reaction of labelling protein GbpA with fluorescent dye rhodamine

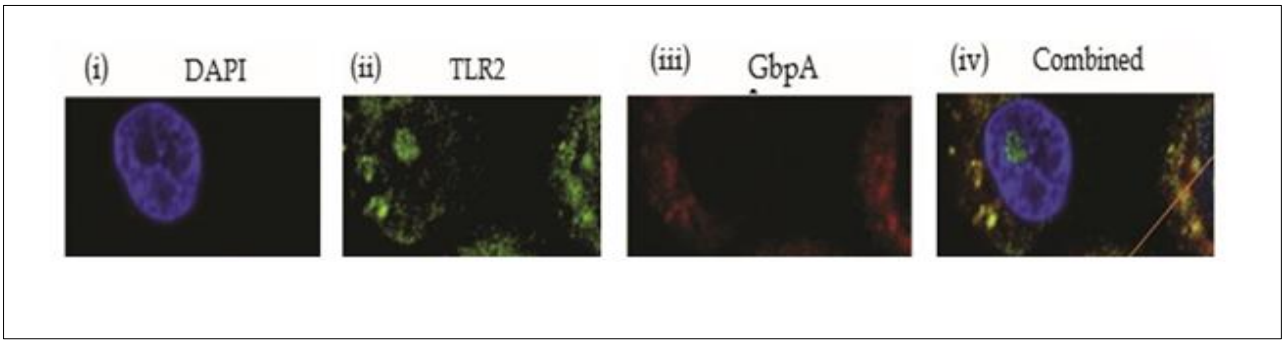

Fig. 2: GbpA binds with TLR2 on host cell surface.

Confocal images of HT-29 cells treated with rhodamine- tagged GbpA (red fluorescence). Green fluorescence corresponds to TLR-2 on cell surface. DNA in HT-29 cells was stained blue with DAPI. Co-localization of GbpA and TLR-2 was shown in the enlarged version of the merged image. The original magnification was 63X. Bar, 20µm.

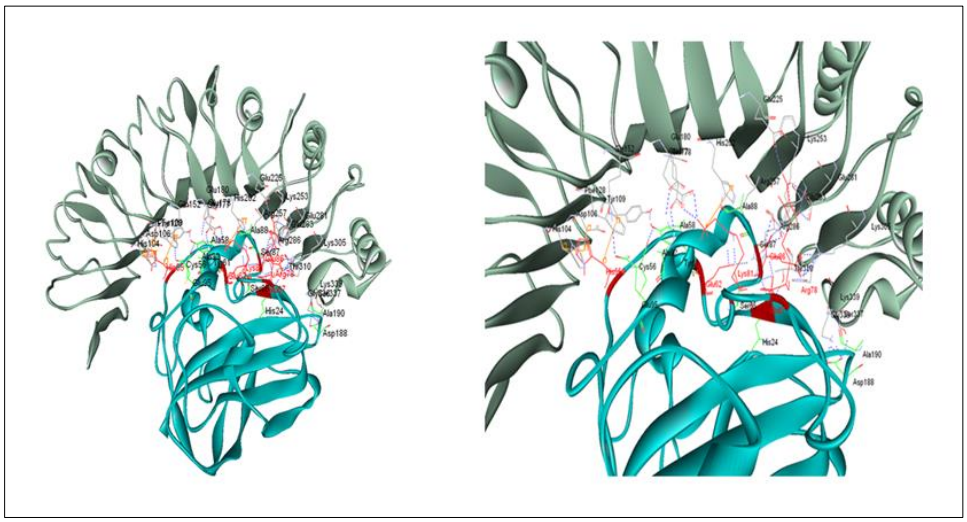

Fig. 3: The concave surface of TLR2 is involved in GbpA binding

In silico model of TLR2-TLR1 heterodimer (PDB: 2Z81) and GbpA (PDB: 2XWX) complex. Image is showing H-bond interaction between GbpA (Magenta) and TLR2 (Green). H-bonds are represented by blue dotted lines.

