UDC 577.218+29 https://doi.org/10.7124/FEEO.v32.1551

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STRUCTURAL PROFILE OF LIGAND-BASED INHIBITION OF BACTERIAL FtsZ

Aim. The idea of the study was to compare and generalize RCSB Protein Data Bank and ChEMBL data in order to establish the structural and biological relationship of experimentaly proved effectors of FtsZ with binding sites. ***Methods.*** Literature and database search. Comparison of protein and ligand structures. Protein structure modeling, MD, structural superimposition, etc. ***Results***. The experimental protein-ligand complexes structures of bacterial FtsZ were revised. The structural superimposition of experinental PDB and full-atomic AlphaFold2 models of bacterial FtsZs confirmed their significant structural similarity. Three protein-ligand binding sites were identified by structural alignment. The rating based on database (RCSB Protein Data Bank, ChEMBL, DrugBank, BindingDB, PubChem), patente and literature information on FtsZ-ligand interactions identify perspective sites and main reference compounds. ***Conclusions.*** It was identifyd 3 main protein-ligand binding regions in FtsZ: I. Nucleotide Binding Domain (Ia. Site of GTP/GDP and Ib. MB3 site); II. Site of inter-domain cleft (IDC) and III. Site of coumarin bindig (4HC = 4-hydroxycoumarin). It was indicated that benzamide-binding site, located in the region of inter-domain cleft of FtsZ, demonstrate highest site- and target-specificity.

Keywords: FtsZ, Z-ring, bacteria, inhibitors, ligand, binding site.

FtsZ (Filamenting temperature sensitive mutant Z) is the major cytoskeletal protein of bacterial cytokinesis [1, 2]. It forms a specific ring structure (Z-ring), that constricts and initiate division of the cell in bacteria [3]. Being a cytoskeletal homolog of eukaryotic tubulin, FtsZ plays a highly conserved and foundational role in cell division and has been the primary molecular target of ligand-dependent inhibition of cell division in bacteria [4]. From the standpoint of current knowledge, FtsZ contains two main drug-binding pockets: the GTP-binding site (= nucleotide binding domain, NBD) located at the interface between polymeric subunits [5], and the inter-domain cleft (IDC) [6], thats located between the N-terminal and C-terminal segments of the core globular part of molecule [4, 7]. The last one located in the cleft beneath the H7 helix and adjacent to the T7 loop of FtsZ molecule and olso known as allosteric site [4, 8]. It have been shown, that substances, like PC190723 (3-((6-chlorothiazolo[5,4-b]pyridin-2-yl)methoxy)-2,6-difluorobenzamide), bind in IDC region, provoke allosteric shift, causing deformations in the nucleotide binding domain [8]. At the same time, there is an opinion, that compounds that bind instead to the GTP binding site are much less useful as potential antimicrobial therapeutics because they are potentially more cytotoxic to mammalian cells, due to the high similarity of sites in FtsZ and tubulin [4]. Nevertheless, even in the case of the GTP binding site, in some cases quite good results can be achieved [9]. In particular, effective and patented NBD targeted inhibitors of FtsZ are known, e.g. some compounds were developed using the method of combinatorial modification of GTP/GDP molecules. However, there is no doubt that, the inter-domain cleft demonstrate much less sequence and structural similarity with tubulin, making it a better potential target for drugs that are less toxic to eucaryotes.

In the last 20-30 years, a number of natural and synthetic IDC inhibitors have been identified. To a certain extent, success in the search for FtsZ effectors is associated with reliable experimental data acquisition, as well as with aplication of new methods of Computer Aided Drug Design (CADD) [10]. In turn, the actual output of such CADD methods as molecular docking, pharmacophore search, etc. considerably depends on quality of the initial data on target binding sites and correct of reference compounds. In other words, compounds, for which reliable data on the mechanisms of formation of the ligand-protein complex and biochemical confirmation of the effectiveness of interaction and inhibition (Ki, Kd, IC50 and EC50) are already exist [11, 12]. At present, the main sources of such information are the RCSB Protein Data Bank (*www.rcsb.org*) and ChEMBL [13]. The first one contains experimentally confirmed structural files of macromolecules, often with ligands, and is a source of irrefutable structural facts of ligand-

protein interactions, and secondly, it gives us the information about the site and active conformation(s) of the ligand. The second contains data of biochemical experiments, which, however, are not always accompanied by structural data.

