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Characterization of Gamma-Glutamyl Transpeptidase from Helicobacter Pylori: An In-Silico Study

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Abstract
Gamma-glutamyl transpeptidase (ggT) of *H. pylori* origin has been documented as frequently associated with human gastroduodenal infections. Inhibition of this virulence factor may help in reducing the incidence of gastric cancer. Current study was performed to explore the characteristics of ggT enzyme. Sequence of protein was retrieved from Uniprot database and analyzed through SOPMA tool, SWISSMODEL server, STRING and QUICKGO tools. The alpha helix, extended strand and random coil contents were demonstrated as 33.33, 16.75 and 49.91, respectively. The tertiary (3D) structural analysis revealed complex folding and monomeric nature of protein. The glnA, P5CDH, pepA, gdhA, ansA, dapE, VacA, gatA, cysM and tuf were found as proteins that interact with ggT. The GO analysis showed glutathione hydrolase, biosynthetic and catabolic activities alongwith acyltransferase and transferase functions of ggT. This enzyme plays role in colonization of stomach cells by *H. pylori*. Exploring its characteristics might help in designing therapeutic approaches for inhibition of ggT, thus preventing bacterium from colonizing the gut.

Keywords: Gastric cancer, Helicobacter pylori, gamma-glutamyl transpeptidase, SWISSMODEL, inhibition

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Introduction

The gamma-glutamyl transpeptidase (ggT) is an enzyme of *Helicobacter pylori* that catalyzes the degradation of glutathione and glutamine in host gastric cells leading to decreased level of these compounds in human body (Ricci et al. 2014). Due to the deficiency of glutathione and glutamine, inflammation occurs which leads to secretion of interleukin-8 from the stomach mucosal cells which disturb the T-cell function and affect immunity (Wüstner et al. 2017). This enzyme also accelerates proliferation in gastric cells resulting in stomach cancer. Due to these effects of ggT, it is considered a major virulence factor of *H. pylori* (Gong et al. 2010). This enzyme is also documented as the diagnostic biomarker for the confirmation of *H. pylori* infections (Wang et al. 2017).

Molecular mechanism study was carried out through construction of mouse model with stomach cancer. The models were exposed to wild type *H. pylori* and a mutated strain with knocked out ggT, separately. Later system was also supplied with α-ketoglutarate (α-KG). The experiment revealed that ggT from wild type *H. pylori* had reducing effect on gastric glutamine concentration and accelerating effect on expression of histone genes (H3K27me3 and H3K9me3) and ribosomal protein L15 encoding gene (RPL15). This effect caused hypermethylation of histones, INITIATED the Wnt signaling pathway induction, altered the characteristics of stomach epithelium AND energy metabolism. These changes resulted in the incidence of stomach cancer. The mouse model with supplementation of αKG demonstrated the reversal of these effects (Jiang et al. 2024).

Literature documented variable intensity of gastric infections, T-helper 1 (Th1) and T-helper 17 (Th17) responses, by mutated forms of ggT (Oertli et al. 2013). Mutant forms of this virulence factor may be used for pharmacological inhibition of its activity. Another study demonstrated absence of infection in gastric cancer cells exposed to *H. pylori* containing disrupted gene for ggT (Akashi et al. 2019).

Considering this involvement of ggT with gastric cancer, we designed current study to evaluate the secondary (2D) and tertiary (3D) configuration, protein interacting partners and gene annotation of this virulence factor. Getting insights into this protein might help in designing strategies for inactivation of this protein.

Methodology

Uniprot Database

In current study, sequence of gamma-glutamyl transpeptidase (ggT) also known as HP_1118, virulence factor from *H. pylori* was retrieved from UNIPROT database (https://www.uniprot.org, accessed on November 2024). This sequence is shown in Figure 1. Accession no. of this protein is A0AAC8N2G1.

