Bioinformatics analysis of adhesin-binding potential and ADME/Tox profile of anti-\textit{Helicobacter pylori} peptides derived from wheat germ proteins\footnote{This article is a part of the “Structure and function of food proteins and peptides” Special issue.}

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ABSTRACT

Anti-adhesive activity of wheat germ-derived peptides, which is considered as one of the promising strategies for preventing \textit{Helicobacter pylori} infection, was investigated. The underlying mechanism of anti-adhesive action was due to peptides acting as receptor analogues and binding to \textit{H. pylori} adhesin proteins. However, there is lack of information on the nature and strength of this molecular interaction as well as the participating species and drug-likeness of the food-derived bioactive peptides. In this study, the biostability and ADME/Tox (absorption, distribution, metabolism, excretion and toxicity) profile of the anti-adhesive peptides were analyzed using bioinformatic tools, and their binding potential to \textit{H. pylori}'s adhesins estimated by molecular docking. Binding is facilitated by mostly hydrogen bonding and hydrophobic interaction occurring in the active site of the adhesin proteins with affinities ranging from -6.0 to -7.4 and -6.0 to -7.8 kcal/mol for BabA and SabA, respectively. The results indicate highly possible binding capabilities of the peptides to adhesin proteins. Out of 16 peptides studied, 14 bound in the vicinity of the active site of BabA and SabA whereas two different peptides demonstrated allosteric binding. The most hydrophobic peptide, P210 showed strong binding affinity for both BabA and SabA and, therefore, predicted to be the most promising peptide for further development in the prevention, management and treatment of \textit{H. pylori} infection. The selected peptides were shown to be non-toxic, and to have high potential of localized effect of interfering with bacterial adherence. This work provides insights into the anti-adhesive mechanism of peptides and new evidence demonstrating bioactive peptides as promising nutraceutical candidates for preventing \textit{H. pylori} infection.

1. Introduction

\textit{Helicobacter pylori} (\textit{H. pylori}) is a pathogen responsible for gastric-related infections that affects approximately 50% of the world's population (Van Duynhoven and De Jonge, 2001). \textit{H. pylori} has been classified as a class I carcinogen by the World Health Organization (WHO), and approximately 10–15% of those infected will develop peptic ulcer or gastric adenocarcinoma (Sun et al., 2020a). Currently, antibiotics therapy is the most widely used treatment for \textit{H. pylori} infection; however, it has many side effects and induces the emergence of antibiotic-resistance bacteria (Narayanan et al., 2018). Therefore, it is important to explore preventive therapy and alternative treatments for \textit{H. pylori} infection (Yonezawa et al., 2015).

The mode of infection and survival of \textit{H. pylori} within the harsh environment of the gastric mucosa involve the use of its adhesin proteins, such as BabA and SabA, to bind tightly to the sugar moieties of Lewis\textsuperscript{b} and sialyl-Lewis\textsuperscript{s}, respectively. Their binding to the Lewis antigens on the surface of the epithelial cells lining the stomach and duodenum are mainly mediated by networks of hydrogen bonding and does not cause any conformational change in the adhesin protein (Hage et al., 2015; Pang et al., 2014). Hence, anti-adhesive therapy targeting specifically the active site of the adhesin protein is considered as one of the promising strategies for preventing \textit{H. pylori} infection (Sun et al., 2020b). The principle of anti-adhesive therapy is the interference or inhibition of bacterial adherence to host cells by using anti-adhesive agents as receptor analogs or adhesin analogs, subsequently preventing infection (Sun and...}
Table 1. Sequences of 33 bioactive peptides with gastric biostability (resistance against pepsin)*.

| No. | Peptide sequence | No. | Peptide sequence |
|-----|------------------|-----|------------------|
| 2   | DAVYTHEAR        | 193 | RVITMPK          |
| 4   | VOASIAANTWVSGTPQTK | 207 | AVVIHPYYR        |
| 16  | AGGAYMTNASTAVTR  | 210 | VTGAIPI          |
| 36  | VEIANDOQGNR      | 213 | KYMVPVVGK        |
| 42  | DNIQGTTKPAIR     | 218 | HTGSAAGGGGISR    |
| 70  | ISANIAAR          | 220 | KAVVHVPPYR       |
| 73  | PAGNVGEIR        | 236 | KGHAVGDIPGVR     |
| 86  | TIVQQVEAYR       | 247 | RVNQAVYVIATSTK   |
| 89  | IISSIEQK         | 249 | MDDNNTVGGSR      |
| 90  | IGGGTVPGVR       | 251 | VPKPAGNVGEIR     |
| 97  | DNIQGITPAIR      | 253 | QAQEINVAEIR      |
| 102 | DNIQGITK         | 255 | ITPPHGNSGVR      |
| 115 | MISVTPGR         | 258 | DAPSAATPTGAR      |
| 141 | GHAVGDIPGVR      | 262 | RQGNTARSBR       |
| 165 | NVYYGVAP/AQK     | 264 | VTMVEIE          |
| 184 | VPINPSGDR        | 269 |                 |

* The peptides were hydrolyzed in silico with pepsin at physiological pH 1.3 using ExPaSy Peptide Cutter.

