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(Research Article)

In Silico Molecular Modelling and Docking of Quercetin-γ-Cyclodextrin Inclusion Complex on SGLT of *Vibrio parahaemolyticus*

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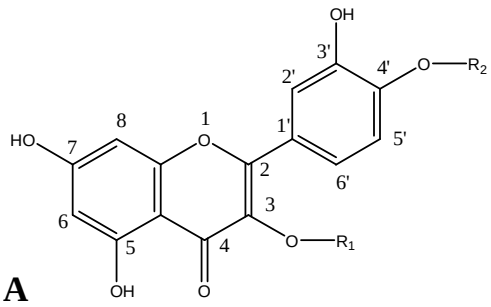
ABSTRACT

Quercetin glucosides are transported across intestinal epithelium by active transport. Is not significantly transported by sodium linked glucose transporter 1 (SGLT1) and shows lesser intestinal absorption than its glucosides. Quercetin complexes with cyclodextrins have been described to increase its solubility. It has long been assumed that cyclodextrins improve its intestinal absorption by releasing it near intestinal epithelium and making quercetin available for passive diffusion. In silico molecular modeling and docking studies provide an accurate and cost effective method to understand these interactions. X-ray crystal structure of sodium dependent glucose transporter isolated from Vibrio parahaemolyticus was used as a model for docking studies. The test ligands including quercetin-γ-cyclodextrin, quercetin-3,4'-glucoside, quercetin-3-glucoside, and quercetin were docked in predicted active site of Vibrio parahaemolyticus sodium linked glucose transporter (vSGLT). Validation of potential mean force method used for docking was performed by docking glucose, a natural ligand of vSGLT. It was found that quercetin glucosides and quercetin-γ-cyclodextrin docked analogously with predicted active site of vSGLT and their docking scores were comparable. Quercetin docked at a different location with less docking score. The results point towards possible mimicking of glucose moieties by γ-cyclodextrin in mechanism of active transport of quercetin and its glycosides by SGLT.

Key Words: Quercetin, Glycosides, Sodium linked glucose transporter protein (SGLT), γ-Cyclodextrin, Docking, *Vibrio parahaemolyticus.*

INTRODUCTION

In nature, quercetin exists as glucosides. Structures of two common glucosides of quercetin namely, quercetin-3,4'-diglucoside and quercetin-3-glucoside are shown in **Fig. 1A.** Quercetin moiety without these attached glucose moieties is called quercetin aglycone **(Fig. 1A).** Quercetin is sparingly soluble in water, which has limited its absorption upon oral administration[1]. In addition, it is chemically unstable, especially in aqueous alkaline medium [2]. The compound is also known to undergo extensive metabolism in the gut and the liver following absorption[3], and the resulting metabolites still retain some biological activity [4]. All these characters lead to an extremely low bioavailability of quercetin (based on the unchanged quercetin) in humans.



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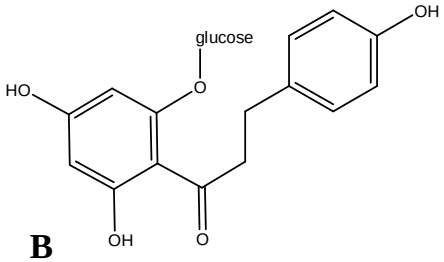

B

Compound	Name	R_1	R_2
I	Quercetin-3,4'-diglucoside	glucose	glucose
II	Quercetin-3-glucoside	glucose	H
III	Quercetin	H	H

Figure 1A: The structures of quercetin-3, 4'-diglucoside (compound I), quercetin-3-glucoside (compound II) and quercetin (compound III). Quercetin -3, 4'-diglucoside (compound I) has two glucose moieties attached at positions 3 and 4' of the general structure. Quercetin-3-glucoside (compound II) has one glucose moiety attached at position 3 of the general structure. Quercetin compound III) has no glucose moieties attached and hence it is also called quercetin aglycone.

Figure 1B: Structure of phlorizin, a highly potent inhibitor of SGLT1. Note the similarities in structures leading to affinity towards SGLT1.

Flavonoids are polyphenolic secondary metabolites present in plants. Many of these compounds are bioactive and play a protective role in the plants. They are widely considered to be potentially beneficial to health by virtue of their antioxidant activities [5] and their capacity to inhibit enzymes such as cyclooxygenase and protein kinases involved in cell proliferation and apoptosis [6]. Dietary flavonoids occur mainly as glycosides, some of which can be absorbed in the human small intestine.

The mechanism of uptake of flavonoids into intestinal epithelial cells and their transfer into the circulation are of great interest. The flavonol quercetin occurs in plants predominantly as glycosides, which are water soluble and chemically stable (Formica and Regelson 1995). It has been described in literature that quercetin glycosides are poorly absorbed in the small bowel and that the most likely route of intestinal transport is by passive uptake of the relatively lipophilic aglycone in the colon after hydrolysis of the glycosides by the large intestinal microflora [7]. However, a study showed that in humans, quercetin glucosides were absorbed within 30 min of ingestion, apparently in preference to quercetin aglycone [8]. To explain this, it was proposed by Haollman *et al.* [9] that the glucose moiety may enable flavonoid glycosides to be transported by the sodium-dependent glucose transporter 1 (SGLT1).

