Rutin as a potential inhibitor to target peptidoglycan pathway of Staphylococcus aureus cell wall synthesis

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Abstract

Bacterial cell wall synthesis pathway “Peptidoglycan Synthesis” is implicated in the development of antibiotics against various pathogens and provides an interesting target for therapeutic intervention. A medicinally valuable flavonoid “Rutin”, have been shown to be effective against various pathogenic bacteria. Hence in this study, the prospective role of Rutin interaction in the capability against peptidoglycan synthesizing enzymes has been investigated, where the binding pattern of rutin with Mur ligases, MraY, FemX, FemA, FemB, and PBP2 have been studied. However, among them, Rutin interacted better with PBP2, which catalyze two important steps (transglycosylation and transpeptidation) of cell wall synthesis using TGase and TPase domains respectively. The interaction pattern and stability of Rutin towards both domains were studied individually through docking and simulation studies, which suggested that Rutin affinity towards TGase domain was more and similar to the reference molecule Moenomycin, compared to the TPase domain. Thus, were reported herein, Rutin as a novel structural class for inhibitor development based on the results which provided better insights on the interactional studies against S. aureus by targeting TGase domain of PBP2.

Introduction

The Staphylococcal cell wall is complex, dynamic, multilayered structures that play a variety of protective and adaptive roles for cell viability by providing the rigid exoskeleton which is essential for stabilizing the cell membrane against high internal osmotic pressures [1]. The major conserved component of the cell wall is peptidoglycan, a macromolecule consisting of linear glycan chain interlinked by short peptides [2]. Synthesis of peptidoglycan units is a multistep enzymatic process which can be divided into four phases (Figure 1) [3]: The first phase of peptidoglycan synthesis begins in the cytoplasm of the bacterial cell with the synthesis of UDP-Mur NAc pentapeptide, catalyzed by the cytoplasmic enzymes Mur ligases (MurA, B, C, D, E, F, G- which are functionally different from each other) [4,5]. The first committed step catalyzed by MurA enzyme with the transfer of enolpyruvyl moiety from phosphoenolpyruvate (PEP) to uridinediphosphate (UDP)-N-acetylmuramyl pentapeptide. Further, MurC, MurD, MurE, and MurF catalyze the addition of L-Ala, D-Glu, L-Lys and D-Ala-D-Ala respectively onto acetylmuramic acid. Further, MurG by adding UDP-MurNac-pentapeptide and GlcNAc to a membrane-bound acceptor. While, Lipid II formation is catalyzed by MurB (UDP-Mur NAc) [6]. Second phase includes membrane-associated steps, where formation of lipid I and lipid II are initiated by MraY [7] and MurG [8]. MraY catalyzes the formation of lipid I by transferring the MurNac-pentapeptide and GlcNAc to a membrane-bound acceptor. While, Lipid II formation is catalyzed by MurG by adding UDP-GlcNAc to lipid I and formed membrane bound disaccharide pentapeptide. Further in the third phase, disaccharide pentapeptide is modified by attaching the sequential addition of pentaglycine chain to the lysine residue of the pentapeptide by three homologous peptidyl transferases namely FemX/FmhB [9], FemA [10], and FemB [11]. Finally, in fourth phase, membrane bound disaccharide pentapeptide residue is transferred to the outside of the cell membrane to form linear peptidoglycans via transglycosylase and transpeptidase process, catalyzed by penicillin binding protein (PBP).

Based on the essentiality of cell wall for bacterial cell integrity, β-lactam antibiotics have been successfully targeted to inhibit the cell wall synthesis. However, these drugs besides producing enormous side effects have also made the organisms resistant to them. Since, natural compounds were reported to have pharmacological or biological activity that can be of therapeutic benefit in treating resistant strains, the present study was focused with the aim to study the interaction potentials of one of the flavonoid compound “Rutin” with other cell wall synthesizing enzyme through structure based molecular docking and simulation studies.