Table 2. Predicted binding sites and affinities of anti-adhesive peptides with H. pylori adhesins BabA (PDB: 4ZH0) and SabA (PDB: 4O5J).

| No. | Peptide sequence | BabA peptide binding | SabA peptide binding | No. of H-bonding | Active torsion |
|-----|------------------|----------------------|----------------------|------------------|---------------|
| -    |                  | Binding affinity (kcal/mol) BabA Residues involved within 5 Å | H-bond distances (Å) | | |
| 2    | DAVYTHEAR        | -7.1                  | A-110, N-111*, G-112, F-122, I-150, E-151, K-154*, K-155, N-157, E-158, A-159, I-162, Y-185*, Y-187, N-196, C-197, Q-200, V-201, T-202, G-203, K-214, I-215, Q-216*, T-217, I-218, D-219, G-220, T-222, I-248, N-250*, A-264, Q-265*, T-268, L-269 | 6 | 1.9-2.5 | 42 | 7.0 | L-94, W-97*, N-103, F-105, S-131, V-122, Q-123*, G-124, Q-145, Y-148, D-149, K-152*, K-153, E-155*, D-157, L-158, Q-159, A-160, T-163, S-165, K-168, P-332, N-334, P-335, Y-336, R-337, Q-338 | 4 | 2.4-2.6 | 42 |
| 70   | ISANIAAR          | -6.7                  | K-77, A-78, N-79, V-83, Q-84*, L-87, N-91, L-167, G-170, L-171, L-395, A-396, T-397, C-398, Q-410, G-411*, A-413, P-414, C-426, A-427, Y-428, V-429, G-430, Q-431, T-432, T-434, N-435**, N-438, E-440, H-442 | 5 | 2.2-2.8 | 29 | 7.3 | Y-65, S-68, F-69, P-70, N-72, T-77, T-78*, Q-79, S-80, P-81, F-83, N-84, Q-87*, T-91, Q-162, T-163, N-170, N-171, L-172, Q-338*, Q-344, E-345*, T-348, N-351**, N-352, Y-355, Y-356, R-359 | 7 | 2.0-2.8 | 29 |
| 73   | PAGNVGEIR        | -6.9                  | K-114, K-119, I-121, N-123*, N-124*, E-125, S-130, T-131, S-132, T-134, Q-161, Q-164, T-165, K-168, N-162, V-183, T-184, V-185, T-186, R-188, V-232, N-239, T-240, T-241, Y-245, E-247*, F-414, G-415**, T-416, V-417*, T-418 | 6 | 2.1-2.4 | 31 | 7.2 | L-94, W-97, S-98, G-102, N-103, F-105, S-121, V-122, Q-123*, G-124, Y-148, K-152*, A-155, E-156, Q-159, T-163, S-165*, K-168, S-330, P-332, T-333, N-334**, P-335, R-337, Q-338 | 6 | 2.0-2.6 | 31 |
| 86   | TIVQQVEAYR       | -7.4                  | N-109, A-110, N-111, G-112, S-149, I-150, E-151, K-154**, K-155*, E-158, A-159, I-162, Y-185, Y-187, N-196*, C-111 | 11 | 2.0-2.7 | 44 | 6.9 | L-94, W-97, S-98, A-101, G-102, N-103, Y-104, Q-123, G-124, K-152*, K-153, E-156, D-157, L-158, Q-159*, A-105 | 5 | 2.1-2.6 | 44 |

(continued on next page)
### Table 2 (continued)