Phlorizin, which is structurally related to the flavonoids **(Fig. 1B)**, is a highly efficient inhibitor of SGLT1 and is not transported across the cell membrane [10]. Furthermore, flavonoid glucosides are substrates for hydrolysis by lactase phlorizin hydrolase [11], an enzyme localized on the outer surface of the small intestinal brush border membrane [12].

Intestinal transport of quercetin and its glycosides has been studied in rat intestines [7]. It was reported that quercetin glucosides **(Fig. 1A)** interact more with SGLT1 as compared to quercetin. The order of affinity towards SGLT1 was found in order quercetin-3,4'-diglucoside > quercetin-3-glucoside > quercetin [7]. A direct evidence for role of SGLT1 in transport of quercetin-3-glucoside in isolated rat jejunum was reported in subsequent years [13]. The mechanism of interaction between quercetin glucosides and SGLT1 remains unresolved till date. To identify this, *in silico* docking study is a powerful tool. Virtual mechanism screening has advantages over *in vitro* or *in vivo* analysis within cost and time with the same accurate results [14]. Docking study was considered necessary to provide an *in silico* model for understanding mechanism of interaction among quercetin glucosides and SGLT. However, Literature shows no docking studies of quercetin and quercetin glucosides with SGLT1 or SGLT2.

In the development of pharmaceutical products, β-cyclodextrins, a category of pharmaceutical excipients with unique solubilizing properties, have been widely used to tackle low solubility and low membrane permeability of drug molecules [15-18]. However, β-cyclodextrin itself is poorly water soluble and it is disadvantageous to use it for complexation and solubilization of quercetin [18]. On the other hand, γ-cyclodextrin has advantages such as higher solubility and larger internal cavity [18;19]. γ-cylocdextrin has been shown to be well tolerated, non toxic and metabolized in a manner similar to starch and dextrins [19]. The US FDA has assigned a GRAS (generally recognized as safe for use in food and pharmaceuticals) status to γ-cyclodextrin [20]. Hence, it was postulated that complexation with γ-cyclodextrin would improve biological membrane permeability of quercetin. γ-Cyclodextrin has been shown to increase solubility of quercetin by formation of an inclusion complex [1;18]. Effect of this inclusion complexation on intestinal mucosal permeation and mechanism of enhancement of bioavailability of aglycone quercetin remains an open question. Most feasible means of predicting and understanding ligand protein in interactions is *in silico* docking studies. However, literature shows no docking studies of quercetin-γ-cyclodextrin inclusion complex on SGLT1.

Glucose transporters are membrane proteins which exist on the cell surface and incorporate glucose into cells. The glucose transporter family can be divided into two groups. One is the facilitative glucose transporter (GLUT) family and the other is the Na+/glucose cotransporter (SGLT) family. The SGLT family consists of 3 isoforms, distributed in the intestine and kidney. SGLT plays an essential role in the absorption of glucose from food in the intestine and the reabsorption of glucose from urine in the kidney. SGLT1 has limited tissue expression and is found essentially on the apical membranes of small intestinal absorptive cells (enterocytes) and renal proximal straight tubule (S3 cells) [21]. Roles of SGLT1 and SGLT2 are represented in **Fig. 2.**

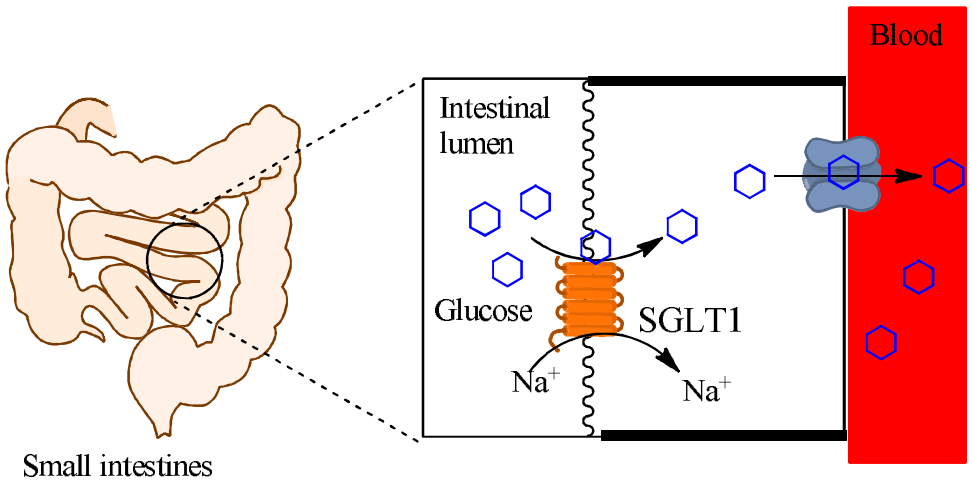

Figure 2: Illustration showing location and role of SGLT1. Note that SGLT1 is predominantly distributed in intestinal lumen (brush border) and involved in glucose transport.

SGLT2 is predominantly expressed on the apical membrane of renal convoluted proximal tubules (S1 and S2 cells) [22]. It is currently accepted that in the kidney, SGLT2 (low affinity, high capacity) transports the bulk of plasma glucose from the glomerular filtrate. Any remaining glucose is then recovered by SGLT1 (high affinity, low capacity), thus preventing glucose loss in urine.