Materials and methods

Homology modeling and structure refinement

Although eleven enzymes including Mur ligases, MraY, FemX, FemA, FemB, and PBP2 enzymes are found to be involved in the synthesis of peptidoglycan in S. aureus, among them crystal structure were revealed to be present for only four enzymes; MurB (PDB 1HSK), MraY (PDB 4C13), FemA (PDB 1LRZ), and PBP2 (PDB 2OLV) [12,13], necessitated the tertiary structures for other proteins (Mur A, C, D, F, G, MraY, Fem X, Fem B) to be modeled. Therefore, using Modeller 9.15 which included three steps as template selection, target-template alignment and model building, the models were predicted by retrieving the amino acid sequence of those proteins from the...
UniProt database and subjecting them to BLASTp search against the PDB database. Templates were selected for each of the protein on the basis of structural hits and retrieved from the Protein Data Bank (Table 1). Thereafter, 3-D model was generated, and best model was selected based on the lowest discrete optimized protein energy (DOPE) value [15] and further validated using SAVES (Structure Analysis and Verification Server) server [16, 17] (Table 2).

**MD simulation study**

All generated models were simulated using GROMOS96 43a1 force field for 5000 ps for each of the modeled proteins and their energies were minimized using the steepest descent approach in the GROMACS 4.5.5 package and equilibrated (NVT and NPT) for 1000 ps to perform the final MD. The lowest potential energy (PE) conformations were selected from the simulation trajectory by plotting the Root Mean Square deviation (RMSD) graph and used for further analysis.

**Molecular docking study**

All cell wall synthesizing proteins were prepared for docking using Schrodinger protein preparation wizard, where proteins with the crystal structure (Mur B, E, FemA, and PBP2) were preprocessed and prepared by removing the water and other hetero- molecules (OPLS2005 force field) [18]. Grid for ligand docking was generated around the active site for each protein. Since, PBP2 catalyses transglycosylation and...
transpeptidation by TGase and TPase domain respectively, both of these domains were considered for the docking studies. Moreover, active site residues for all proteins were selected with the help of literature survey (Table 3). Finally, the prepared ligand-Rutin (ZINC053683228) was docked to each proteins using Glide package [19] available in Schrodinger software and analyzed manually. On the basis of docking results, best receptor-ligand complex was selected for MD simulation studies.

MD Simulations for the Docked Complexes

The best G-score protein-ligand complexes were further evaluated for their structural stability in dynamic condition through MD simulations approach in GROMACS 4.5.5 [20]. Protein-ligand complex was solvated (cubic box) in SPC216 water model and ions were added for neutralization. Finally minimized (Steepest descent = 500 steps followed by conjugate gradient = 500 steps) and equilibrated (NVT and NPT for 1000 ps) system was taken for MD simulation (15000 ps) at constant temperature (300 K) and pressure (1 atm).

Results and discussion

Three-dimension structure of the modelled proteins

The 3-D structures of modelled proteins generated five models for each of the proteins, however best model was selected based on the lowest DOPE value (Table 2) and validated using SAVES (Table 2), which indicated the predicted model to well correlate with the structural features defined in its sequence and allowed for further study (Table 3) depending on their reliability.

Binding affinity analysis

The interaction affinity of Rutin with other peptidoglycan synthesizing enzymes (Table 4) revealed Mur ligases to obtain G-score ranging between -4.585 to -7.088, however, affinity of Rutin towards MurD was more (G-score, -7.088) in comparison to other Mur ligases, which formed six H-bonds with bond lengths ranging from 1.8296 to 2.32826 Å, while MurF exhibited lowest affinity (-4.585) by forming three H-bonds (bond lengths between 1.99531 to 2.30373 Å). Similarly, homologous peptidyl transferases (FemX, A, and B) interacted with Rutin producing G-scores of -6.857, -5.973, and -5.526 each by making three, seven and six H-bonds respectively. Interestingly, transmembrane protein MraY interacted well (five H-bonds with the bond lengths of 1.82063 to 2.48561 Å) with -5.345 G-score.