MRRSFLKTIGLGVIALFLGLLNPLSAASYPPIKNTKVGLALSSHPLASEIGQKVLE
EGGNAIDAAVAIGFALAVVHPAAGNIGGGGFAVIHLANGENVALDFREKAPLKA
TKNMFLDKQGNVVPKLSEDGYLAAGVPGTVAGMEAMLKKYGTKKLSQLIDPAI
KLAENGYAISQRQAETLKEARERFLKYSSSKKYFFKKGHLDYQEGDLFVQKDLA
KTLNQIKTLGAKGFYQGQVAELIEKDMKKNGGIITKEDLASYNVKWRKPVVGS
YRGYKIISMSPPSSGGTHLIQILNVMENADLSALGYGASKNIHIAAEAMRQAYA
DRSVYMGDADFVSVPVDKLINKAYAKKIFDTIQPDTVTPSSQIKPGMGQLHEGS
NTTHYSVADRWGNAVSVTYTINASYGSAASIDGAGFLLNNEMDDFSIKPGNPN

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LYGLVGGDANAIEANKRPLSSMSPTIVLKNNKVFLVVGSPGGSRIITTVLQVISN
VIDYNMNISEAVSAPRFHMQWLPDELRIEKFGMPADVKDNLTKMGYQIVTKPV
MGDVNAIQVLPKTKGSVFYGSTDPRKEF

Figure 1: Sequence of ggt retrieved from Uniprot database

Sopma Tool

For secondary (2D) configuration prediction, SOPMA tool available at NPS@: Network Protein Sequence Analysis server (https://npsa.lyon.inserm.fr/cgi- bin/secpred_sopma.pl, accessed on November 2024) was employed. To perform analysis, three parameters considered were window width (17), similarity threshold (8) and number of (3).

Swissmodel Server

SWISSMODEL server (https://swissmodel.expasy.org, accessed on November 2025) was employed to retrieve the 3D configuration of ggT protein and for Ramachandran prediction of structure validity. Parameters computed for structure validation included MolProbity and clash score, ramachandran favoured and outliers, rotamer outliers, B-beta deviations, bad bonds and angles, cis prolines and twisted non-prolines.

String Tool

STRING tool for interacting genes or proteins retrieval (https://string.-db.org, accessed on November 2024) was consulted to identify the protein molecules that have tendency to interact with ggT protein.

Quickgo

To perform the gene ontology (GO) annotations, QUICKGO tool (https://www.ebi.ac.uk/QuickGO/annotations, accessed in November 2024) was consulted. This analysis helped us in predicting the molecular and biological functions of ggT virulence factor.

Results

Prediction of Secondary (2D) Configuration

The 2D configuration analysis demonstrated the 33.33, 16.75 and 49.91% amino acids as component of alpha helix, extended strand and random coil, respectively (Table 1 and Figure 1).

Table 1: Prediction of 2D Configuration of ggT Virulence Factor Through SOPMA Tool

No.	Attribute of 2D Configuration	Amino Acids	
		Number	% age
1	Alpha helix	189	33.33
2	Extended strand	95	16.75
3	Random coil	283	49.91

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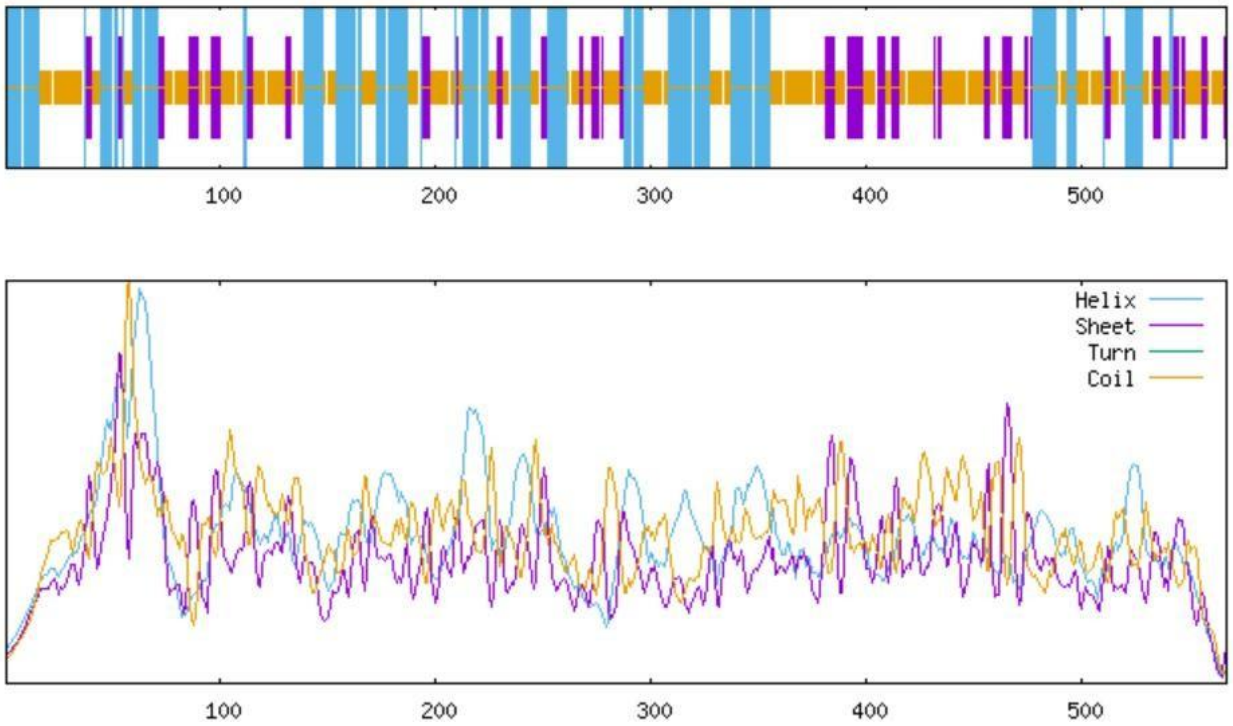