Intestinal absorption and renal reabsorption are both mediated by the coordinated functions of Na^+/K^+ ATPase and SGLT. In the basolateral membrane of the epithelium, Na+/K+ ATPase pumps out Na+ thereby decreasing intracellular concentration of Na+ and creating an inward-oriented Na+-gradient. In apical plasma membrane, SGLT cotransports glucose and Na+ using the Na+ gradient. SGLT inhibitors (mainly SGLT2 inhibitors) should reduce hyperglycemia by increasing urinary glucose secretion [23]. Available literature shows no reports of computational modeling and docking of quercetin and its glycosides on SGLT1.

Exact x-ray crystal structure of human SGLT1 is not available. A bacterial member of the SGLT1 family is the Na^+/glucose symporter (SglS) of *Vibrio parahaemolyticus* [24;25]. The amino acid sequence of the protein has 31% identity and 75% similarity with the human SGLT1 [24]. Owing to its close functional and evolutionary relationships with mammalian SGLT1, the *Vibrio SglS* gene product is refered as vSGLT. There is evidence to infer that members of the Na^+/glucose cotransporter family share a similar transport mechanism [26-29]. A secondary structure model of vSGLT has been proposed based on sequence comparisons with other family members whose secondary structure models have been supported by experimental analysis [30-33]. In this model **(Fig. 3),** vSGLT is composed of 14 transmembrane spans, with the hydrophilic N terminus located in the periplasmic space. The ultimate C-terminal hydrophobic domain forms the 14th transmembrane span with the C terminus at the periplasmic surface of the membrane.

In a previous study, the protein was expressed in *Escherichia coli* [34]. Systematic replacement of amino acid residues showed that amino acid residues Gly 151 and Gln 428 were found necessary for glucose transport activity. Other amino acid residues Leu 147, Leu 149, Ala 423 and Gln 425 were not found necessary for the activity. Split version of vSGLT was generated by co-expression of the N-terminal (N_7) and C-terminal (C_7) halves of the transporter. The split vSGLT maintained Na^+-dependent glucose transport activity. It was concluded that the mechanism of Na^+/glucose transport by vSGLT is similar to mammalian SGLTs [34].

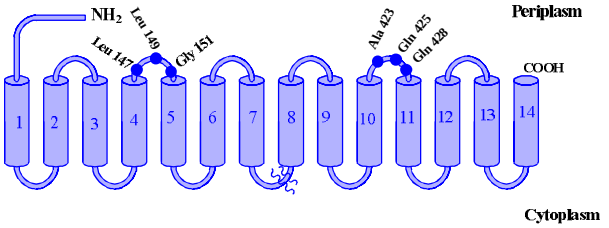

Figure 3: Drawn structure of vSGLT. Note that the protein contains 14 transmembrane domains. The protein was expressed in *Escherichia coli* in a study [34]. Systematic replacement of amino acid residues have shown that amino acid residues Gly 151 and Gln 428 were found necessary for glucose transport activity. Other amino acid residues Leu 147, Leu 149, Ala 423 and Gln 425 were not found necessary for the activity.

The purpose of the present study is to find whether quercetin-γ-cyclodextrin interacts in with SGLT1 in a similar manner as quercetin glucosides. It was postulated that active site of vSGLT could be predicted by fuzzy oil drop technique. This active site could be used as a model of human SGLT1 for docking quercetin glycosides, quercetin and quercetin-γ-cyclodextrin.

Vibrio parahaemolyticus is a curved, rod-shaped, Gram-negative bacterium found in water and causes gastrointestinal illness in humans [35]. *V. parahaemolyticus* is oxidase positive, facultatively aerobic, and does not form spores. Like other members of the genus *Vibrio*, this species is motile, with a single, polar flagellum [36]. X-ray crystal structure of vSGLT has been described [37].

MATERIALS AND METHODS

Software

In the present work, all the ligands used were made using Chemdraw 3D Ultra 8.0. Before the docking calculation of

the ligands, the structures were fully optimized. Argus Lab 4.0 was used to perform all the docking techniques. The crystal structure of vSGLT (PDB ID 3DH4) was downloaded from Protein Data Bank (http://www.rscb.org/) as PDB file.

Prediction of Ligand Binding Site of vSGLT

The fuzzy oil drop is a mathematical model which is used to predict localization of area responsible for ligand binding or protein-protein complex formation. The mathematical model is based on characteristics of spatial distribution of hydrophobicity in a protein molecule. It has long been used for recognition of ligand binding site in proteins [38]. It is assumed that hydrophobicity changes from protein interior (maximal hydrophobicity) to exterior (close to zero level of hydrophobicity) according to the three-dimensional Gauss distribution. It is generally accepted that the core region is not well described by a spheroid of buried residues surrounded by surfaces residues due to hydrophobic channels that permeate the molecule. Therefore the simple comparison of theoretical (idealized according to Gauss function) and empirical spatial distribution of hydrophobicity in protein gives the opportunity to identify the regions with high deviation versus the ideal model. Those regions recognized by high hydrophobicity density differences seem to reveal functionally important sites in proteins. The model has been found to be verified positively for prediction of 3D coordinates of major cold shock protein (chain A) (PDB ID: 1NMF), a downhill protein [39]. The model has also been validated for small peptides representing various functional groups [40]. A study has described a method for prediction of ligand binding site based on location of a region of unusual hydrophobicity in a protein structure [38].

