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ORIGINAL RESEARCH PAPER

Inhibitory effect of theaflavin-3,3′-digallate can involve its binding to the "stem" domain of α-hemolysin of Staphylococcus aureus

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ABSTRACT

Infections caused by *Staphylococcus aureus* are currently a worldwide threat affecting millions of individuals. The pathogenicity of *S. aureus* is associated with numerous virulence factors, including cell surface proteins, polysaccharides, and secreted toxins. The pore-forming α-hemolysin, known as α-toxin, is produced by nearly all virulent strains of *S. aureus* and is implicated in several diseases including skin and soft tissue infections, atopic dermatitis, and pneumonia. There are currently no vaccines available for the prevention of *S. aureus* infections and the efficacy of available antibiotics has been fading. In this study we examined the mode of antihemolytic activity of theaflavin-3,3′-digallate against α-hemolysin of methicillin-resistant *S. aureus* by molecular docking using AutoDock Vina as the molecular docking tool. The theaflavin-3,3′-digallate docked the molecular sequence of the Hla (PDB ID:7ahl). The scores of the top 10 binding modes obtained were between −9.0 and −8.5 kcal mol^{-1}, and the best binding mode was −9.0 kcal mol^{-1}. Direct binding sites of theaflavin-3,3′-digallate to the "stem" domain of Hla were revealed which primarily targeted of the residues Met113, Thr117, Asn139. The disclosure of this potential binding mode warrants further clinical evaluation of theaflavin-3,3′-digallate as an anti-hemolytic compound in order to practically validate our results.

KEYWORDS

Staphylococcus aureus, α-hemolysin, theaflavin, molecular docking

INTRUDUCTION

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium identified as an important cause of infection in hospitals and in communities worldwide (1). Its ubiquitous presence is also considered as being highly virulent in medical and non-medical settings, and mainly associated with skin and soft tissue infections (SSTI), sepsis and pneumonia (2). High virulence of *S. aureus* strains is in part related to expression of phenol soluble modulins (PSMs) and alpha-hemolysin (α-hemolysin, α-toxin, Hla) as virulence factors (3). Hla is a pore-forming exotoxin with cytolytic activity toward various cell types, such as keratinocytes, epithelial cells, erythrocytes and leucocytes, and is lethal to animals like rodents and rabbits (4).

Hla is secreted as a water-soluble, 34 kDa monomer that binds to lipid parts of host cell membranes, which subsequently undergoes oligomerization resulting in membrane-inserted heptameric 238 kDa form that generates membrane pores about 2 nm in diameter (5). The amino acid sequence of Hla revealed that this protein contains no cysteine but has an abundance of glycine residues (6). The structure of Hla resembles the mushroom-shaped homo-oligomeric heptamer, comprising protomers of 100 Å in length and 100 Å in diameter, with three distinct domains: cap, rim, and stem. The stem domain (52 Å in length, 26 Å in diameter) is a 14-strand anti-parallel β-barrel that outlines the lytic transmembrane channel.

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The interior of the β-barrel is primarily hydrophilic, while its exterior has a hydrophobic belt. The cap domain (comprising β sandwiches and amino latches of each protomer) is a hydrophilic domain that overhangs from the extracellular surface. The rim domain extends from the underside of the cap domain in proximity to the outer leaflet of the cell membrane.

Theaflavins is a common class of flavonoids abundant in black tea (7). They demonstrate a variety of metabolic effects, such as anti-carcinogenic, anti-septic, anti-diabetic, and have anti-oxidative properties (8). Our recent study documented the effect of theaflavin-3,3′-digallate (TF3) on the activity, production, secretion of Hla by *S. aureus* USA300. TF3 as a natural dietary flavonoid, accounts for about 40% in black tea with multiple biological activities, including anti-inflammatory, antioxidant, antiviral, and antibacterial characteristics (9). We found that TF3 have anti-hemolytic effect protecting keratinocytes against Hla-triggered cell death and inflammation. Its effect on skin barrier function while beneficial in relieving skin injury occurs without affecting *S. aureus* growth or viability, thus not exhibiting direct anti-*S. aureus* activity, which could slow the progress of antibiotic resistance (10).

Pharmacokinetic and pharmacodynamic studies conducted earlier indicate that nano-formulation of TF3 could increase its bioavailability and thus be an attractive candidate for further study against *S. aureus* infections. Also, the combination of TF3 with the inhibitors of Hla, could form a first-line approach for the treatment of *S. aureus* infections as well (11). Here we investigated the molecular mechanism of TF3 binding to Hla that could corroborate our earlier findings and eventually form the foundation for further therapeutic applications and study.

MATERIAL AND METHOD

Structure preparation

The molecular sequence of the Hla (PDB ID:7ahl) was downloaded from the protein database (www.rcsb.org).

