Molecular Graphics Approach to Bacterial AcrB Protein – β-Lactam Antibiotic Molecular Recognition in Drug Efflux Mechanism

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Abstract

Quantum-chemical calculations on 16 β-lactams and four more substrates (dequalinium DEQ, ciprofloxacin CPF, ethidium ET and rhodamine 6G or RHG) of the AcrB component in the AcrAB-TolC bacterial efflux pump have been performed. Molecular graphics methods were used on the drugs molecular structures, available crystal structures of AcrB protein and its complexes with the four drugs. Electronic features (dipole moment components and polarizabilities) as well as various steric properties related to substrate principal axes and molecular boxes showed to be related with stereoelectronic properties of possible receptors and recognition sites of AcrB (vestibules, the pore, central cavity) as well as of the outer leaflet of the inner membrane. Similarities between the four drugs and β-lactams enabled, when combined with the experimental AcrB - drug geometry, prediction of β-lactam positions in the AcrB central cavity. These predictions are in reasonable accordance with literature and reveal preferred drug orientation during the efflux process.

About this work

AcrAB-TolC is one of the most important bacterial multidrug efflux pumps. Six crystal structures containing this pump (from *E. coli*), among which four structures have ligands complexed to the pump (DEQ, CPF, ET, RHQ) exist today. These four complexes, together with previously published QSAR results for 16 β-lactams (see the related poster), and analogies between the ligands and the β-lactams are used in this work to elucidate probable AcrB - β-lactam stereoelectronic relationships at qualitatite and quantitative level. As the final conclusion, a more general and complete β-lactam efflux mechanism is proposed. The results and conclusions are supported by figures and corresponding figure captions, which are numbered in a logical sequence. *n*-Hexane, erythromycin and rifampicin are also studied in this work.

Further information:

1) Ferreira M. M. C., Kiralj R., "*QSAR study of β-lactam antibiotic efflux by the bacterial multidrug resistance pump AcrB*", *J. Chemometr.*, 18 (2004) 242-252. The publication is available online at: http://pcserver.iqm.unicamp.br/~marcia/Pub71.pdf

2) R. Kiralj, M. M. C. Ferreira, *J. Chemometr.* and *J. Mol. Graph. Mod.*, submitted.

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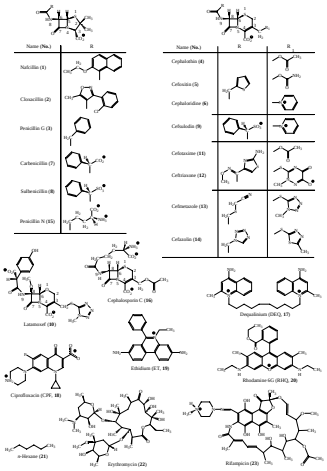
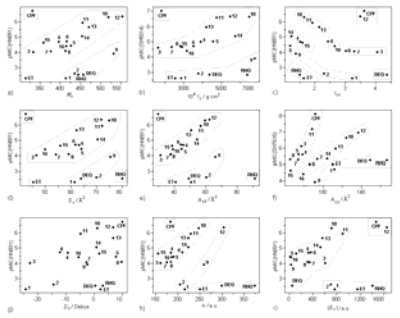
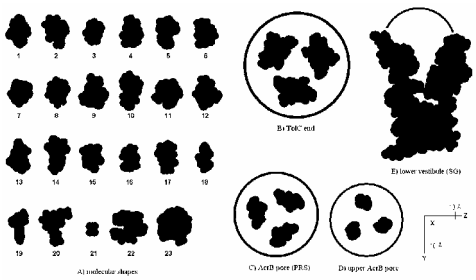
FAPESP



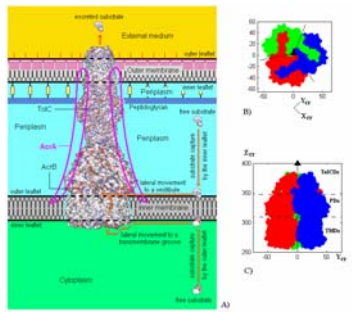
1. Chemical structures of studied AcrAB-TolC substrates: β-lactam antibiotics (1-16) with atomic numbering, dequalinium (17), ciprofloxacin (18), ethidium (19), rhodamine 6G (20), *n*-hexane (21), erythromycin (22) and rifampicin (23).