| No. | Peptide sequence | BabA peptide binding | Residues involved within 5 Å | No. of H-bonding | Active torsion | BabA peptide binding | Residues involved within 5 Å | No. of H-bonding | Binding distances | Active torsion |
|-----|------------------|----------------------|-----------------------------|-------------------|--------------|----------------------|-----------------------------|-------------------|------------------|--------------|
| 89  | ISSIEQK          | -6.3                 | N-109, A-110, N-111, G-112, I-150, I-151, N-152, K-154*, K-155*, L-156, A-159, L-162, Y-185, Y-187, V-200, Q-201, K-214*, Q-216, T-217, I-218, D-219, T-225, I-227, N-250, L-252, A-261, A-264, Q-265*, S-267, T-268, N-271, T-272, N-275 | 4 | T-77, T-78*, Q-79, S-80, P-81, F-83, N-84, Q-87, Q-162*, S-164, K-168, Q-169, G-169, N-170, N-171, L-172, Q-338*, N-341*, Q-344, E-345, T-348, K-350, N-351*, N-352*, S-354, Y-355, V-356, T-368* | 10 | 1.9-2.5 | 36 |
| 102 | DNIQGITK         | -6.5                 | N-109, A-110, N-111*, G-112, S-149, I-150, E-151, K-154*, K-155, E-158, A-159, L-162, Y-185, Y-187, V-201, Q-202, T-202, G-203, K-214, Q-216*, T-217, I-218, D-219, T-225, I-227, N-250, L-252, A-261, A-264, Q-265, S-267, T-268* | 6 | 2.0-2.5 | 39 | -6.9 | L-94, W-97*, S-98, G-102, N-103, F-105, V-122, Q-123*, Y-148, D-149, K-152, Q-153, A-155, E-156*, Q-159*, A-160, Q-162, T-163*, N-164, S-165, K-168*, G-169, Y-322, K-324, P-332, N-334, F-335, Y-336, R-337, Q-338 | 7 | 1.9-2.6 | 39 |
| 115 | MISVTGPR         | -6.5                 | A-110, N-111, F-122, E-151, K-154, K-155, N-157, E-158, A-159, L-162, Y-185, Y-187, V-201, Q-202, T-202, K-214, Q-216*, T-217*, I-218, I-219, T-225, I-227, N-250, L-252, A-261, A-264, Q-265, S-267, T-268* | 4 | 2.0-2.3 | 30 | -7.0 | L-94, W-97*, S-98, G-101, G-102, N-103, S-121, V-122, Q-123*, Q-145, Y-148, D-149, K-152*, A-155, E-156*, Q-159*, A-160, T-163, S-165, K-168, P-332, T-333, N-334*, P-335**, Y-336, R-337 | 8 | 1.9-2.4 | 30 |
| 184 | VHPNPSPGDR       | -6.9                 | G-101, Y-102*, V-103, T-104, Q-105*, C-106, G-107, K-119, I-121, N-123, N-124, G-127, Y-128*, R-129, S-130, T-131, S-132*, I-133*, T-134**, C-135, S-136*, L-137, T-186, T-188, T-241, Y-245, E-247, Y-279, H-281 | 10 | 1.8-2.4 | 32 | -6.5 | Q-42, A-44, S-45, Q-48, S-49*, N-52, S-61, S-62, N-64*, Y-65, L-66, S-68, N-72, Y-355, Y-356, R-359, D-361, A-362, A-363, S-365**, R-368*, D-369, N-376, E-379 | 5 | 2.3-2.5 | 32 |
| 193 | RVTIMPK          | -6.6                 | R-63, N-329, E-330, H-331, E-332*, Q-333, T-334, T-335**, F-336, G-337, G-338, N-339, F-345, P-347, F-348*, T-349, D-350, A-351, S-352*, F-353, A-354, G-356, M-357, L-460*, V-461, N-462, F-463 | 7 | 2.2-2.5 | 31 | -7.7 | L-94, W-97*, S-98, G-102, N-103, Y-104, V-122, Q-123, G-124, N-125, K-152*, E-156, Q-159*, A-160, T-163, N-164, S-165, K-168, G-169, P-326, A-328*, G-329, S-330, T-331, P-332, T-333*, N-334*, P-335, V-336, R-337, Q-338 | 6 | 2.1-2.4 | 31 |
| 207 | AVVBHVPPYR       | -7.3                 | N-109, A-110, N-111, G-112, E-151, N-152, K-154*, K-155*, E-158, A-159, I-162, Y-185, T-187*, N-196, V-201, T-202 | 5 | 2.2-2.6 | 32 | -6.9 | L-94, S-98, G-102, N-103, Q-123*, K-152**, K-153, A-155, E-156**, D-157, Q-159, A-160, T-163, S-165, K-168, A-170, Q-171, A-173 | 7 | 1.9-2.5 | 32 |
Table 2 (continued)