Theoretical fuzzy-oil-drop [38]

The *fuzzy-oil-drop* is described by three-dimensional Gauss function. Gauss function usually interpreted as a probability distribution and is assumed to represent the hydrophobicity distribution. If the j-th point described by cartesian coordinates (X_j, Y_j, Z_j) belongs to a box with its center at the origin of the coordinate system (0, 0, 0) the expected hydrophobicity value $\tilde{He}_j$ for this point, is calculated as follows:

$$\tilde{He}_j = \frac{1}{\tilde{He}_{sum}} \exp\left(\frac{-(x_j-\bar{x})^2}{2\sigma_x^2}\right) \exp\left(\frac{-(y_j-\bar{y})^2}{2\sigma_y^2}\right) \exp\left(\frac{-(z_j-\bar{z})^2}{2\sigma_z^2}\right)$$

The hydrophobicity maximum localized in a center of ellipsoid decreases in form of distance-dependence according to three-dimensional Gauss function. The parameter - mean value, for which Gauss function reaches its maximum is localized in (0, 0,0) point in coordinate system. The second parameter - standard deviation represents the size of drop (the values of three standard deviations determines the size of drop: (σ_x, σ_y, σ_z), and depends on the length of polypeptide under consideration. $\tilde{He}_{sum}$ is the sum of theoretical hydrophobicity for all analyzed grid points. Each *j*-th grid point (X_j, Y_j, Z_j) is characterized by the $\tilde{He}_j$ value, which represents the idealized degree of hydrophobicity in the *fuzzy-oil-drop.*

Observed fuzzy-oil-drop [38]

The observed hydrophobicity distribution within the *fuzzy-oil-drop* is calculated using the simple sigmoid function proposed to quantitavely describe the hydrophobic interactions. The $\tilde{j}$-th point collects hydrophobicity $\tilde{Ho}_j$ as follows:

$$\tilde{Ho}_j = \begin{cases} \frac{1}{\tilde{Ho}_{sum}} \sum_{i=1}^{N} \tilde{H}_i^T \left[1 - \frac{1}{2}\left(7\left(\frac{r_{ij}}{c}\right)^3 - 9\left(\frac{r_{ij}}{c}\right)^4 + 5\left(\frac{r_{ij}}{c}\right)^6 - \left(\frac{r_{ij}}{c}\right)^8\right)\right], & r_{ij} \le c \\ 0, & r_{ij} > c \end{cases}$$

where *N* is the total number of residues in the protein under consideration, $\tilde{H}_i^T$ denotes the hydrophobicity of the *i*-th residue according to the normalized scale of hydrophobicity for amino acids, r_{ij} denotes the separation of the *j*-th grid point and the effective atom of the *i*-th residue, and *c* denotes the hydrophobic cutoff and has the fixed value of 9.0A^0. This means that only residues with $r_{ij} \le c$ influence the *j*-th point. $\tilde{Ho}_{sum}$ is the sum of observed hydrophobicity for all analyzed grid points. Using $\frac{1}{\tilde{Ho}_{sum2}}$ is a normalizing coefficient the observed hydrophobicity can be compared to the theoretical hydrophobicity described previously. The application of this function requires the parameters of hydrophobicity attributed to each amino acid.

The PDB file containing 3D co-ordinates of vSGLT obtained from RSCB protein data bank was submitted to fuzzy oil drop model server at website (http:// www.bioinformatics.cm-uj.krakow.pl/activesite/) for determination of ligand binding site. The calculated ligand binding site co-ordinates were saved on hard disk of a computer having Intel Core2Duo™ microprocessor and Windows7™ operating system as PDB file.

Docking Protocol

In the automated Argus Lab 4.0 system, using a generic algorithm with a fast-simplified Potential of Mean Force (PMF) carried docking of synthesized ligands into active site of HMT. It was assumed that the protein and the ligand docked non-covalently. The standard PMF implementation used UFF potential for this purpose. The docking was carried with flexible ligand into a rigid protein active site. The general procedure for the docking process started with the addition of energy minimized target ligand on the enzyme. The active site and the ligands were specified in the program.

Docking of quercetin in hydrophobic core of γ-cyclodexrin

To construct an *in silico* molecular model of quercetin-γ-cyclodextrin inclusion complex, a 15X15X15 A^o box was constructed around the γ-cyclodextrin. Quercetin was docked in the hydrophobic core of the molecule. The docking score was recorded and the docked quercetin-γ-cyclodextrin was observed for its structure and orientation of quercetin in the cavity. The whole procedure of docking was repeated until a constant value of docking score was achieved. Concluding docking results were parameterized in terms of docking score in kcal/mol.

Docking of test ligands on predicted ligand binding site of vSGLT

Using a 30 X 30 X 30 A^o box located around center of predicted ligand binding site, quercetin, quercetin-3-

glucoside and quercetin-3,4'-glucoside were docked on predicted ligand binding site of vSGLT. The docking score of best ligand-protein docking pose was recorded. The docked ligands complexed with vSGLT active site were interpreted by visual examinations of hydrogen bonding or hydrophobic interactions of the ligands with amino acid residues of the active site.

Validation of Docking Model

To establish the relevance of docking model to ensure that it yielded reproducible results, the docking model was required to be validated. Before docking test ligands, known ligands of SGLT1 were docked on the predicted binding site of vSGLT. Glucose was selected as it has been described as natural ligand of SGLT1 [23]. Phlorizin, which is structurally related to the flavonoids (**Fig. 1B**), has been described in literature as highly efficient inhibitor of SGLT1 [10]. The docking score of best ligand-protein docking pose was recorded. The procedure was repeated thrice and root mean square deviation (RMSD) of docking results was determined. The docked ligands complexed with vSGLT active site were interpreted by visual examinations of hydrogen bonding or hydrophobic interactions of the ligands with amino acid residues of the active site.