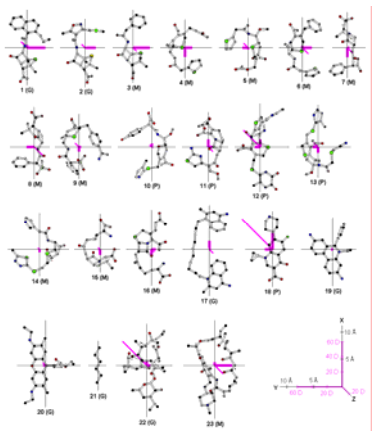
2. Representative pMIC - molecular descriptor plots important for elucidation of the efflux mechanism. M_r - relative molecular mass; I_x - 1st moment of inertia; I_{yx} - ratio of 2nd and 1st moments of inertia; S_x, A_{yz}, A_{xy} - steric descriptors defining the size of molecular box; D_y - Y-component of dipole moment; α – polarizability; $|\beta_x|$. – absolute X-component of hyperpolarizability β. The relationships are linear or nonlinear, and the samples frequently form clusters with similar behavior. β-Lactams and the other compounds show similar trends in most cases.



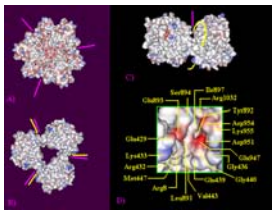
3. A) Shapes of 1-23 viewed along the principal axis X. The shapes of pump channels and grooves: B) the TolC end; C) the pore entrance; D) the upper pore channel; E) the south groove of a vestibule. Molecules that have compatible shapes with the pump profiles may be good efflux substrates.



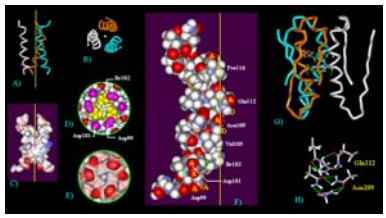
4. A): Combined 3D - schematic representation of AcrAB-TolC pump in *E. coli*. Two main drug efflux mechanism are shown, one starting in the periplasm, and other in the cytoplasm. B) and C): Blue, red and green AcrB protomers around the symmetry axis.



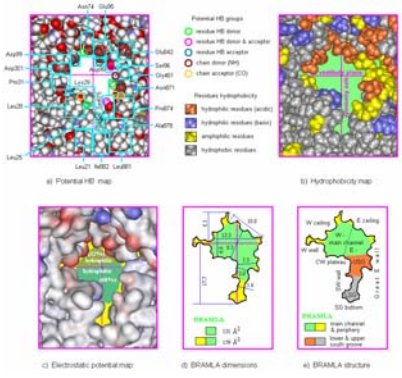
5. Molecules 1-23 with principal axes and dipole moment components. Molecules are marked as good (G), moderately good (G) and poor (P) substrates of the AcrAB-TolC efflux pump. Visual relationships between the dipole moment components and biological activity is supported quantitatively also (correlations).



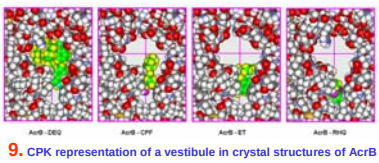
6. Electrostatic potential representation: A) of the pore domains (PDs), and B) the transmembrane domains (TMDs) viewed upwards and downwards the vestibules plane, respectively. C) The transmembrane groove (TMG) circular shape and position at the TMDs surface. The position of the vestibules (pink line) and the TMGs (yellow circle). D) The TMG pore at its entrance, with essential residues.



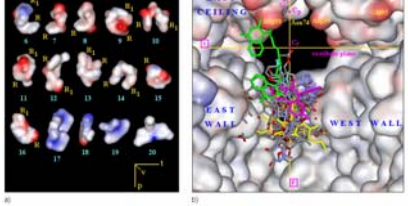
7. The structure of the pore channel viewed perpendicularly to (A) and along with (B) the symmetry axis. C) The pore channel interior excluding a pore helix. The structure of the pore recognition site represented by CPK model (D) and electrostatic potential (E). F) A pore helix from a protomer represented by CPK model and hydrogen bond donor (D's) and acceptor (A's) groups from residues (yellow) and the polypeptide chain (green). G) Three RND conserved motifs called A including the pore with hydrogen bonding residues that keep the pore to be tight. H) These residues with their hydrogen bond network.