DISCUSSION

Recent studies have started elucidating role of GbpA in eliciting host responses [Bhowmick R *et al*., 2008, Mandal S *et al*., 2016]. In this study we evaluated the interaction of GbpA with TLR2. Here we have shown the binding of GbpA with TLR2 through cell imaging and molecular docking analysis. TLR2 has been well documented as a sensor molecule for bacterial ligands where it was found to trigger immune responses in host cells [Swaminathan V *et al*., 2013, Furrie E *et al*., 2005, Soong G *et al*., 2004 and Faure E *et al*., 2001]. Lipoproteins, lipoteichoic acid (LTA), Outer membrane proteins (Omp) and peptidoglycan (PGN) from different bacteria have been reported to induce host cellular responses via TLR2 [Segawa T *et al*., 2013, Villarino R.R *et al*., 2016, Nahid M.A *et al*., 2013, Cervantes-Barragan L *et al*., 2009, Kinsner A *et al*., 2006, Fisette P.L *et al*., 2003, Kang SS *et al*., 2015, Bhowmick R *et al*., 2014]. Previous studies have reported that intestinal cells are not responsive towards TLR2 bacterial ligands [Melmed G *et al*., 2003]. However, a number of studies have revealed that TLR2 was involved in eliciting immune response via MyD88-dependent or independent pathways in HT29 intestinal cells [Lee KD *et al*., 2010, Gao Q *et al*., 2012, Cario E *et al*., 2004].

The molecular docking analysis produced a hypothetical model showing the amino acid residues involved in the interaction in GbpA-TLR2/1 complex. This was done based on a previous study where the tri-acylated lipopeptide has been reported to induce the formation of a ‘‘m” shaped heterodimer of TLR1 and TLR2 [Jin M.S *et al*., 2007]. The structure prediction revealed that the domain-1 of GbpA was involved in binding with the concave surface of TLR2.

CONCLUSION

Through this work we were able to show for the first time the interaction between GbpA and TLR2 at a molecular level. The cell imaging showing the binding of GbpA protein with host cell surface receptor TLR2 was an important illustration reinforcing the previous findings. The molecular docking analysis further helped to study the molecular interaction between the two counterparts and revealed the key amino acids residues engaged in the interaction. However, this structural prediction needs deeper analysis employing site directed mutagenesis of key amino acids of GbpA and TLR2.

Conflict of Interest: The authors declared no conflict of interest.

REFERENCES

- Bhowmick, R., Ghosal, A., Das, B., Koley, H., Saha, D. R., Ganguly, S., ... & Chatterjee, N. S. (2008). Intestinal adherence of Vibrio cholerae involves a coordinated interaction between colonization factor GbpA and mucin. *Infection and immunity*, *76*(11), 4968-4977.
- Bhowmick, R., Pore, D., & Chakrabarti, M. K. (2014). Outer membrane protein A (OmpA) of Shigella flexneri 2a induces TLR2-mediated activation of B cells: involvement of protein tyrosine kinase, ERK and NF-κB. *PLoS One*, *9*(10), e109107.
- Cario, E., Gerken, G., & Podolsky, D. K. (2004). Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C. *Gastroenterology*, *127*(1), 224-238.
- Cervantes-Barragán, L., Gil-Cruz, C., Pastelin-Palacios, R., Lang, K. S., Isibasi, A., Ludewig, B., & López-Macías, C. (2009). TLR2 and TLR4 signaling shapes specific antibody responses to Salmonella typhi antigens. *European journal of immunology*, *39*(1), 126-135.
- Faure, E., Thomas, L., Xu, H., Medvedev, A. E., Equils, O., & Arditi, M. (2001). Bacterial lipopolysaccharide and IFN-γ induce Toll-like receptor 2 and Toll-like receptor 4 expression in human endothelial cells: role of NF-κB activation. *The Journal of Immunology*, *166*(3), 2018-2024.
- Fisette, P. L., Ram, S., Andersen, J. M., Guo, W., & Ingalls, R. R. (2003). The Lip lipoprotein from Neisseria gonorrhoeae stimulates cytokine release and NF-κB activation in epithelial cells in a Toll-like receptor 2-dependent manner. *Journal of Biological Chemistry*, *278*(47), 46252-46260.
- Furrie, E., Macfarlane, S., Thomson, G., Macfarlane, G. T., & Microbiology & Gut Biology Group, Tayside Tissue & Tumour Bank. (2005). Toll-like receptors-2,-3 and-4 expression patterns on human colon and their regulation by mucosal-associated bacteria. *Immunology*, *115*(4), 565-574.
- Gao, Q., Qi, L., Wu, T., & Wang, J. (2012). Clostridium butyricum activates TLR2-mediated MyD88-independent signaling pathway in HT-29 cells. *Molecular and cellular biochemistry*, *361*, 31-37.
- Harris, G., KuoLee, R., & Chen, W. (2006). Role of Toll-like receptors in health and diseases of gastrointestinal tract. *World journal of gastroenterology: WJG*, *12*(14), 2149.
- Jin, M. S., Kim, S. E., Heo, J. Y., Lee, M. E., Kim, H. M., Paik, S. G., ... & Lee, J. O. (2007). Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. *Cell*, *130*(6), 1071-1082.
- Kang, S. S., Noh, S. Y., Park, O. J., Yun, C. H., & Han, S. H. (2015). Staphylococcus aureus induces IL-8 expression through its lipoproteins in the

human intestinal epithelial cell, Caco-2. *Cytokine*, *75*(1), 174-180.