The interaction of Rutin with both TGase and TPase domains of PBP2, although revealed highest G-scores (-8.297 and -8.057) compared to other proteins, the scores were approximately same with major differences mediated by the number of H-bonds (Figure 2), each with ten and two (bond lengths from 1.8878 to 2.32564 Å and 1.94745 and 1.94973 Å respectively). Thus, based on this observation, further these complexes with the complexes formed with the reference molecules (inhibitor) of Moenomycin and Cefotiboprole (Table 5 and Figure 3). Although, TGase domain interacted with Rutin and Moenomycin (-8.297 and -8.0137 G-scores), a greater number of H-bonds (ten) were formed with rutin compared to Moenomycin (eight). However, interacting residues (as Lys163, Glu232 and Asp156) were common in both suggesting the binding efficiency of Rutin similar to the reference compound. In addition, π-π, π-cation and π-sigma interactions observed showed Rutin to form four π-cation interactions (one with Lys155 and Lys168 and two by Lys163), which was significantly absent in Moenomycin. On the other hand unlike TGase, TPase domain exhibited interaction with the reference compound Cefotiboprole and comparison with Rutin complexes, although revealed G-scores to be on par with each other (-7.924 and -8.057), Rutin complex exhibited only two H-bonds proving it less efficient as the key residue Ser398 that interacted with Cefotiboprole (2.0567 Å) and their π-π interactions were unobserved. Thus, the studies indicated the affinity of Rutin towards TGase than TPase domain. Interestingly, Rutin followed the same kind of interaction pattern in the TGase domain and interacted more efficiently compared to Moenomycin while, its interaction was very poor with TPase domain compared to Cefotiboprole. Hence, in order to ascertain the reasons for these variations, all these complexes were further analysed under dynamic conditions.

Table 1. Details of the templates used for modeling the structures of the Proteins

| Proteins | Protein UniProt ID | Protein Length (Numbers of amino acids) | Template (PDB ID) | Query Coverage (%) |
|----------|--------------------|-----------------------------------------|------------------|-------------------|
| MurA     | P84058             | 421                                     | 3SG1             | 99                |
| MurC     | O31211             | 437                                     | 1GOQ             | 96                |
| MurD     | P0A091             | 449                                     | 3L7T7A           | 97                |
| MurF     | K7XML5             | 452                                     | 3ZM5             | 95                |
| MraY     | P01CR8             | 321                                     | 4J72             | 94                |
| MurG     | A5ISU9             | 356                                     | 1FKK             | 91                |
| FemB     | Q2FYR1             | 419                                     | 1LIRZ            | 99                |
| FemH     | Q2FVZ4             | 421                                     | 1LIRZ            | 96                |

Table 2. Validation of the modeled proteins

| Proteins | ERRAT Score | VERIFY3D (%) | RMSD between template & modeled structure (Å) | Residues in allowed region | DOPE value |
|----------|-------------|--------------|---------------------------------------------|---------------------------|------------|
| MurA     | 83.495      | 97.86        | 0.099                                       | 97.60%                    | -49255.6   |
| MurC     | 70.163      | 88.98        | 0.776                                       | 94.90%                    | -46056.9   |
| MurD     | 79.365      | 91.09        | 0.123                                       | 96.20%                    | -52871.2   |
| MurF     | 70.045      | 89.16        | 0.263                                       | 97.00%                    | -46772.6   |
| MraY     | 96.141      | 95.01        | 0.161                                       | 95.00%                    | -44797.5   |
| MurG     | 93.966      | 94.02        | 0.544                                       | 97.20%                    | -47435     |
| FemB     | 74.572      | 82.83        | 0.366                                       | 96.60%                    | -48329.2   |
| FemH     | 97.304      | 98.1         | 0.494                                       | 95.00%                    | -40185.6   |
Table 3. Structure of proteins involved in peptidoglycan synthesis