The idea of this study was to compare and generalize the current information from RCSB Protein Data Bank and ChEMBL data in order to establish the structural and biological relationship of proved FtsZ effectors with binding sites. Despite priority of these resources, we also took into account reliable experimental data from other web-resources, such as PubChem [13], DrugBank [14], BindingDB (*www.bindingdb.org*) [15], etc. and literature, to clarify some controversial points. In fact, the actual number of known FtsZ effectors significantly exceeds the number of compounds deposited in these databases. However, PDB and ChEMBL provide the most confirmed structural and biochemical information. Based on this, we hope, to clarify the binding sites for compounds, for which the sites is not available yet. On the other hand, the absence of obvious homologies and matches, will be an argument for us to search for new sites of protein-ligand interaction. It's no doubts about such sites existence, because it argued by bioinformatic studies, and larger number of ligand-protein interaction sites in tubulin.

Materials and methods

The objective of the study was search and identification of sites of protein-ligand interaction between FtsZ proteins and effectors. The object of the study was the spatial structures of FtsZ proteins and their effectors with proven activity. Databases such as RCSB Protein Data Bank (www.rcsb.org), ChEMBL (www.ebi.ac.uk/chembl/) [16], PubChem (https://pubchem.ncbi.nlm.nih.gov/) [13], and DrugBank (go.drugbank.com) [14] were used as the sources of molecular, structural and biological activity information. Amino acid sequences of the studied proteins were obtained from UniProtKB (www.uniprot.org) [17]. The main source on the chemical properties and namenclature of ligands was the PubChem database. The information from PubChem was used for standardization of IUPAC (https://iupac.org/) names of compounds.

All visualizations and analysis of 3D-structures of proteins and ligands were performed using PyMOL v.1.5.0.5 software (*www.pymol.org*).

Results and discussion

Correct information on the efficiency and mechanisms of ligand-protein interactions is a key term for the successful design and combinatorial synthesis of new effective antibacterial agents. Despite significant virtualization and the application of structural bioinformatics, the succsess of any CADD screening considerably depend on the initial laboratory experiments. In doing so, primary importance belong to structuraly solved complexes, and biochemical data on the dynamics and efficiency of interaction. The primary source of such information is the number of experimentally validated databases, of which ChEMBL and RCSB Protein Data Bank are the key ones. For today, ChEMBL containe 417 compounds, for which direct interaction with FtsZ were confermed based on biochemical tests (265 compound) and homology (152 compounds). At the same time, these compounds belong to different chemical gropes, and their molecular weight (MW) varies from 130.15 (CHEMBL1097445) to 1449.27 g·mol-1 (Vancomycin, CHEMBL262777). The specificity and efficiency of binding of this compounds varies considerably, and confirmed by the Ki, Kd, IC50, and EC50 values of biochamical assays, executed on recombinant FtsZ from *Bacillus subtilis* (strain 168), *Bacillus subtilis*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

On the other hand, due to the high costs and complexity of research, only 32 FtsZ protein-ligand complexes have been deposited in the RCSB Protein Data Bank, which contain both a native substrate (GTP / GDP) and various effectors and probes (for example, a fluorescent probe DVX (in 6KVQ)). In most cases, the structural data deposited in the RCSB Protein Data Bank is consistent with data from a DrugBank. In total, our revision identified 15 unique ligands, cocrystallized with FtsZ. On the other hand, the RCSB Protein Data Bank data only partially overlap with the data from ChEMBL. At the same time, the data of ChEMBL have much in common with the data presented in BindingDB. Thus, on the one hand, for a number of compounds, we have irrefutable structural data on complex formation (data from the RCSB Protein Data Bank), on the other hand, we have irrefutable data on biological activity against FtsZ, but the binding site is still unknown.

PDB and ChEMBL related articles were found only for eight substances: native substrate – GTP/GDP (PDB: 3VOB, 5XDT, 5XDU, 6KVQ, 6RVP, 6Y1U, 6Y1U, 6YD5, 6YD6 / CHEMBL384759), as well as for synthetic agents: 4HC (6Y1U, 6Y1V) CHEMBL301141; 9PC (3VOB, 4DXD) / CHEMBL511201; GP2 (7OJZ) /

CHEMBL1164951); MB3 (5XDT, 6RVP, 6YD5, 6YD6) / CHEMBL12543; OLQ (6YD1) / CHEMBL453452; ZI6 (5XDU, 5XDV) / CHEMBL3098779; ZI7 (5XDT) / CHEMBL3909654. Relevant ChEMBL information for PDB structures: 01G (2R75), DVX (6KVQ), G2P (1W58, 1W5F), GCP (7OMJ, 7OMP, 7OMQ), OM8 (6YD5), OMW (6YD6), and ZI1 (6KVP), are currently missing.