- Kinsner, A., Boveri, M., Hareng, L., Brown, G. C., Coecke, S., Hartung, T., & Bal-Price, A. (2006). Highly purified lipoteichoic acid induced pro-inflammatory signalling in primary culture of rat microglia through Toll-like receptor 2: selective potentiation of nitric oxide production by muramyl dipeptide. *Journal of neurochemistry*, *99*(2), 596-607.
- Kirn, T. J., Jude, B. A., & Taylor, R. K. (2005). A colonization factor links Vibrio cholerae environmental survival and human infection. *Nature*, *438*(7069), 863-866.
- Lee, K. D., Guk, S. M., & Chai, J. Y. (2010). Toll-like receptor 2 and Muc2 expression on human intestinal epithelial cells by Gymnophalloides seoi adult antigen. *Journal of Parasitology*, *96*(1), 58-66.
- Mandal, S., & Chatterjee, N. S. (2016). Vibrio cholerae GbpA elicits necrotic cell death in intestinal cells. *Journal of Medical Microbiology*, *65*(8), 837-847.
- Meibom, K. L., Li, X. B., Nielsen, A. T., Wu, C. Y., Roseman, S., & Schoolnik, G. K. (2004). The Vibrio cholerae chitin utilization program. *Proceedings of the National Academy of Sciences*, *101*(8), 2524-2529.
- Melmed, G., Thomas, L. S., Lee, N., Tesfay, S. Y., Lukasek, K., Michelsen, K. S., ... & Abreu, M. T. (2003). Human intestinal epithelial cells are broadly unresponsive to Toll-like receptor 2-dependent bacterial ligands: implications for host-microbial interactions in the gut. *The Journal of Immunology*, *170*(3), 1406-1415.
- Montgomery, M. T., & Kirchman, D. L. (1992). Role of chitin-binding proteins in the specific attachment of the marine bacterium *Vibrio harveyi* to chitin. *Appl. Environ Microbiol*, *59*, 373-379.
- Nahid, M. A., Yao, B., Dominguez-Gutierrez, P. R., Kesavalu, L., Satoh, M., & Chan, E. K. (2013). Regulation of TLR2-mediated tolerance and cross-tolerance through IRAK4 modulation by miR-132 and miR-212. *The Journal of Immunology*, *190*(3), 1250-1263.
- Romero, R. V., Hasan, S., Faé, K., Holubova, J., Geurtsen, J., Schwarzer, M., ... & Sebo, P. (2016). Bordetella pertussis filamentous hemagglutinin itself does not trigger anti-inflammatory interleukin-10 production by human dendritic cells. *International Journal of Medical Microbiology*, *306*(1), 38-47.
- Segawa, T., Saeki, A., Hasebe, A., Arimoto, T., Kataoka, H., Yokoyama, A., ... & Shibata, K. I. (2013). Differences in recognition of wild-type and lipoprotein-deficient strains of oral Streptococci in vitro and in vivo. *Pathogens and Disease*, *68*(3), 65-77.
- Soong, G., Reddy, B., Sokol, S., Adamo, R., & Prince, A. (2004). TLR2 is mobilized into an apical lipid raft receptor complex to signal infection in airway epithelial cells. *The Journal of clinical investigation*, *113*(10), 1482-1489.
- Swaminathan, V., Prakasam, S., Puri, V., & Srinivasan, M. (2013). Role of salivary epithelial toll-like receptors 2 and 4 in modulating innate immune responses in chronic periodontitis. *Journal of periodontal research*, *48*(6), 757-765.
- Wong, E., Vaaje-Kolstad, G., Ghosh, A., Hurtado-Guerrero, R., Konarev, P. V., Ibrahim, A. F., ... & van Aalten, D. M. (2012). The Vibrio cholerae colonization factor GbpA possesses a modular structure that governs binding to different host surfaces. *PLoS pathogens*, *8*(1), e1002373.