| S. No | Protein Name | 3-D Structure of Modeled Protein | Active Site Residues | References |
|-------|--------------|---------------------------------|----------------------|------------|
| 1     | MurA         | ![Image](image1.png)             | Leu13, Pro114, Gly115, Gly116, Cys117, Ser118, Ile119, Gly120, Ala121, Arg122, Pro123 | [21]       |
| 2     | MurB*        | ![Image](image2.png)             | Asp71, Tyr175, Arg176, Arg213, Ser226, Arg259, Glu296 | [22]       |
| 3     | MurC         | ![Image](image3.png)             | Tyr154, Asp174, His175, Arg298, Tyr313, His315, Thr344, His343, Arg347, Ala417 | [23]       |
| 4     | MurD         | ![Image](image4.png)             | Leu17, Lys19, Ser20, Gly21, Asn38, Thr330, Arg355, Ser424, Phe431 | [24]       |
| 5     | MurF*        | ![Image](image5.png)             | Ser30, Tyr45, Val47, Asn151, Thr152, Thr153, Ser179, Arg187, Arg383, Asp406, Ser456, Glu460 | [25]       |
| 6     | MurG         | ![Image](image6.png)             | Ile31, Phe45, Glu48, Asn49, Phe55, Ile145, Asn336, Ser338, Thr340, Glu368, Asn369 | [26]       |
| 7     | MraY         | ![Image](image7.png)             | Asp117, Asp118, Asp265, His324, His325, His326 | [27]       |
| 8     | MurG         | ![Image](image8.png)             | Asn128, Arg164, Ile245, Ala264, Gln288, Gln289, Glu269 | [8]         |
| 9     | FemX/Fm      | ![Image](image9.png)             | Lys36, Gly40, Tyr41, Leu103, Tyr224, Tyr316 | [9]         |
| 10    | FemA*        | ![Image](image10.png)            | Lys36, Gly40, Tyr41, Leu103, Tyr220, Phe224, Ala326 | [9]         |
| 11    | FemB         | ![Image](image11.png)            | Arg36, Gly40, Phe41, Leu103, Lys220, Tyr224, Ser326 | [9]         |
| 12    | PHP2*#       | ![Image](image12.png)            | Glu114, Glu144, Ala146, Glu152, Lys155, Thr156, Arg167, Lys168, Glu171, Tyr196, Pro231, Gin232, Val233, Asn235 (TGase Domain) | [28]       |

Yellow and green ribbon indicates the template and target protein structure respectively. *Proteins, for which crystal structure is available. **Protein contains two catalytic domains (TGase and TPase domains).
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Figure 2. Comparative H-bond interaction pattern in the TGase and TPase domains of PBP2

Here (A) represent PBP2 structure with the two domains TGase and TPase shown as cartoon; (B) Moenomycin, (C) Rutin, (D) Cefobiprole and (E) Rutin interaction provided the details of the binding mode with TGase and TPase domain of PBP2 respectively. White ribbon represents the protein backbone. Pink, cyan and green stick represents Moenomycin, Cefobiprole and Rutin respectively where, blue dotted line indicates H-bonds.

Table 4. Interaction pattern of the Rutin with the proteins involved in peptidoglycan synthesis

| Proteins | G-score, Kcal/mol (Rutin) | Residues involved in H-bond | No of H-bond |
|----------|---------------------------|-----------------------------|--------------|
| MurA     | -6.461                    | ARG95, ALA96, ASP308, ASP308, ASN23, ASN23 | 6            |
| MurB     | -5.394                    | GLU72, GLU72, GLU171, VAL65 | 4            |
| MurC     | -5.417                    | SER12, TYR313, ALA417, GLU150, GLU150 | 5            |
| MurD     | -7.088                    | ASN145, SER168, ASN78, GLU166, LYS328, GLY80 | 6            |
| MurE     | -5.913                    | ARG187, ASN407, HIS181, TYR351, GLU382, ALA150 | 6            |
| MurF     | -4.385                    | SER33, SER33, SER33 | 3            |
| MurG     | -5.345                    | SER108, ASN166, SER108, GLN269, GLN269 | 5            |
| FemA     | -5.973                    | LYS33, LYS33, GLN154, ARG228, GLU36, VAL152, TYR69 | 7            |
| FemB     | -5.526                    | LYS106, GLN155, THR152, TYR70, SER153, ASP37 | 6            |
| FmhB/X   | -6.857                    | LYS374, LEU319, GLY356 | 3            |