The structural alignment of full-atomic FtsZ models built in AlphaFold2 it was performed: A5Z1V5_BACIU (UniProtKB: A5Z1V5) from *Bacillus subtilis* (strain 168), FTSZ_BACSU (UniProtKB: P17865) from *Bacillus subtilis*, FTSZ_ECOLI (UniProtKB: P0A9A6) from *Escherichia coli*, FTSZ_MYCTU (UniProtKB: P9WN95) from *Mycobacterium tuberculosis*, FTSZ_PSEAE (UniProtKB: P47204) from *Pseudomonas aeruginosa*, FTSZ_STAAU (UniProtKB: P0A031) from *Staphylococcus aureus* (Fig.1a). It was namely those recombinant proteins, that were used in ChEMBL biochemical experiments and commercially available for new testing. PyMOL alignment of previously optimized (in Gromacs) 3D-models, confirmed their significant structural similarity. For example, RMSD rate of globular part of the FtsZ was less than 1 and varied within 0.150–0.767, while the alignment of the AlphaFold model of A5Z1V5_BACIU and the 2VAM PDB structure from *B. subtilis* showed RMSD=0.579. This fully confirms the validity of ligand clustering by structural alignment of the polypeptide chains of the molecular target.

Subsequent structural alignment of the complexes deposited in the RCSB Protein Data Bank revealed three key groups of ligands, united by sites: I. Nucleotide Binding Domain (NBD); II. Site of inter-domain cleft (IDC) and III. Site of coumarin bindig (4HC = 4-hydroxycoumarin). (Fig. 2) In addition, NBD include two subsites: Ia. Site of GTP/GDP and Ib. Site of MB3 (MB3 = 1-methylpyrrolidin-2-one). (Fig. 2, Ia and Ib)

The binding specificity of ligands from identified groups was assessed based on facts of their interaction with tubulin and alternative molecular targets. The analysis, was based on data from RCSB Protein Data Bank, ChEMBL, DrugBank, BindingDB, patents and literature. The evaluation was prossesed in accordance with: 1) the fact of interaction with FtsZ (PDB/ ChEMBL); 2) the fact of interaction with α-, β or γ-tubulin; 3.1) the fact of interaction with alternative molecular targets (based on PDB and ChEMBL data), 3.2) alternative interactions based on literature and bioinformatic prediction. The generalized results are presented in the Table (see the Table 1).