| No. Peptide sequence | BabA peptide binding | Residues involved within 5 Å | No. of H-bonding | H-bond distance (Å) | Active torsion | BabA peptide binding | Residues involved within 5 Å | No. of H-bonding | Binding distances (Å) | Active torsion |
|----------------------|----------------------|-----------------------------|------------------|-------------------|---------------|----------------------|-----------------------------|------------------|---------------------|---------------|
| 202, K-214, Q-216, T-217, I-218, D-219, G-220, I-227, I-248, N-250, A-264, Q-265, S-267, T-268, N-271, T-272, N-275* | 6 | 2.0-2.6 | 46 | -6.3 | 321, Y-322, P-332, T-333, N-334, P-335, Y-336*, R-337, Q-338 |
| KMEVPCIVK - 6.5 | N-109, A-110, N-111, G-112, S-149, I-150, E-151, K-154, K-155, E-158, A-159, I-162, Y-185, Y-187, N-196, Q-200, V-201*, T-202*, G-203, V-204, K-212, K-214, I-215, Q-216*, T-217, I-218, D-219, G-220*, K-221, I-227, N-250, L-252, A-264, Q-265*, T-268 | 4 | 2.1-2.4 | 21 | -7.8 | L-94, W-97, S-98, G-102, N-103, Q-152**, A-155, E-156, Q-159, A-160, T-163, N-164, S-165, K-168, P-326, A-328, G-329, S-330*, T-331, P-332, T-333, N-334*, P-335*, Y-336, R-337, Q-338 |
| VTGAIPT -7.0 | G-101, Y-102*, V-103, T-104*, Q-105, C-106, K-119, I-121, N-123, G-127, Y-128, S-130*, T-131, S-132, I-133, T-134*, C-135, S-136, T-188, S-190, T-241, V-243, Y-245, E-247, H-281, A-282 | 2 | 1.9-2.1 | 39 | -6.0 | L-94, W-97, S-98, G-102, N-103, Q-152**, A-155, E-156, Q-159, A-160, T-163, N-164, S-165, K-168, P-332, T-333, N-334**, P-335, Y-336, Q-337 |
| KAVVBVPR -6.7 | N-109, A-110, N-111, G-112, S-149, I-150, E-151, K-154*, K-155, L-156, E-158, A-159, I-162, Y-185, Y-187, N-196, Q-200, V-201, T-202, K-214, Q-216, T-217*, I-218, D-219, G-220, T-225, I-227, N-250, L-252, A-264, Q-265, S-267, T-268, N-271, T-272, Q-319 | 6 | 2.1-2.6 | 40 | -6.6 | L-94, W-97, S-98, G-102, N-103, P-105, S-121, V-122, Q-123*, G-124, Q-145, V-148, D-149, K-150, K-152, K-153, A-155, E-156**, Q-159, S-165, K-168, P-332, T-333, N-334**, P-335, Y-336, Q-337 |
| MDNNTVGGS -7.0 | G-101, Y-102, V-103, T-104, Q-105, C-106*, K-119, I-121, N-123, N-124*, E-125, C-127, Y-128, R-129, S-130, T-131, S-132*, I-133, T-134*, C-135, S-136, T-186, T-188, C-189, S-190*, V-203, N-239, T-240, P-241, V-243, S-244, Y-245*, E-247, H-281, A-282, T-249 | 6 | 2.1-2.6 | 40 | -6.6 | L-94, W-97, S-98, G-102, N-103, P-105, S-121, V-122, Q-123*, G-124, Q-145, V-148, D-149, K-150, K-152*, K-153, A-155, E-156*, D-157, Q-159, A-160, T-163, S-165, P-332, N-334*, P-335*, Y-336, R-337, Q-338 |
| RQGNTRSR -6.0 | A-110, N-111, I-150, E-151**, N-152, F-153, K-154, K-155, L-156, N-157, E-158*, A-159, Y-185, Y-187, S-199, Q-200**, V-201, T-202, Q-203, K-214, Q-216, T-217*, I-218, D-219, I-227, N-250*, A-261, A-264, Q-265, T-268, N-271, T-272, N-275 | 8 | 2.0-2.9 | 40 | -6.2 | E-123, N-124*, C-125, S-136, G-137, I-138, E-139, M-214, W-215, K-216, N-217, G-228*, A-229, I-230, T-231**, S-232, T-233*, N-234, Q-238, Y-239, A-240*, V-241, N-243, N-244*, L-256, S-259, I-310, P-311, E-313, Q-314* |
| VTMVVEE -6.0 | N-109, A-110, N-111**, G-112, S-149, I-150, E-151, K-154*, K-155, E-158, A-159, Y-185, Y-187, N-196, S-199, Q-200, V-201, T-202, G-203, V-204, K-212, K-214, I-215, Q-216*, T-217, I-218, D-219, G-220, I-227, N-250, L-252, A-264, Q-265*, T-268, N-271, T-272, N-275 | 6 | 2.1-2.5 | 32 | -6.8 | L-94, W-97, S-98, G-102, N-103*, Y-104, F-105, Q-123, G-124, K-152*, A-155, E-156, Q-159*, A-160, T-163, S-165, K-168, A-221, Y-222, K-224, P-332, N-335, P-336, Y-337, R-338, Q-339 |

(continued on next page)
| No. | Peptide sequence | BabA peptide binding | SabA peptide binding |
|-----|------------------|----------------------|----------------------|
|     |                  | Binding affinity (kcal/mol) BabA | Residues involved within 5 Å | No. of H-bonding | Active torsion |
|     |                  | BabA | No. of H-bonding | H-bond distance (Å) | BabA | No. of H-bonding | H-bond distance (Å) | BabA |
| 214 | Q-216*, T-217, I-218, D-219, I-248, N-250, Q-265, T-268 | 334* | P-335, Y-336, R-337, Q-338 | |

# Selected as representative anti-adhesive peptides.
* Indicates H-bond donor which could be *, **, *** for 1, 2, 3 donors respectively.
/ indicates H-bond acceptor which could be /, /, / for 1, 2, 3 acceptors respectively.

BabA Binding site: Vicinity of C189, G191, N194, N206, D233, S234, S244, and T246.
SabA binding site: Vicinity of S-80, P-81, W-97, Y-148, K-152, Q-159, and Q-162.