Ligand preparation

The three-dimensional structure of TF3 in sdf format was downloaded from pubchem (https://pubchem.ncbi.nlm.nih.gov/compound/135403795) and OpenBabel was used to convert it into a mol2file for further processing (12).

Molecular modeling

In this study, AutoDock Vina was selected as the molecular docking tool (13). MGLTools 1.5.6 was used to add hydrogens and give Kollman charge for the protein from 7ahl, then the 7ahl.pdbqt file was generated as a receptor file. With reference to the compound structure of 3m4e, a complex structure of *S. aureus* Hla and beta-cyclodextrin, the center coordinates of the docking box x, y, z were defined as 35.5, 31, 26 based on the binding position of beta-cyclodextrin, and the box size was set to $30 \times 30 \times 30$ Å, to contain the entire pocket area. The ligand_prepare.py script in the molecular docking package was used to deal with the mol2 file of the ligand TF3. The flexible bond was set by default, and Gasteiger charge was added to generate the ligand pdbqt file. The exhaustiveness value of the search parameter was set to 10 and defined to output top 10 ranking conformations according to docking scores. The default values were selected for the rest of the parameters.

RESULTS AND DISCUSSION

Evaluation of the binding of TF3 to the Hla via molecular docking revealed the direct interaction between TF3 and Hla. From the view of the heptamer down, TF3 is wrapped in the Hla binding pocket and possesses a large contact surface with Hla residues (Fig. 1A). Further binding mode analysis showed that TF3 binds within the stem domain of *S. aureus* Hla, forming hydrophobic interactions and hydrogen bonds with multiple residues on the protein.

The scores of 10 binding modes of TF3 with *S. aureus* Hla obtained by molecular docking are shown in Table 1. The Vina docking score was based on the experimental binding free energy value as the fitting object, and the unit was kcal mol^{-1}. The scores of the top 10 binding modes were between −9.0 and −8.5 kcal mol^{-1}, and the best binding mode was −9.0 kcal mol^{-1}.

Several hydrogen bonds were found to be formed between the hydroxyl groups of TF3 and the residues in the stem domain of *S. aureus* Hla, such as Asn139, Phe120, Thr117 from chain B, Thr115, Asn139, Val140 from chain C, Thr117, Ser141 from chain D, and The145 from chain E. At the same time, TF3 forms hydrophobic interactions with Met113, Thr117, Asn139, Val140, Ser141 from chain C, Met113, Thr115, Ile142 from chain D, and Gly143 from chain E (Fig. 1B-C). From the view of the heptamer parallelly to the sevenfold axis, Asn139/Met113/Thr117 binding residues overhang the cylindrical channel of Hla, and thus may play important roles in the hemolytic activity of Hla, and are the target binding sites for TF3, in turn stabilizing the binding cavity of Hla and affecting its lytic activity. This demonstrated binding mode could reveal the molecular basis for the biological activity of TF3 against Hla.

The impact of Hla on the severity of *S. aureus* infections are largely recognized, since this toxin is a highly conserved virulence factor in *S. aureus* and highly available to the immune system (14). Based on the findings presented here, TF3 is projected to bind to “stem” of Hla. This part of Hla is recognized as directly involved in the pore-forming process and detrimental for the hemolysis process and hemolytic activity of this toxin *per se*. Interestingly, Ragle et al. have reported about Hla modified β-cyclodextrin compound preventing Hla-induced lysis of human alveolar epithelial cells, but not the formation of the heptameric Hla (15). Studies of Wang et al. demonstrated that myricetin, a