RESULTS AND DISCUSSION

The present study attempted to find whether quercetin-γ-cyclodextrin binds with SGLT1 in a manner similar to that of quercetin glucosides. Since x-ray crystallographic structrures of human SGLT1 or SGLT2 are not available, the x-ray crystal structure of sodium linked glucose tranporter protein of *Vibrio parahaemolyticus* (vSGLT) was chosen as a model for docking studies.

Prediction of Active Site of vSGLT

The active site of vSGLT was predicted with fuzzy oil drop technique. Obtained active site showed at least two distinct domains. The enthalpy change ($\Delta\hat{H}$ value) was found 5.001e-04 J. A 3D model was generated by replacing temperature factor (61-66) with normalized $\Delta\hat{H}$ per residue. The predicted active site contained 1980 residues. Redundant amino acid residues were automatically eliminated by the server. The Gly 151 residue was relocated to new location Gly 161 while the Gln 428 was relocated to Gln 430 in the revised sequence. The obtained 3D ribbon structure of the ligand binding site of vSGLT is shown in **Fig. 4**.

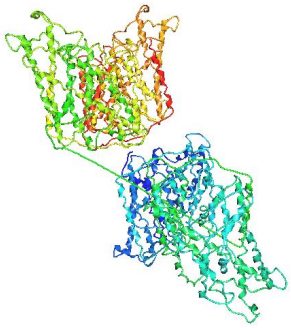

Figure 4: Obtained predicted active site of vSGLT from Fuzzy oil drop™ server. Note that tw0 distinct domains in the structure. The domains correspond to split domains (at C_7 N_7) described in literature [32;33].

Docking of Quercetin in Hydrophobic Core of γ-Cyclodextrin

Crystal structure of *Thermoactinomyces vulgaris* R-47 amylase 2 co-crystallized with γ-cyclodextrin was downloaded from RCSB protein data bank as a PDB file (PDB ID: 1VFU). Thermoactinomyces vulgaris R-47 alpha-amylase 2 (TVAII) has specificity towards γ-cyclodextrin. The cocrystallized structure contained two γ-cyclodextrin molecules. One of the γ-cyclodextrin molecules was selected by highlighting the miscellaneous sequences 901GLC to 908GLC and hiding the unselected sequences. The original structure obtained from RCSB protein data bank and obtained structure of γ-cyclodextrin is shown in **Fig. 5A and Fig. 5B.**

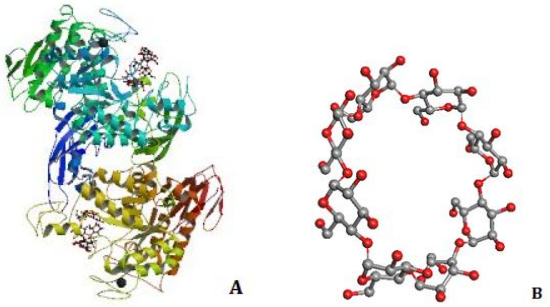

Figure 5A: Crystal structure of *Thermoactinomyces vulgaris* R-47 amylase 2 enzyme co-crystallized with γ-cyclodextrin (source: RCSB protein data bank, PDB ID: 1VFU). Note that the enzyme is complexed with two γ-cyclodextrin molecules as seen in the figure.

Figure 5B: Obtained structure of γ-cyclodextrin. Note the orinentation with torroidal hydrophobic cavity and outward pointing hydroxyl groups.

Structure of quercetin was constructed with Chemdraw ultra 8.0 ™ software and exported to Chem3D ultra™ software for energy minimization. Energy minimized structure of quercetin was imported in Arguslab 4.0 software [41] for docking. The docked structure of quercetin in hydrophobic cavity of γ-cyclodextrin is shown in **Fig. 6.** Quercetin docked in the γ-cyclodextrin cavity with its planar benzopyrone part into the hydrophobic cavity. Best ligand-substrate docking pose had docking score of -6.81603 KCal/mole. Absence of hydrogen bonds with γ-cyclodextrin confirmed the hydrophobic nature of the binding.

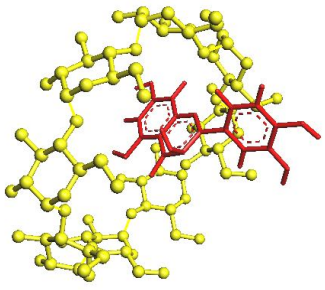

Figure 6: Obtained docked structure of quercetin (red) in hydrophobic core of γ-cyclodextrin (yellow). Note that quercetin docked into the core with its benzopyrone part in the hydrophobic cavity and its 2-phenyl group pointing outside the core.

Docking of Test Ligands in the Predicted Active Site of vSGLT

To find docking scores and docking conformations of quercetin-γ-cyclodextrin inclusion complex, quercetin, quercetin-3-glucoside and quercetin-3,4'-glucoside with predicted active site of vSGLT, docking studies were performed. Briefly, the docked structure of quercetin-γ-cyclodextrin was selected and defined as a ligand in the Arguslab 4.0™ software. Structures of quercetin, quercetin-3-glucosides and quercetin-3,4'-glucoside were drawn and with Chemdraw ultra 8.0 ™ software and exported to Chem3D ultra™ software for energy minimization. Energy minimized structures were then imported in Arguslab 4.0.