Figure 1. (a) Binding of pepsin-resistant wheat germ peptides P86 at the vicinity of the active site of H. pylori adhesin BabA (b) interacting amino acids within 3Å and hydrogen bond pattern (c) charge environment and hydrophobicity/hydrophilicity of the binding pocket in the Kyte-Doolittle scale with colors ranging from dodger blue for the most hydrophilic to white 0.0 to orange red for the most hydrophobic.
tools to evaluate potential pepsin-resistant DWGPH peptides that bind to the active sites of BabA and SabA at high affinity, which could prevent bacteria adherence to Lewis antigens of the gastric mucosa, and to study the ADME/Tox (absorption, distribution, metabolism, excretion and toxicity) profile of the peptides.

2. Materials and methods

2.1. Anti-adhesive peptide library

A database of 267 peptides identified in defatted wheat germ protein hydrolysate with anti-adhesive property against *H. pylori* was retrieved from our recent study (Sun et al., 2020a).

2.2. Biostability assessment by PeptideCutter

The anti-adhesive peptides were hydrolysed *in silico* with pepsin using ExPASy PeptideCutter (https://web.expasy.org/peptide_cutter/). Pepsin is a stomach enzyme with optimum activity at pH 1.3. The hydrolysis tool generated a map of the peptide sequence with indication of pepsin cleavage sites and resulting fragments. Thirty-three out of the 267 peptides showed resistance against pepsin degradation in the gastric phase of digestion and therefore were chosen for subsequent evaluations.

2.3. Molecular docking of anti-adhesive peptides to adhesin proteins

Among the 33 pepsin-resistant peptides, only 16 with maximum length of 10 amino acids were selected for docking due to size constraint of the docking program. The crystal structures of the two *H. pylori* adhesins (BabA and SabA) were retrieved from the RCSB Protein Data Bank (PDB), with PDB code 4ZH0 and 4O5J, respectively. Chimera UCSF software version 1.15 (Pettersen et al., 2004) and Autodock Vina package version 1.1.2 (Trott and Olson, 2012) were used to generate peptide structures and perform molecular docking study. Polar hydrogen atoms and Gasteiger charges were added, and non-standard amino acid residues were ignored prior to docking. All structures were minimized to eliminate internal clashes and optimize structure with minimum energy. Water and ligands crystalized alongside the adhesin proteins were removed prior to docking simulation to minimize interference or blocking of the active site. The whole protein was selected as potential binding site and grid box dimensions for estimating peptide-BabA (4ZH0) binding were center: 14.3954, 12.2729, 43.3475; and size: 59.4532, 43.0248, 99.6112 while that of peptide-SabA (4O5J) binding were center: 90.697, 22.0579, 44.3475; and size: 33.4532, 22.0248, 44.6112.
The docking scores for binding affinity values were selected as the best-ranked docking pose of peptides. Intermodel hydrogen bonds were determined by relaxing the constraints by 0.4 Å and 20°.

2.4. In silico drug-likeness evaluation

The drug-likeness of the 16 docked peptides were assessed in silico using SwissADME (http://www.swissadme.ch/index.php). SwissADME is a free and validated web tool that is user-friendly to support non-experts in drug discovery. SwissADME allows for the prediction and evaluation of physiochemical and pharmacokinetics (based on ADME parameters) of small molecules (Daina et al., 2017). In addition, ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred/index.html) was used as a method for predicting the potential toxicity of the wheat germ protein-derived antiadhesive peptides.

3. Results and discussion

3.1. Bioactive peptides with gastric stability

DWGPH was reported to reduce adherence of *H. pylori* to a human gastric cell line, and 267 peptides were identified from the bioactive fraction that were bound to *H. pylori* (Sun et al., 2020a). This anti-adhesive property was hypothesized to be due to peptides binding to *H. pylori* via adhesins such as BabA and SabA, thus preventing *H. pylori* from binding to host cells. The peptides should not be hydrolyzed by pepsin in stomach, which could lead to the loss of binding activity and anti-adhesive activity against *H. pylori*. To select the pepsin-resistant peptides for the docking study, the initial database of 267 peptides were hydrolyzed in silico. As shown in Tables 1 and 3 peptides maintained intact sequences after pepsin activity in silico. The pepsin-resistant activity is important because the peptides must remain structurally intact in the stomach where their anti-adhesive activity is needed. The structural properties including molecular weight, pI, instability index, aliphatic index, net charge, boman index, hydrophobicity, Hmoment (α-helix), and Hmoment (β-sheet) of all peptides were analyzed in our previous work (Sun et al., 2020a,b). There are no structure-activity relationships among these 33 peptides.