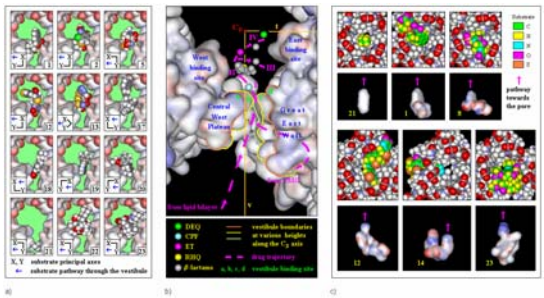
8. Vestibule and its 2D representation BRAMLA (BRAzil Map-Like Area). a) Potential hydrogen bonding groups from amino-acid residues and polypeptide chains. b) The hydrophobic character of the essential residues. c) The electrostatic potential map. d, e) BRAMLA area characterized by its dimensions (d) and distinct regions of both its interior and exterior (e).



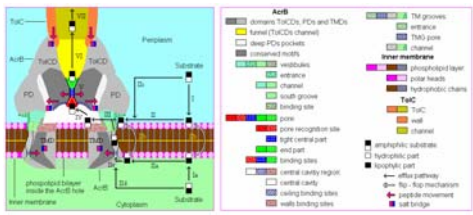
9. CPK representation of a vestibule in crystal structures of AcrB complexed by substrates DEQ, CPF, ET and RHQ. The substrates are colored differently from the vestibule. It is obvious that substrate structure affects its binding mode.



10. a) Electrostatic potential of 1-20 in the central cavity. Most of drugs expose positive, amphiphilic or hydrophobic heads (R or R_1) toward predominantly negatively charged ceiling and the pore. b) 16 β-Lactams in predicted positions/orientations in the central cavity, with experimental location of DEQ (green), CPF (light blue), ET (pink) and RHQ (yellow). Important charged residues are labeled at the pore region. The drug position parameters were predicted from their molecular properties and crystal structures of the four complexes.



11. a) Representative drug molecules at the vestibule entrance. The orientation of substrates on their way towards the vestibule channel (see the orientation of the XY axes) is qualitative. b) Probable drug pathway through the vestibule and binding to the central cavity/vestibule binding sites. Electrostatic potential of the TMDs as viewed downwards the symmetry axis. Both experimental and predicted positions of center of mass reveal four possible pathways in the binding area: I - binding to the vestibule binding sites a-d (E and SW walls at the vestibule end); II - drug binding to the W binding site in the central hole; III - drug positioning in the middle of the binding area including interactions with two or three binding sites; IV - binding to the E binding site. It is supposed that a drug molecule moves along these trajectories until reaching its best binding position. c) Selected pore tripeptide - drug complexes. The substrates are colored differently to be distinguished from the pore residues. Free and bound substrates are oriented in such a way that the most hydrophobic fragment (R in β-lactams) goes first into the PRS pocket.



12. The AcrB mediated drug efflux from a Gram-negative bacteria cell. The efflux component AcrA is excluded because of the clarity. AcrB, TolC and the inner membrane components are colored differently. AcrB is viewed from the direction of the removed protomer, showing thus two symmetrically related protomers. The efflux process can be divided into steps I - VII. Hydrophilic/lipophilic orientation of an amphiphilic drug molecule with respect to the macromolecular systems (the pump, the inner membrane) is also visible. Conserved motifs, salt bridges and principal peptide movements are marked with appropriate symbols. This efflux mechanism is more detailed and general than that one from Figure 4 (which was based on literature). The mechanism consists of a series of allosteric effects initiated by proton influx, braking of salt bridges and hydrogen bonds, changing of pump conformation and opening its efflux channel components, binding drugs in the central hole and extruding them through the pore and TolC. Drug molecular properties significantly affect the efflux and interaction with inner membrane.