Figure 1: Prediction of 2D configuration of ggT virulence factor from H. pylori, through SOPMA tool

Prediction of Tertiary (3D) Configuration

The 3D configuration analysis revealed that the enzyme comprised of single domain with complex folding (Figure 2). Major part of structure comprised of alpha helix and beta strands were a few. The GMQE score observed was 0.92 and sequence identity was 100.

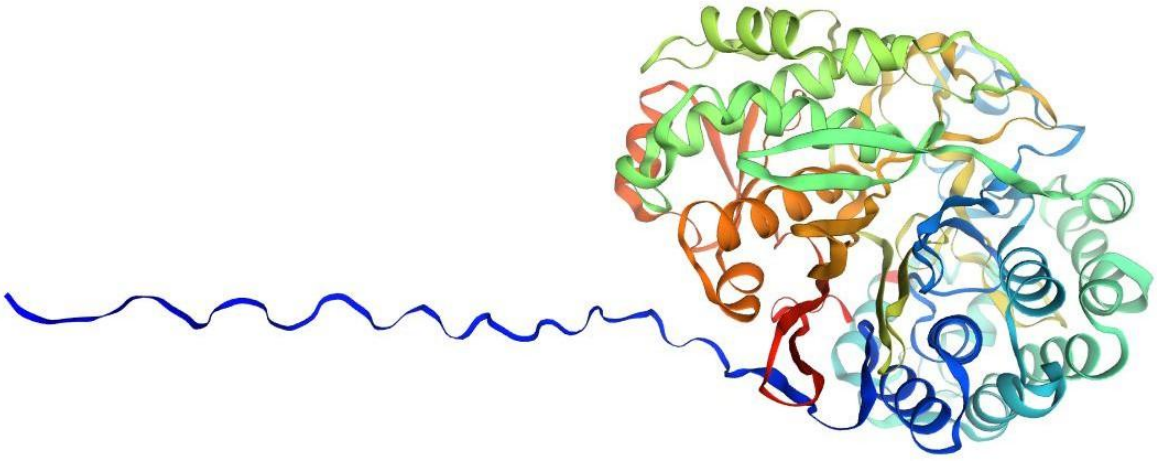

Figure 2: Assessment of tertiary (3D) configuration of ggT virulence factor from H. pylori, based on SWISSMODEL server

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The structure generated through SWISSMODEL server was validated via Ramachandran plot (Figure 3).

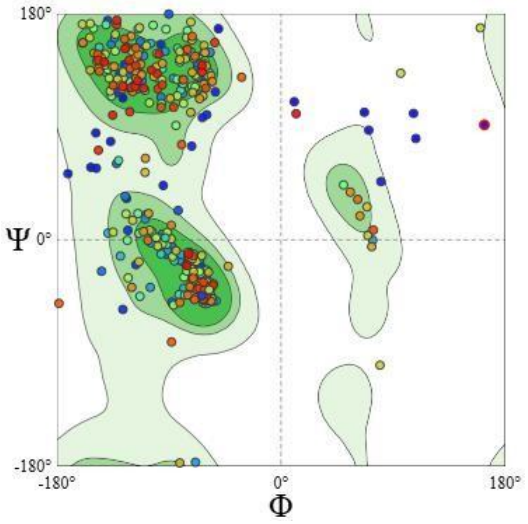

Figure 3: Validation of 3D structure of ggT predicted through SWISSMODEL server through Ramachandran plot