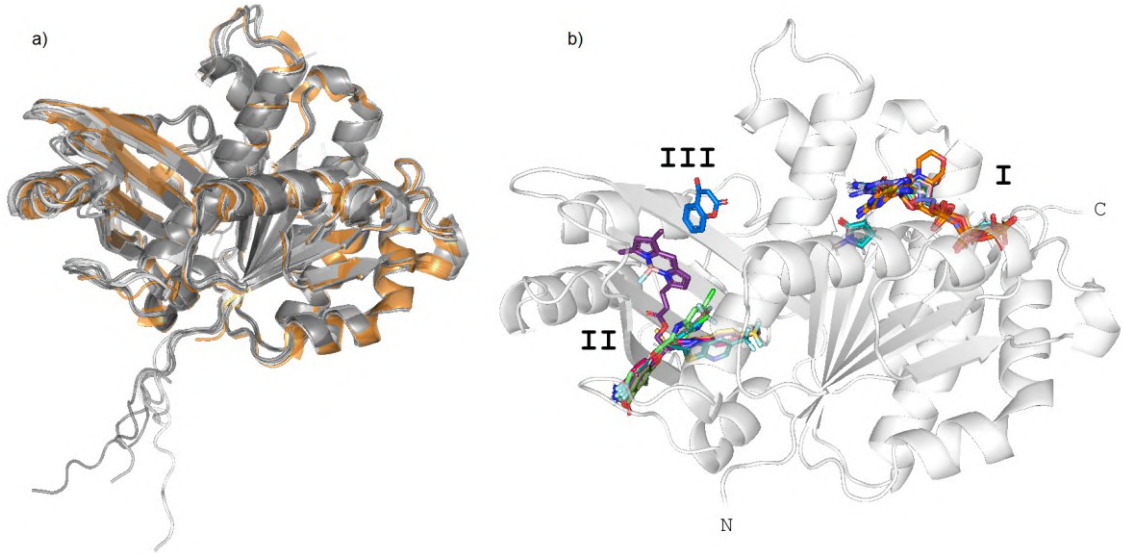

Fig. 1. Identification of the key ligand binding sites with bacterial FtsZ based on structural superimposition of complexes deposited in the RCSB Protein Data Bank: a) Structural superimposition of full-atomic models of FtsZ (marked in gray: *Bacillus subtilis*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) constructed using AlphaFold2 and experimental PDB-structure 2VAM from *B. subtilis* (marked in orange) [18]; b) Clusters of the ligands, co-crystallized with FtsZ deposited in Protein Data Bank represented on the surface of a full-atom model of FtsZ from *B. subtilis*.

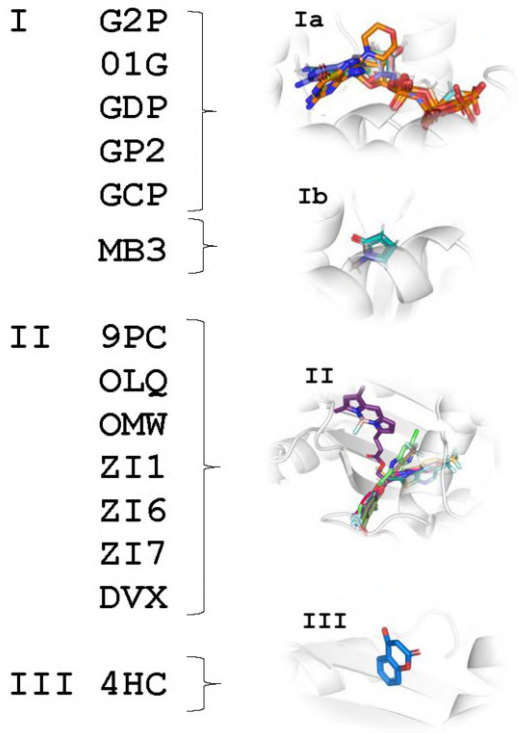

Fig. 2. Three protein-ligand binding sites on the "surface" of bacterial FtsZ, identified by structural alignment of the complexes deposited in the RCSB Protein Data Bank: I) Nucleotide Binding Domain (Ia) Site of GTP/GDP and Ib) MB3 site (MB3 = 1-methylpyrrolidin-2-one)); II) Site of inter-domain cleft (IDC); III) Site of coumarin bindig (4HC = 4-hydroxycoumarin).

Table 1. The rating of FtsZ-ligand interactions, based on databases and literature analysis, in order to identify perspective sites and reference compounds

PDB-Ligand ID	**FtsZ** (PDB/ ChEMBL)	**Tubulin (PDB/ ChEMBL)**	**Alternative targets**	
			PDB/ChEMBL	**etc.***
Site Ia				
G2P	+	+	+/+	+
01G	+	-	+/-	+
GDP	+	+	+/+	+
GP2	+	+	+/+	+
GCP	+	+	+/+	+
Site Ib				
MB3	+	-	+/+	+
Site II				
9PC	+	-	-/-	+
OLQ	+	-	-/-	+
OMW	+	-	-/-	-
ZI1	+	-	-/-	-
ZI6	+	-	-/-	+
ZI7	+	-	-/-	+
DVX	+	-	-/-	-
Site III				
4HC	+	-	+/+	+