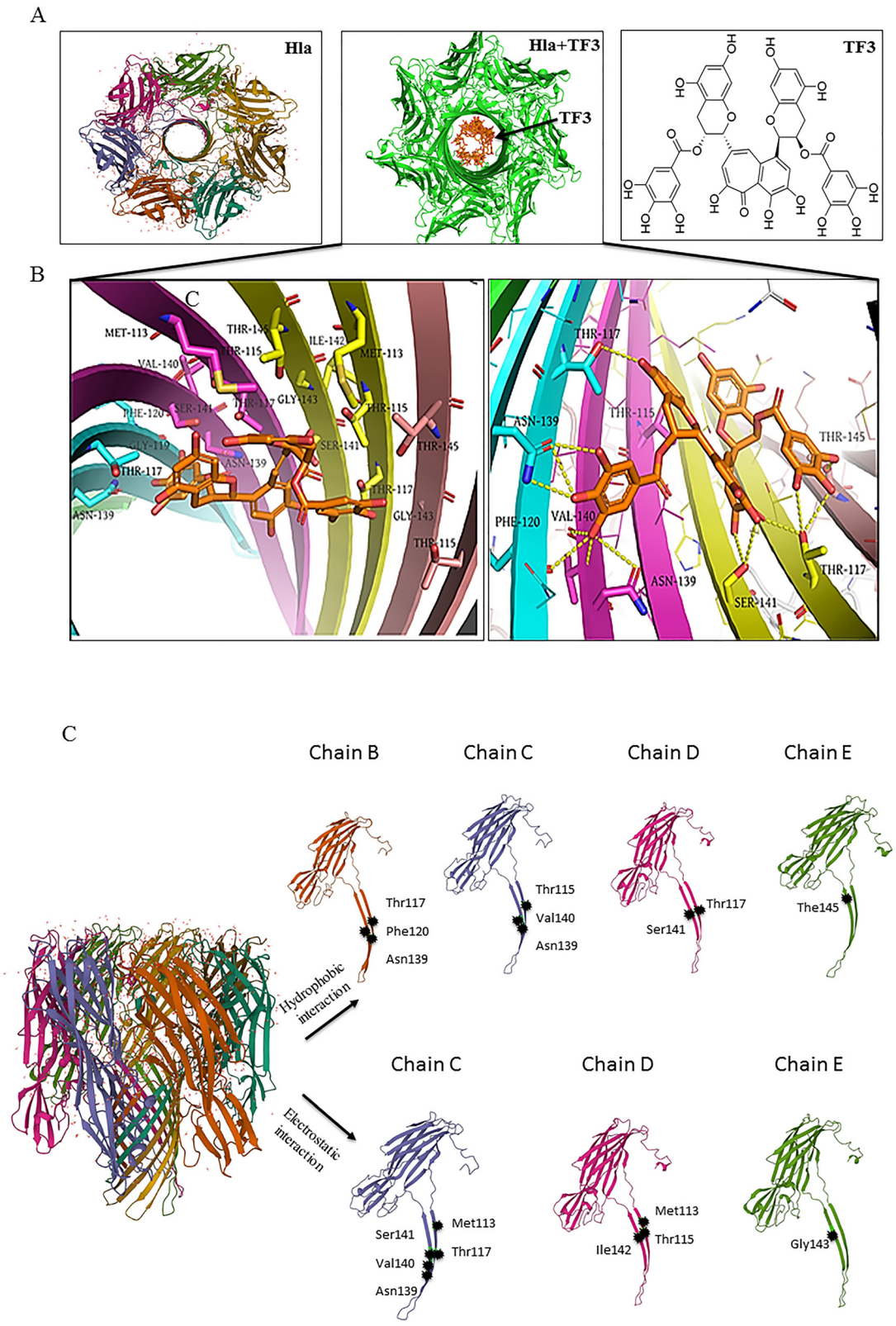

Fig. 1. **Binding of TF3 to Hla of *S. aureus*.** (A). Binding mode distribution of TF3 within Hla from top view. Heptameric Hla is represented by the green cartoon model, and TF3 is represented by the orange stick model. (B) Best binding mode of TF3 from top view (left panel) and side view (right panel) where Hla is shown in cartoon model (B chain in blue, C chain in purple, D chain in yellow, and E chain in pink), and TF3 is shown in orange stick models (C in orange and O in red). The residues within 4Å nearby TF3 are depicted in stick models, and hydrogen bonds are shown by yellow dashed lines. (C) Schematic representation of hydrogen and electrostatic binding interactions of TF3 with particular residues on the "stem" of Hla

Table 1. Docking scores for 10 binding modes obtained by molecular docking

Models	Docking score (kcal mol^{-1})
Model1	−9.0
Model2	−8.8
Model3	−8.7
Model4	−8.7
Model5	−8.6
Model6	−8.6
Model7	−8.6
Model8	−8.6
Model9	−8.5
Model10	−8.5

natural flavone, inhibits Hla hemolytic activity and prevents *S. aureus*-mediated cell injury (16). Similarly, Wang et al. published findings showing that curcumin also has anti-hemolytic properties and targets the "stem" region of Hla (17). In addition, Dong et al. reported that inhibition of Hla production by apigenin leads to protection of lung cells from the Hla-induced injury (18). Finally, Gua et al. published a study about honokiol, a natural polyphenol, that reduces secretion of Hla by *S. aureus* and inhibits Hla-mediated inflammatory responses (19). Thus, this type of inhibitory mechanism, as presented here, could facilitate the development of new and more effective anti-hemolytic agents.

CONCLUSION

Major finding of this study is direct evidence that the binding sites of TF3 to the "stem" domain of Hla primarily involve the residues Met113, Thr117, Asn139. This binding enforces the concomitant change in the conformation of the "stem" region which is restricted to this part of Hla only. Outcome of our study imply that further search and development of inhibitors aimed at staphylococcal virulence exoproteins could be based on the agents with TF3-based structure as the inhibitors of Hla activity.

Ethics statement: Not applicable.

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