Docking of quercetin-γ-cyclodextrin

Structure of best ligand-substrate pose of quercetin with γ-cyclodextrin obtained by docking as described above was docked on predicted active site of vSGLT. Quercetin-γ-cyclodextrin docked at a site near center of two domains of vSGLT with docking score of -11.2410 Kcal/mole. This docking score was more than quercetin alone (-6.3251 Kcal/mole) and quercetin-3-glucoside (-8.4376 Kcal/mole) and was equivalent to quercetin-3,4'-diglucoside (-10.3517 Kcal/mole). The order of docking scores was quercetin-γ-cyclodextrin > quercetin-3,4'-diglucoside > quercetin-3-glucoside > quercetin.The docked structure of quercetin-γ-cyclodextrin in predicted active site of vSGLT is shown in **Fig. 7.**

Close examination of docked structure revealed hydrogen bonding interactions with amino acid residues of predicted active site as shown in **Table 1.** The docking location was similar to characteristic docking location observed with quercetin glucosides. The involvement of hydrogen bonding interactions of γ-cyclodextrin's hydroxyl groups with Gly 161 and Gln 430 among other amino acid residues was seen. Hydrogen bonding with these amino acid residues was observed with glucose and 3-hydroxy group in quercetin glucosides. These observations supported the hypothesis that cyclodextrin docked the SGLT in a similar manner to the glucose moiety of quercetin glucosides.

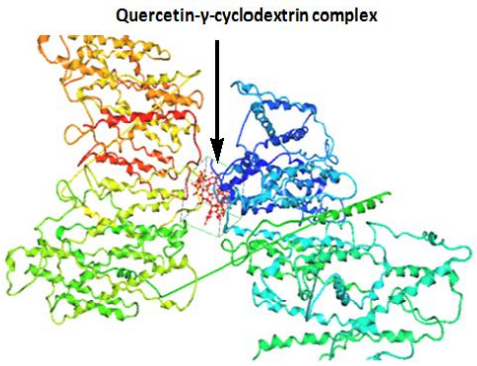

Figure 7: Obtained docked structure of quercetin-γ-cyclodextrin complex (red) with predicted active site of vSGLT. Note that binding location of quercetin-γ-cyclodextrin similar to characteristic binding location of quercetin glucosides.

Docking of quercetin-3,4'-glucoside

Quercetin-3,4'-glucoside docked at center of two domains of predicted active site of vSGLT with docking score of -10.3517 Kcal/mole. Close examination of docked structure revealed hydrogen bonding interactions with amino acid residues of predicted active site shown in **Table 1.** 3-glucose moiety of quercetin-3,4'-glucoside molecule was found involved in hydrogen bonding interactions with amino acid residues Gly 161 and Gln 430 among others. This observation was notably similar to that observed with docking of glucose and quercetin-3-glucoside. The 4'-glucose moiety showed an extended hydrogen bonding interaction with amino acid residues Leu 146. The best docking pose of quercetin-3,4'-diglucoside was similar to that observed with quercetin-3-glucoside **(Fig. 8)**.

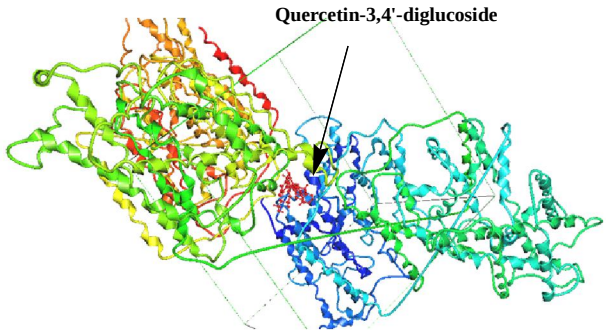

Figure 8: Obtained docked structure of quercetin-3,4'-diglucoside with predicted active site of vSGLT. Note that the similarity in docking locations of quercetin-3,4'-diglucoside and quercetin-3-glucoside.

Docking of quercetin-3-glucoside

Quercetin-3-glucoside docked at center of two domains of predicted active site of vSGLT with docking score of -8.4376 Kcal/mole. Close examination of docked structure revealed hydrogen bonding interactions with amino acid residues of predicted active site have been shown in **Table 1.** Glucose moiety at 3-position of quercetin-3-glucoside molecule was found involved in hydrogen bonding interactions with amino acid residues Gly 161 and Gln 430 among others. This hydrogen bonding interaction was observed with glucose also. The best docking pose observed with quercetin-3-glucoside has been shown in **Fig. 9**. The results suggested that 3-glucose moiety in the structure of quercetin-3-glucoside interacted with predicted active site of vSGLT in a manner similar to that of glucose.

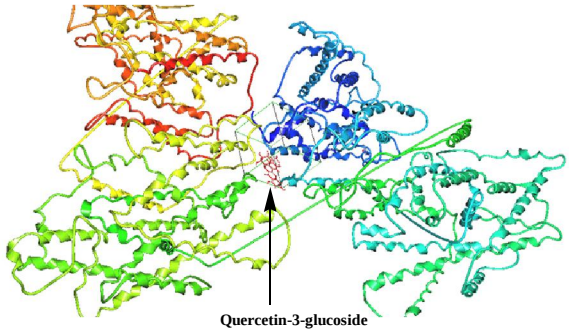

Figure 9: Obtained docked structure of quercetin-3-glucoside (red) with predicted active site of vSGLT. Note that the similarity in docking locations of and quercetin-3-glucoside and quercetin-3, 4'-diglucoside.