Ramachandran plot showed the values of 1.00 and 0.12 for MolProbity and clash scores, respectively. 92.74% amino acids were found in favoured region of the plot and 2.65% amino acids outlied the allowed region. The B-beta deviations observed were 7. Number of bad bonds and bad angles was 0 out of 4388 and 33 out of 5931, respectively. The cis prolines were 3 out of 27. Twisted non-prolines were 5 out of 539 (Table 2).

Table 2: Validation of 3D Structure of ggT, Generated Through SWISSMODEL Server, via Ramachandran Plot

#	Parameters computed	Scores
1	MolProbity score	1.00
2	Clash score	0.12
3	Ramachandran favoured	92.74%
4	Ramachandran outliers	2.65%
5	Rotamer outliers	0.66%
6	B-beta deviations	7
7	Bad bonds	0 / 4388
8	Bad angles	33 / 5931
9	Cis prolines	3 / 27
10	Twisted non-prolines	5 / 539

The ramachandran outliers amino acids sequence was comprised of phenylalanine, arginine, leucine, alanine, methionine, leucine, asparagine, threonine, tryptophan, leucine, proline, lysine, arginine, serine and lysine. Two threonine and one leucine residue was found in rotamer outliers. C-beta deviations comprised of two arginines, one methionine, one asparagine, one leucine, one glycine and one lysine residue (Figure 4).

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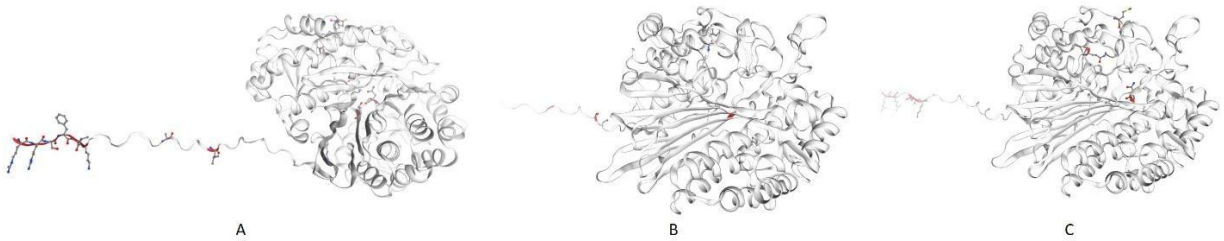

Figure 4: Ramachandran validation of structure generated by SWISSMODEL server
(A) Ramachandran outliers (B) Rotamer outliers (C) C-beta deviations

Prediction of Protein Interacting Partners

Protein legends with highest scores observed were glutamine synthetase (glnA), delta-1-pyrroline-5-carboxylate dehydrogenase (P5CDH or HP_0056), aminopeptidase a/i (pepA) and glutamate dehydrogenase (gdhA). Scores observed were 0.953, 0.947, 0.930 and 0.927, respectively. In addition, six proteins partners. i. e., L-asparaginase II (ansA) (0.555), succinyl- diaminopimelate (dapE) (0.534), vacuolating cytotoxin (VacA) (0.512), Glu-RNA amidotransferase subunit A (gatA) (0.489), cysteine synthetase (cysM) (0.478) and translation elongation factor EF-Tu (tuf) (0.477) were also observed (Figure 5).