3.2. Binding affinities and substantive binding sites of peptides to *H. pylori*’s adhesins BabA and SabA

The substantive binding sites of the pepsin-resistant peptides as well as the strength and nature of their interaction with *H. pylori* adhesins, BabA and SabA, are presented in Table 2, Figures 1, 2, 3, and 4. The
binding affinity (kcal/mol) was calculated based on the sum of binding forces such as electrostatic interactions, van der Waals force, hydrogen bonding, entropy and conformational state of ligands (Pantsar and Poso, 2018). The binding affinities of sixteen peptides to BabA and SabA range from -6.0 to -7.4 and -6.0 to -7.8 kcal/mol, respectively. These values indicate medium to strong binding of the peptides to the adhesins, with the larger magnitude negative numbers implying that a relatively low concentration of the peptide is adequate to maximally occupy a ligand-binding site on the adhesin proteins and trigger a physiological response. Also, higher negative value of the binding affinity indicates spontaneous formation of a more stable complex. Previous studies have shown that BabA binding to the Lewisb antigens does not cause any conformational changes in the adhesin protein (Hage et al., 2015). Therefore, it is imperative to screen bioactive peptides that could bind the active site of BabA at high specificity and affinity, as allosteric binding might not cause any conformational change in the protein that could inhibit its binding with gastric mucosa antigens. Hence, the site of peptide interaction is crucial to inhibit the interaction of adhesin proteins with the Lewis antigens in the gastric mucosa.

The binding site of BabA is situated within the β-strand motif and binding is mainly facilitated by networks of hydrogen bonding between two fucose residues (Fuc1 and Fuc4), one galactose residue Gal5) and an N-acetylglucosamine residue (GlcNAc3) of the hexasaccharide form of the Lewisb antigen and a total of eight amino acid residues of BabA (C-189, G-191, N-194, N-206, D-233, S-234, S-244 and T-246) (Hage et al., 2015). The crystal structure of soluble extracellular adhesin domain of SabA (PDB 4O5J) was used in this study and the binding site is within the vicinity of S-80, P-81, W-97, Y-148, K-152, Q-159 and Q-162. In Table 2, our study showed that in both BabA and SabA, 14 peptides bound in the vicinity of the active site of the adhesin proteins whereas P70 and P193 in BabA and P184 and P262 in SabA demonstrated allosteric binding. Some of the 14 peptides (P2, P86, P207, P210, P249; and P2, P70, P73, P115, P193, P210) bound in the region of the active site of BabA and SabA, respectively, with appreciable affinity and hydrogen bonding network to form more stable complex. Hence, these peptides would be more effective at preventing H. pylori infection in the gastric wall as the active site of the adhesin proteins would be largely pre-occupied with peptides. It has been reported previously to bind selectively to sialyl-Lewisx and Lewisx antigens but not to Lewisb, Lewisb or Lewisy (Pang et al., 2014). There was no strong correlation between the strength/stability of the binding at the vicinity of the active site and allosteric site. The various peptides binding at the allosteric site of BabA (P70 and P193) and SabA (P184 and P262) showed relatively low affinity and stability. However, while peptides binding in the vicinity of the active site of BabA (P2, P86, P207, P249) and SabA (P2, P70, P73, P115, P193) showed strong binding affinity and stability, other peptides such as P89, P102, P115, P213, P264

Figure 4. (a) Binding of pepsin-resistant wheat germ peptides P262 at the allosteric site of H. pylori adhesin SabA (b) interacting amino acids within 3Å and hydrogen bond pattern (c) charge environment and hydrophobicity/hydrophilicity of the binding pocket in the Kyte-Doolittle scale with colors ranging from dodger blue for the most hydrophilic to white 0.0 to orange red for the most hydrophobic.
for BabA and P102, P213, P220, P249 for SabA, that equally bound in the vicinity of the active site, demonstrated relatively low affinity and stability. Therefore, the site of the interaction could not be completely attributed for the various energy differences.

Interestingly, P86 demonstrated the highest affinity for BabA, which could be due to favourable electrostatic, Van der Waals and hydrophobic interactions within the active site of the protein as well as well-ordered 11 hydrogen bonding patterns in BabA-P86 complex involving the residues K154, K155, N196, V201, K214, I215, T217, D219 and Q265 (Table 2, Figure 1a, b & c) within a bond distance of 2.0–2.7 Å. Although hydrogen bonding played a role in SabA binding to glycerol involving K152 and Q159 of the adhesin protein (Pang et al., 2014), the highest binding affinity of P210 to both adhesin proteins in their various active sites, is attributed for the various energy differences.

Likewise, P193 had the closest affinity for SabA as P210 but it bound only in the allosteric site of BabA. Therefore, P210 having shown great binding affinity for both adhesins proteins in their various active sites, is predicted as the most promising peptide in the database for further development as a nutraceutical against H. pylori infection. It is also noted that binding affinity alone does not determine potency. Potency is a complex interplay of both binding affinity and ligand efficacy, which is the ability of the ligand to produce biological response upon binding to the target receptor (Kenakin, 2006). As such, in order to further select best candidates for validation, more studies will need to look into ligand efficacy as well as to incorporate information of aliphatic and instability compared to other peptides with higher number of hydrogen bonding network (Table 2). The strong binding potential of P210 to both adhesin proteins could be attributed to strong hydrophobic interaction. Figure 3b & c showed that P210 interacted appreciably with hydrophobic amino acids and was almost completely engulfed into the hydrophobic core of the protein as this peptide is the most hydrophobic among the reported peptides (Sun et al., 2020a). Overall, P86 had the strongest affinity for BabA but did not show the same effect on SabA.