Docking of quercetin

To compare docking scores and docking conformations of quercetin, quercetin glucosides and quercetin-γ-cyclodextrin complex, quercetin was docked in the predicted active site of vSGLT. Quercetin docked in the predicted active site of vSGLT showed a low docking score (-6.3251 Kcal/mole). The docking location of quercetin was different from glucose **(Fig. 10).** The amino acid residues of vSGLT near docked glucose structure are shown in **Table 1.** Notable difference was observed in the amino acid residues in vicinity of glucose and that of quercetin.

The results support the hypothesis that quercetin interacted with vSGLT in different way than glucose and quercetin glucosides. The glucose moieties in the structures of quercetin-3-glucoside and quercetin-3,4'-glucoside interacted with the predicted active site similarly to glucose. The glucopyranose moieties of γ-cycodextrin in quercetin-γ-cyclodextrin showed similar docking orientation as that of glucose.

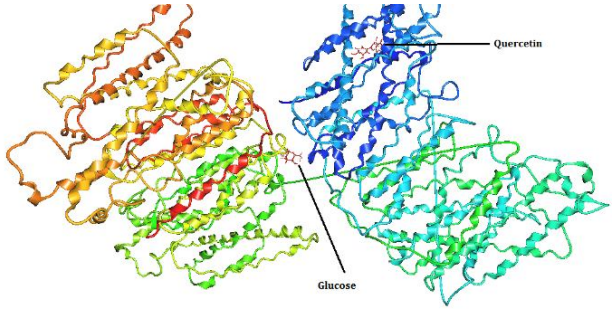

Figure 10: Obtained docked conformations of quercetin (right top) and glucose (center). Note that the docking locations are entirely different.

Validation of docking model

Docking of Glucose

To assess the validity of the docking model, glucose was docked in predicted active site of vSGLT. Glucose is a natural substrate of SGLT family. It was hypothesized that glucose would show high docking score and characteristic docking pose in predicted active site of vSGLT.

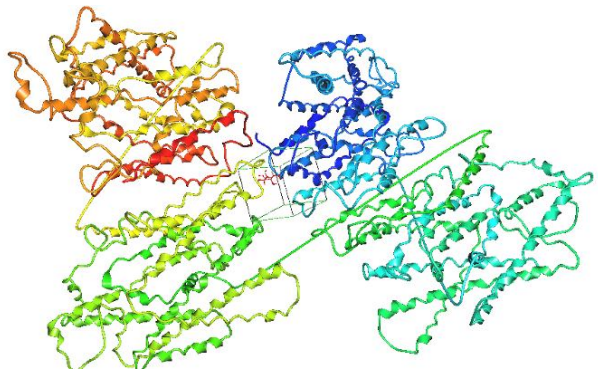

Figure 11: Obtained docked structure of glucose (red) in predicted active site of vSGLT. Note that the characteristic docking location is same as that found in quercetin glucosides and quercetin-γ-cyclodextrin.

The docked structure of glucose in predicted binding site of vSGLT is shown in **Fig. 11.** Glucose docked the predicted binding site between two domains of the predicted binding site with a docking score of (-9.3267 Kcal/mole). This location entirely differed with quercetin, but was similar to docking location of quercetin-3,4'-glucoside and quercetin-3-glucoside. The root mean square deviation of repeat docking scores was 3.15, which was well within the acceptance criterion (NMT 5).

Table 1: Obtained amino acid residues involved in hydrogen bonding with aglycone part and sugar moiety of test ligands

Sr. No.	Test Ligand	Aglycone Part	Sugar Moiety
1.	Quercetin-γ-cyclodextrin	Ala 126, Ala 174	**Gly 161**, Ala 155, **Gln 430,** Thr 162
2.	Quercetin-3,4'-diglucoside	Leu146	Ala 127, **Gly 161,** Ala 176, **Gln 430,** Val 169
3.	Quercetin-3-glucoside	Ala 156, Asn 171	**Gly 161**, Ala 126, **Gln 430,** Val 167
4.	Quercetin	Gln 23, Asn 18, Ala 17, Ala 46	Not applicable
5.	Glucose	Not applicable	**Gly 161,** Asn 156, Ala 173, **Gln 430**

DISCUSSION

The present study was an attempt to investigate role of γ-cyclodextrin moiety of quercetin-γ-cyclodextrin in its interaction with vSGLT. It was hypothesized that γ-cyclodextrin in quercetin-γ-cyclodextrin complex played a similar role as that of glucose moieties of quercetin glucosides while interacting with vSGLT.

Prediction of active site of vSGLT and subsequent molecular modeling and docking studies performed in the present study indicated that glucopyranose moieties of γ-cyclodextrin in quercetin-γ-cyclodextrin inclusion complex bind the predicted active site of vSGLT. γ-Cyclodextrin and 3-glucose moieties of quercetin glucosides shared the same docking location and hydrogen bonded with amino acid residues Gly 161 and Gln 430 in predicted active site of vSGLT among others. These findings suggested with high level precision that γ-cyclodextrin and glucose moiety on 3-position of quercetin glucoside interact analogously with predicted active site of vSGLT.