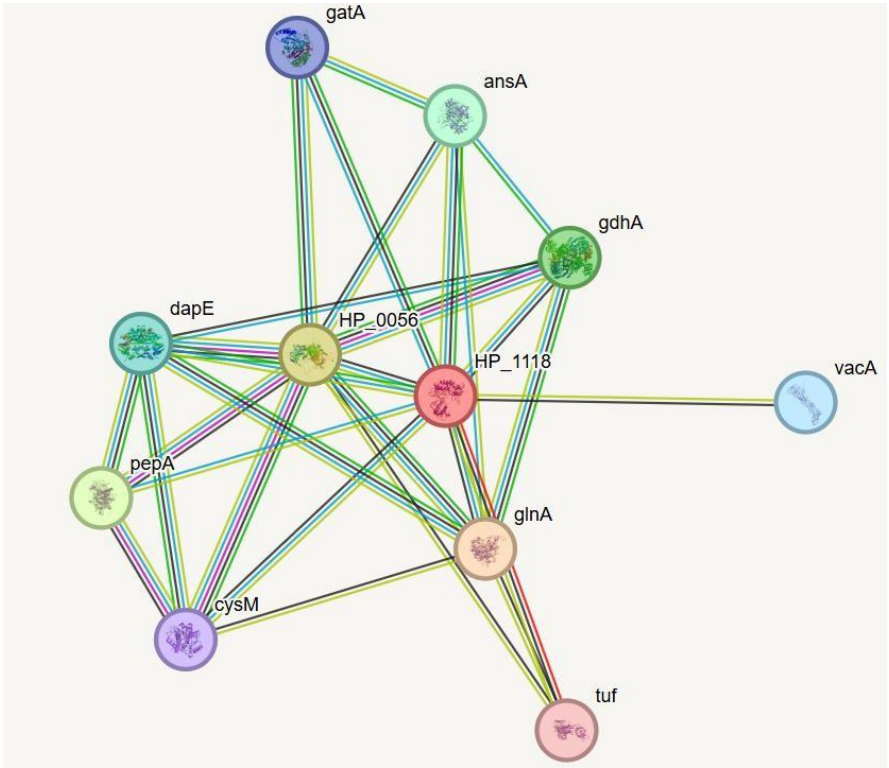

Figure 5: Prediction of protein interaction partners of ggT virulence factor via STRING tool

gatA: glutamine synthetase, ansA: L-asparaginase-II, gdhA: glutamate dehydrogenase, vacA: vacuolating cytotoxin, tuf: translation elongation factor EF-Tu, glnA: glutamine synthetase, HP_0056: delta-1-pyrroline-5-carboxylate dehydrogenase, cysM: cysteine synthetase, pepA: aminopeptidase a/i, dapE:

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succinyl-diaminopimelate desuccinylase

Prediction of Gene Ontology

GO annotations based on QuickGO tool revealed the molecular functions of ggt. i. e., transferase, acyltransferase and hydrolase activity (GO:0016740) and glutathione hydrolase activity (GO:0036374) and biological functions. i. e., glutathione biosynthetic process (GO: 0006750) and glutathione catabolic process (GO: 0006751).

Discussion

The 2D structural analysis predicted alpha helix and beta contents of ggT as 33.33 and 16.75%, respectively. This is in accordance with literature which documents major proportion of this virulence factor to be comprised of alpha and beta parts. However, exact proportion has never been reported so far. Current study demonstrated single domain in 3D structure of ggT enzyme which is inconsistent with literature (Hibi et al. 2004). Previous work demonstrated enzyme comprised of two domains, one heavy with 38kDa and second light with 21kDa (Song et al. 2011). Alongwith these two subunits, there is a signal peptide sequence. This sequence is cleaved through post translation modification (PTMs) (Ricci et al. 2014).

Finding of interaction of P5CDH, pepA, dapE, gatA with ggT is not in line with previous work as no interaction has been reported yet (Kumar et al. 2023). Interaction of ggT with VacA, gdhA, glnA, asnA, cysM and tuf is consistent with previous published work (Chiu et al. 2017, Liu et al. 2024, Maggi et al. 2015, Miller and Maier 2014, Oertli et al. 2013).

The most important interacting protein for ggT is VacA. Major function of VacA is disturbance of the function of host gastric cells through vacuolation. The ggT catalyzes breakdown of glutamine into ammonia (Ling et al. 2015). In gastric cells, ggT interacts with VacA and potentiates its effect.

Mutations might be induced in ggT enzyme to inactivate it and to reduce the colonization potential of *H. pylori*. Additionally, drugs might be designed to target this virulence factor based on the properties explored in current investigation.

Statements and Declarations

Informed Consent: N/A **Ethical**
Approval: N/A

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

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Author contributions: N.H. write up of original draft, S.M.B. formal analysis, A.H.I.A.H. formal analysis, A.K. data curation, A.N. data visualization, F.M. supervision and perceived the idea

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Submission declaration and verification: The manuscript has not been submitted anywhere else and is not under consideration by any other journal.

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Data availability statement: The sequence of ggT documented in current study is available at https://uniprot.org.

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