Abbreviations: molecular weight (g/mol) (MW); number of rotatable bonds (ROTB); hydrogen bond donors (HBD); hydrogen bond acceptors (HBA); estimated solubility (ESOL) with solubility classes (HS - highly soluble, VS - very soluble, MS - moderately soluble, S - soluble); topological polar surface area (TPSA); logarithm of compound partition coefficient between n-octanol and water (CLoGP); Lipinski filter (Lipinski's rule-of-5); gastrointestinal absorption (GIA), P-glycoprotein substrate and CYP3A4 inhibitor.

### Table 3. In silico absorption, distribution, metabolism, excretion and toxicity (ADME/Tox) profile of the selected 16 anti-adhesive peptides generated using SwissADME and ToxinPred

| No. | Sequence | MW (g/mol) | ROBT (n) | HBA (n) | HBD (n) | ESOL Log S | Toxicity (SV) | Lipophilicity (TPSA) (Å²) | Drug likeness (CLogP) | Bioavailability (HBA) | Lipinski filter | GIA | P-glycoprotein substrate | CYP3A4 inhibitor |
|-----|----------|------------|----------|-------|--------|------------|---------------|-----------------------------|---------------------|-----------------|----------------|-----|--------------------------|------------------|
| 2   | DAVTYTETAR | 1162.21    | 45       | 21    | 20     | 0.08       | -1.14         | No                          | 551.09              | -4.02           | 0.17          | No | Low                      | Yes              | No |
| 70  | ISANIAAR  | 814.93     | 34       | 13    | 14     | 0.83       | -1.23         | No                          | 392.24              | -3.07           | 0.17          | No | Low                      | Yes              | No |
| 73  | PANGYGEIR | 912.00     | 38       | 15    | 15     | 0.64       | -1.20         | No                          | 424.42              | -3.64           | 0.17          | No | Low                      | Yes              | No |
| 86  | TIVQQVEAYR | 1206.35    | 49       | 19    | 19     | -0.94      | -1.16         | No                          | 551.06              | -2.52           | 0.17          | No | Low                      | Yes              | No |
| 89  | ISSIEQK   | 917.05     | 40       | 16    | 14     | 1.59       | -0.78         | No                          | 413.89              | -2.46           | 0.17          | No | Low                      | Yes              | No |
| 102 | DNIQGITK  | 887.98     | 38       | 16    | 14     | 2.96       | -1.78         | No                          | 436.76              | -3.57           | 0.17          | No | Low                      | Yes              | No |
| 115 | MISVTGPR  | 860.03     | 34       | 13    | 13     | -0.14      | -1.05         | No                          | 385.89              | -2.07           | 0.17          | No | Low                      | Yes              | No |
| 184 | VPBPNSGDR | 1051.15    | 38       | 17    | 14     | 0.40       | -0.91         | No                          | 461.37              | -4.34           | 0.17          | No | Low                      | Yes              | No |
| 193 | RVTIMFK   | 884.08     | 34       | 12    | 12     | -0.96      | -1.29         | No                          | 362.58              | -1.33           | 0.17          | No | Low                      | Yes              | No |
| 207 | AVVIHVPR  | 1053.26    | 38       | 14    | 14     | -3.76      | -1.22         | No                          | 398.14              | 0.18            | 0.17          | No | Low                      | Yes              | No |
| 210 | VTGAIP    | 669.81     | 24       | 10    | 8      | -1.09      | -0.63         | No                          | 249.36              | -0.31           | 0.17          | No | Low                      | No               | No |
| 213 | KMEVPVCIVK| 1209.52    | 48       | 17    | 14     | -2.21      | -0.20         | No                          | 490.10              | 0.47            | 0.17          | No | Low                      | Yes              | No |
| 220 | KAVVIHVPR | 1181.43    | 45       | 16    | 16     | -3.78      | -1.25         | No                          | 453.26              | 0.07            | 0.17          | No | Low                      | Yes              | No |
| 249 | MDNNTVGGSR| 1050.10    | 45       | 19    | 19     | 3.47       | -1.02         | No                          | 576.36              | -6.68           | 0.17          | No | Low                      | Yes              | No |
| 262 | ROQTARKR  | 1045.11    | 47       | 18    | 23     | 4.49       | -1.03         | No                          | 608.46              | -7.60           | 0.17          | No | Low                      | Yes              | No |
| 264 | VTMEVEE   | 819.96     | 33       | 14    | 11     | -1.32      | -0.66         | No                          | 358.05              | -0.45           | 0.11          | No | Low                      | No               | No |
|     | Rebamipide|            |          |       |        |            |               |                             |                     |                 |              |     |                         |                  |     |
|     | (Negative control) |   |       |       |        |            |               |                             |                     |                 |              |     |                         |                  |     |
|     | 3-sialyllactose |         | 633.55  | 16    | 19    | 13        | 2.01          | N/A                         | 342.92              | -5.76           | 0.11          | No | Low                      | Yes              | No |
indexes of the peptides, which predict peptide stability in lab benchwork for food thermal processing (Sun et al., 2020a).