In general, our data indicate that compounds bound in the site of inter-domain cleft (IDC site / Site 2) demonstrate the highest specificity. In opposed to ligands interacting with sites I (a/b) and III, there are no experimentally confirmed facts of interaction with tubulin or any other molecular

target for any PDB-deposed effectors assosiated with site II. This site is considered by us as a priority one, and the fact that all ligands of this group belong to benzamides allows us to specify the regeon of inter-domain cleft as the priority binding site for compounds of this chemical group: 93C (3-[(6-chloranyl-[1,3]thiazolo[5,4-b]pyridin-2-yl)methoxy]-2,6-bis(fluoranyl)benzamide); OLQ (2,6-difluoro-3-methoxybenzamide); OMW (2,6-bis(fluoranyl)-3-[[3-(trifluoromethyl)phenyl]methoxy]benzamide); ZI1 (3-[(1R)-1-[5-bromanyl-4-[4-(trifluoromethyl)phenyl]-1,3-oxazol-2-yl]ethoxy]-2,6-bis(fluoranyl)benzamide); ZI6 (3-[[5-bromanyl-4-[4-(trifluoromethyl)phenyl]-1,3-oxazol-2-yl]methoxy]-2,6-bis(fluoranyl)benzamide); ZI7 (2,6-bis(fluoranyl)-3-[[6-(trifluoromethyl)-[1,3]thiazolo[5,4-b]pyridin-2-yl]methoxy] benzamid); DVX ([(2R)-2-[3-aminocarbonyl-2,4-bis(fluoranyl)phenoxy]-2-[5-bromanyl-4-[4-(trifluoromethyl)phenyl]-1,3-oxazol-2-yl]ethyl] 3-[2,2-bis(fluoranyl)-10,12-dimethyl-3-aza-1-azonia-2-boranuidatricyclo[7.3.0.0^{3,7}]dodeca-1(12),4,6,8,10-pentaen-4-yl]propanoate). As it was noted earlier, at 1st quarter of 2023, in ChEMBL database deposited 417 compounds interacting with FtsZ. Based on the fact that in the case of the closest homologues of FtsZ – eukaryotic tubulins, we know a greater number of sites of protein-ligand interactions, we fully confident that for FtsZ a number of protein-ligand sites remains unknown. We plan to screen compounds deposited in ChEMBL against the described sites and select the ligands with low-affinity for the sites I, II and III. These compounds will be considered as FtsZ effectors with alternative mechanisms of protein-ligand binding and will be the subject of a special study.

Conclusions

It was identified 3 main regions of protein-ligand binding in FtsZ: I. Nucleotide Binding Domain (Ia. Site of GTP/GDP and Ib. MB3 site); II. Site of inter-domain cleft (IDC) and III. Site of coumarin bindig (4HC = 4-hydroxycoumarin). It was indicated that benzamide-binding site, located in the region of inter-domain cleft, demonstrate highest site- and target-specificity.

The research was performed as the part of the basic research work of the Institute of Food Biotechnology and Genomics of Natl. Acad. sci. of Ukraine: # 0120U100937 – Bioinformatic and molecular-cell studies of the structure and functions of the cytoskeleton of plants.

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СТРУКТУРНИЙ ПРОФІЛЬ ЛІГАНД-ЗАЛЕЖНОГО ІНГІБУВАННЯ БАКТЕРІАЛЬНИХ FtsZ-БІЛКІВ

Мета. Метою дослідження були пошук та встановлення зв’язку між структурно-хімічними характеристиками експериментально доведених ефекторів FtsZ-білків та сайтами їх взаємодії шляхом узагальнення існуючої інформації з банку даних RCSB Protein Data Bank і ChEMBL та їх аналізу із використанням методів *in silico*. ***Методи.*** Аналіз літературних джерел та баз даних. Порівняльний аналіз структур білків, лігандів та їх комплексів. ***Результати.*** Здійснено пошук та аналіз експериментально підтверджених фактів білок-лігандної взаємодії з бактеріальним FtsZ. За результатами аналізу просторових структур експериментальних моделей PDB і повноатомних моделей AlphaFold2 бактеріальних FtsZ доведено їх значну структурну подібність. Визначено три сайти виникнення білок-лігандної взаємодії бактеріальних FtsZ з їх ефекторами. ***Висновки.*** Ідентифіковані 3 основні ділянки зв’язування білок-ліганд у FtsZ: I. Домен зв’язування нуклеотидів (Ia. Сайт GTP/GDP і Ib. сайт MB3); II. Щілина між доменами (IDC), III. Місце зв’язування кумарину (4HC = 4-гідроксикумарин). Також показано, що сайт зв’язування бензаміду, який розташований в області міждоменної щілини, має найвищу сайт- та мішень-специфічність.

Ключові слова: FtsZ, Z-кільце, бактерії, інгібітори, ліганд, сайт зв’язування.