Table 5. Differences in the docking pattern of Rutin at TPase and TGase domain of PBP2

| PBP2-Lig Complex | G-score (Kcal/mol) | H-bond Residues | Bond Length (Å) | Donor Atom | Acceptor Atom | No of H-bond |
|------------------|---------------------|-----------------|-----------------|-----------|--------------|-------------|
| TGase-Rutin      | 8.297               | GLY145, SER147, LYS163, LYS163, GLN232, GLU171, ASP156, ASP156 | 1.8878, 2.32564, 2.24244, 2.07449, 2.04573, 2.21883, 2.05424, 1.88623, 1.80934, 1.45512 | H | 10 |
| Tgase-Moenomycin | 8.0137              | GLN161, LYS163, ARG167, ARG167, GLN322, ASP156, ASP156 | 1.74209, 2.38802, 2.19337, 2.16287, 2.37979, 2.34513, 2.47206 | HE22, HZ1, HZ2, HZ2, HZ3, HZ3, HZ3, HZ3, HZ3, HZ3 | 8 |
| TPase-Rutin      | 8.057               | GLN641, GLN453 | 1.94745, 1.94973 | H23, O23 | 2 |
| TPase-Cefobiprole| -7.924              | LYS401, LYS401, SER398*, TYR437, ASN456 | 2.39515, 2.4851, 2.0567, 1.97877, 2.09275 | HZ1, HZ2, HZ2, HZ2, HZ2 | 5 |

*Key residue, Common interacting residues in comparison to the reference molecule (Moenomycin and Cefobiprole) are bold-faced.
represents the protein backbone. Pink, cyan and green stick represents Moenomycin, Ceftobiprole and Rutin respectively where, blue dotted line indicates H-bonds.

**Evaluation of protein-ligand complex stability through MD simulation**

The stability and behavior of Rutin, Moenomycin and Ceftobiprole complexed with both domains of bound PBP2 and unligated PBP2 were assessed by simulating for 15000 ps and analyzed by plotting the backbone root mean deviation (RMSD), root mean fluctuation of protein backbone (RMSF), radius of gyration (Rg) and H-bonds graph for the period of 15000 ps.

**RMSD**

The RMS deviation plots of PBP2 backbone (Figure 3A,3B) revealed TGase domain of bound form with Rutin and Moenomycin to deviate more compared to the unligated PBP2. The RMS deviation plot shows that the unligated PBP2 equilibrated after ~6000 ps and thereafter deviated between ~0.21 to ~0.31 nm. Whereas, backbone deviation was observed more in reference complex PBP2-Moenomycin and PBP2-Rutin in comparison to the unligated PBP2. The PBP2-Moenomycin complex reached at equilibrium after ~9000 ps and deviated between ~0.3 to ~0.4 nm while, PBP2-Rutin complex equilibrated from ~4000 ps and deviated between ~0.6 to ~0.7 nm. Noticeably, the deviation pattern in PBP2-Moenomycin and PBP2-Rutin was observed to be similar. On the other hand, when TPase domain was occupied by Rutin and Ceftobiprole the RMS deviation plot (Figure 3B) showed less deviation in comparison to the TGase bound PBP2 complexes (PBP2-Rutin and PBP2-Moenomycin). The PBP2-Ceftobiprole equilibrated after ~2000 ps and further deviated between ~0.2 to ~0.3 nm, which was similar as the unligated PBP2. Whereas, PBP2-Rutin complex reached at equilibrium after ~8000 ps and thereafter deviated between ~0.3 to ~0.4 nm, which was more in comparison to the reference complex (Ceftobiprole) and unligated PBP2.