Quercetin docked on predicted active site of vSGLT with low docking score as compared to glucose and quercetin glycosides. The docking location was entirely different and involved hydrogen bonding with different amino acid residues as compared to glucose and quercetin glycosides. The results support and augment earlier finding that quercetin has low affinity towards SGLT1 [7]. These results explain extremely low oral bioavailability of unchanged quercetin as compared to quercetin glucosides in human as described in earlier works [1;3;4]. However, in docking simulations the vSGLT was assumed to have rigid structure. This rarely is the fact in biological systems and remains major limitation of the approach.

Quercetin docked hydrophobic cavity of γ-cyclodextrin with lower docking score of -6.81603 KCal/mole. No hydrogen bonds were observed in best ligand substrate docking pose. The results are supported by the theory that small lipophilic molecules interact with hydrophobic core of cyclodextrins [1]. The observed docked conformation of quercetin-γ-

cyclodextrin showed that benzopyrone part was enclosed in the cavity and phenyl ring was pointing outwards. This conformation was contradictory to that reported with quercetin-β-cyclodextrin which involved phenyl ring (ring C) enclosed in the cavity and benzopyrone part (ring A and ring B) pointing outwards [1]. A possible explanation is larger cavity of γ-cyclodextrin as compared β-cyclodextrin [18], which affords more space to accommodate bulky benzopyrone part (A and B rings) of quercetin.

Quercetin glycosides docked at a location different than quercetin alone. Their docking scores were higher (-10.3517 Kcal/mole for quercetin-3,4'-diglucoside and -8.4376 Kcal/mole for quercetin-3-glucoside) than quercetin alone (-6.3251 Kcal/mole). The scores were comparable to glucose (-9.3267 Kcal/mole). The results are in good confirmation with the fact that glucose is a natural substrate of vSGLT, SGLT1 and SGLT2 [42]. These results also support and augment the reported finding that order of affinity towards SGLT1 sis as follows quercetin-3,4'-diglucoside > quercetin-3-glucoside > quercetin [7]. The same order has been observed in intestinal absorption of flavonol glucosides [43]. The docking score of glucose was found -9.3267 KCal/mole. Glucose and quercetin glucosides shared hydrogen bonding with amino acid residues Gly 161 and Gln 430. It seems notable that amino acid residues Gly 161 and Gln 430 correspond to Gly 151 Gln 428 in the original sequence. These amino acid residues have been reported necessary for glucose transport by SGLT1 [34]. Findings of the present study indicate that glucose moieties of quercetin glucosides and glucose interact analogously with vSGLT.

Quercetin-γ-cyclodextrin complex docked the predicted active site of vSGLT with highest docking score of -11.2410 Kcal/mole. The docking score was more than that observed with quercetin alone (-6.3251 Kcal/mole) and quercetin-3-glucoside (-8.4376 Kcal/mole). The docking score was comparable to that observed with quercetin-3,4'-diglucoside (-10.3517 Kcal/mole). The order of docking scores was quercetin-γ-cyclodextrin > quercetin-3,4'-diglucoside > quercetin-3-glucoside > quercetin. Docking location of quercetin-γ-cyclodextrin **(Fig. 7)** was similar to that of quercetin glucosides and glucose **(Figs. 8, 9 and 11).** It differed from docking location of quercetin **(Fig. 10).**

Glucopyranose moieties in quercetin-γ-cyclodextrin inclusion complexshowed hydrogen bonding interactions with amino acid residues Gly 161 and Gln 430. This trend was also observed with docking of glucose and quercetin glycosides. The results indicate that glucopyranose moieties in quercetin-γ-cyclodextrin inclusion complex interact with vSGLT in a manner analogous to glucose in quercetin glucosides.

Complexation with cyclodextrins increases solubility of quercetin. Cyclodextrins also afford protection from enzymatic degradation of guest molecules in the intestines [16] and increase absorption of intact molecules from brush border of intestinal epithelium. The mechanisms of this increase in absorption remain unknown. It has long been believed that cyclodextrins release complexed guest molecules near brush border of intestinal epithelium in a 'dissolved' state and molecules cross the intestinal epithelium by passive diffusion [44]. Results of the present study indicate that these mechanisms are more complex and also involve interaction of cyclodextrins with membrane transporters such as SGLT. Intestinal absorption of glycosides has been found higher than corresponding aglycones [45]. The complex docked in active site of vSGLT in a favorable pose similar to quercetin glucosides and found to interact in analogous manner with the transporter.

Quercetin glucosides are transported across brush border of intestinal epithelium by SGLT1. However, quercetin is transported to a lesser extent than its glucosides [3;7;13]. The study has contributed to understanding of mechanism of this difference. The results indicate that glucopyranose moiety of quercetin-γ-cyclodextrin complex act similarly to glucose moieties of quercetin glucoside and help the complex to bind with vSGLT. This increases its transport through brush border of intestinal epithelium.

The results need to be further confirmed by expressing SGLT1 and SGLT2 in recombinant model organisms such as *Escherichia coli* and measuring cellular uptake of quercetin-γ-cyclodextrin complex vis-à-vis quercetin glycosides and glucose from the medium. *In silico* docking protocol of quercetin-γ-cyclodextrin complex with predicted active site of vSGLT needs to be modified to accommodate effect of water solvation and flexible substrate.

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