### 3.3. In silico physicochemical properties and drug-likeness

The drug-likeness and medicinal chemistry friendliness of the sixteen peptides with binding activities to \textit{H. pylori} adhesins were predicted and evaluated based on physiochemical and pharmacokinetic properties using SwissADME (Table 3). The pharmacokinetic properties were estimated based on ADME parameters. In this study, 3-sialyllactose sodium salt from human and bovine milk that has been clinically studied extensively as an anti-adhesive agent against \textit{H. pylori} infection was selected as a positive control. Rebamipide, a gastroprotective drug that interferes with \textit{H. pylori} adhesion by acting on gastric epithelial cells rather than \textit{H. pylori}, was chosen as a negative control (Suzuki et al., 1994).

![Bioavailability radar images of the anti-adhesive peptides, rebamipide (negative control) and 3-sialyllactose (positive control) generated using SwissADME. Bioavailability radar displayed six physiochemical properties: lipophilicity, size, polarity, solubility, flexibility and saturation. #Selected as representative anti-adhesive peptides.](image-url)
As shown in Table 3, all peptides had relatively high molecular weight (ranging from 669.81 to 1209.52 g/mol). Additionally, oral bioavailability of a compound could be predicted based on two properties including flexibility (ROTB - the number of rotatable bonds –) and polarity (TPSA - topological polar surface area) (Ji et al., 2020). Compounds with >10 rotatable bonds have been associated with poor oral bioavailability (equated to high molecular weight and structure rigidity), while compounds with low topological polar surface area (between 20 and 130 Å²) tend to have high oral bioavailability (Veber et al., 2002; Mbarik et al., 2019). According to Table 3, all sixteen peptides showed values of TPSA >140 Å² and ROTB >10, indicating that they have poor oral bioavailability. The bioavailability scores of the peptides were also low (0.11 & 0.17), similar to 3-sialyllactose (0.11) and much lower than Rebamipide (0.56), in compliance with predicted oral bioavailability.

Bioavailability radar images of the peptides were shown in Figure 5.

Figure 5. (continued)
molecule by six physiochemical properties: lipophilicity, size, polarity, solubility, flexibility and saturation (Daina et al., 2017). The closer the parameter to the centre of radar, the smaller the value. The selected peptides generated very similar bioavailability radar images, specifically in size, polarity and flexibility parameters. Four peptides (P207 - AVVHVFPYR, P210 - VTGAIPI, P213 - KMEVPPYIKV and P220 - KAVVHVFPYR) showed higher lipophilicity, as they were also shown to be hydrophobic in the primary database (Sun et al., 2020a; van de Water-beemd et al., 1994). The radar images also resembled that of 3-sialyllactose (in flexibility and polarity parameters), considering the similarities in physiochemical properties to the peptides, and different from that of Rebamipide (higher lipophilicity, smaller size, and much higher saturation).

All sixteen DWPGH-derived bioactive peptides were shown to be non-toxic, with negative SVM (support vector machines) scores, ranging from -0.07 to -2.10. They also did not pass the Lipinski rule-of-five, to -2.10. They also did not pass the Lipinski toxic, with negative SVM (support vector machines) scores, ranging from 

Rebamipide (higher lipophilicity, smaller size, and much higher in physiochemical properties to the peptides, and different from that of tose (in hydrophobic in the primary database (Sun et al., 2020a; van de Water-beemd et al., 1994)).

AVVIHVPYR, P210 - VTGAIPI, P213 - KMEVPPYIKV and P220 - KAVVHVFPYR) showed higher lipophilicity, as they were also shown to be hydrophobic in the primary database (Sun et al., 2020a; van de Water-beemd et al., 1994). The radar images also resembled that of 3-sialyllactose (in flexibility and polarity parameters), considering the similarities in physiochemical properties to the peptides, and different from that of Rebamipide (higher lipophilicity, smaller size, and much higher saturation).

4. Conclusion

This study confirmed the interactions between DWPGH-derived anti-adhesive peptides and the two dominant H. pylori adhesins, BabA and SabA, using molecular docking simulation. The peptides occupy the binding pocket of BabA and SabA, which is possibly responsible for their anti-adhesive activity against H. pylori since binding at allosteric site does not cause conformational changes in the protein. This work provided new insights into the anti-adhesive mechanism for continuous discovery and identification of readily available, cost-effective, and highly potent anti-adhesive agents in the future. Moreover, 33 out of 267 DWPGH-derived peptides were bioactive in the gastric digestion phase in silico. The ADME/Tox profile indicated that these peptides have no toxicity, low bioavailability, and poor intestinal absorption. Taken together, the findings demonstrate that the wheat germ peptides, especially P210 (VTGAIPI), have strong potential as nutraceutical candidates for preventing H. pylori infection.

Declarations

Author contribution statement

Chi Dang, Ogadimma Okagu: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Xiaohong Sun: Conceived and designed the experiments; Analyzed and interpreted the data.

Chibuike C. Udenigwe: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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