**RMSF**

In order to analyze the residual atomic fluctuations of the backbone atoms in the presence of Rutin at TGase and TPase domain, RMSF plots were generated for all complexes. The RMS fluctuation of individual amino acid in both domain complexes, which is shown in figure 3A,3B, revealed that the major movement took place at TGase domain which is present at the N-terminal region, with respect to the TPase domain. Comparative analysis of RMS fluctuation for both domain complexes (TGase and TPase complexes) showed that in the presence of ligand (Moenomycin and Rutin) at TGase domain (between 100 to 250 residues) (Figure 3A) backbone fluctuation was less in comparison to the TPase occupied complexes (Figure 3B). On the other hand, TPase domain, which is present at C-terminal (350 to 600 residues), was observed to be stable in both complexes. Based on the fluctuation pattern of PBP2 backbone in both complexes it was suggested that the Rutin stability at TGase domain was more in comparison to the TPase domain.

**Radius of gyration**

Further, to analyze the compactness TGase and TPase domain of PBP2 backbone in the presence of Rutin with respect to the
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The stabilization of protein–ligand complexes also depends on the intermolecular H-bonds, therefore the stability of the H-bonds formed at TGase and TPase domain of PBP2 with Rutin and their inhibitors (Moenomycin and Ceftobiprole respectively) throughout the simulation were computed and shown in figure 4. The H-bond plot showed Rutin formed three and one H-bonds (shown as red in the Figure 4A,4B) at TGase and TPase domains respectively while the reference compound Moenomycin and Ceftobiprole formed ~4 and ~3 H-bonds at TGase and TPase domain respectively throughout the simulation period (Figure 4C,4D). Here, (A) and (B) represents the RMSD of PBP2 (C) represent Moenomycin and Rutin while (D) Ceftobiprole and Rutin interaction pattern in TGase and TPase domain respectively during the simulation period (15000 ps).

**Discussion**

The previous study on the Rutin binding affinity towards the PBP2a active site [29,30], provided the information of Rutin efficiency against MRSA. In order to investigate its capability towards the other peptidoglycan synthesizing enzymes, molecular docking and simulation studies were performed and analyzed. Enzymes MurA, MurB, MurC, MurD, MurE, MurF, MurG, MraY, Fem X, Fem A, Fem B, and PBP2 were found to be involved in peptidoglycan synthesis [31]. Among them, only MurB, MurE, FemA, and PBP2 proteins crystal structure were available in the PDB, hence other proteins tertiary structures were modeled and the lowest potential energy (PE) conformations were selected for further studies. Using molecular docking approach, it was observed that the Mur ligases, trans membrane protein MraY, and homologous peptidyl transferases (FemX, FemA, and FemB) active site interacted with Rutin by obtaining G-score in between -4.585 and -7.088, which was observed to be less in comparison to the PBP2.

Studies has revealed that *S. aureus* contains four types of PBPs namely PBP1 [32], PBP2 [33], PBP3 [34]and PBP4 [35] and among them PBP2 was observed to be essential for the cell viability in sensitive *S. aureus* strain [33]. Since PBP2 catalyzes two important steps (transglycosylation and transpeptidation) of peptidoglycan synthesis with the help of two different catalytic sites namely TGase and TPase domain, hence Rutin affinity was analyzed for both of the domains. The molecular docking studies showed that the Rutin G-score for both domains (TGase and TPase) was approximately similar, but interestingly Rutin formed ten (bond length ranged between 1.8878 to

**Figure 4.** The plots represent the stability of the Protein-ligand Complexes mediated by the Hydrogen bonds
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