Designing DapE- Inhibitors through Molecular Dynamic Simulations

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Abstract

Development of new classifications of inhibitors are essential in order to control infectious diseases. Currently the only antibiotics available follow two approaches: inhibition of cell wall remodeling and protein synthesis. DapE is part of the succinylase biosynthetic pathway and is important to the production of lysine and mDap (meso-diaminopimelate) both of which synthesize protein and bacterial peptidoglycan cell wall remodeling. Due to increasing bacterial resistance, a new method of treatment is critical through inhibition of the enzymatic activity of DapE. There is no evidence of DapE in mammals, therefore, inhibition should be selective in thwarting bacterial growth. High-Throughput Screening (HiTS) was utilized to screen over 33,000 different compounds which identified several inhibitors of DapE. Computational chemistry uses the x-ray determined structure of DapE to perform molecular docking. The application of this process could lead to the discovery of other possible inhibitors as new antibiotics.

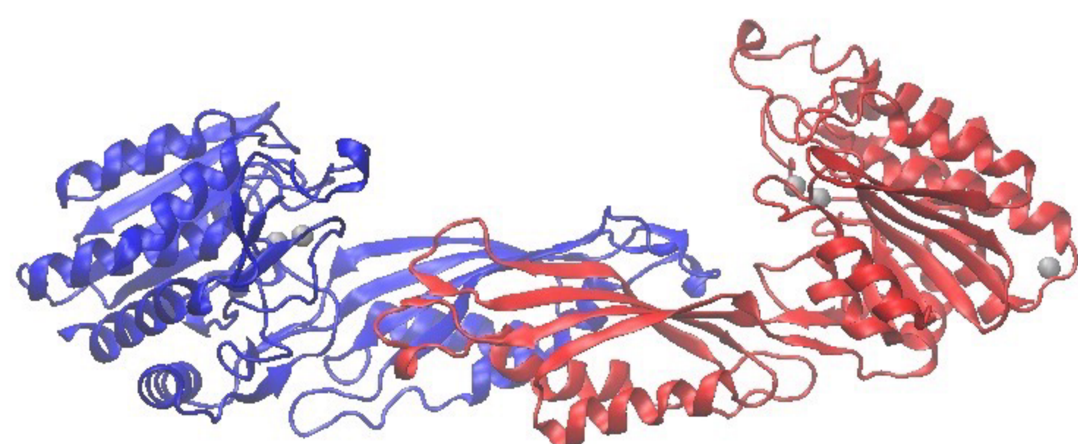

Figure 1. DapE is in red and blue; Zn ions in silver.

Background

A plausible bacterial target that is both present in Gram-negative and some Gram-positive bacteria is the DapE-encoded N-succinyl-L, L-diaminopimelic acid desuccinylase which is apart of the lysine biosynthetic pathway. This pathway contains several different enzymes that could provide probable drug targets. Lysine and meso-diaminopimelate (mDAP) are products from this pathway that are needed to produce protein and peptidoglycan cell wall synthesis. Lysine cannot be synthesized in humans, so it is an essential amino acid meaning that it must be consumed. The deletion of the DapE gene in mDAP/lysine biosynthetic pathway is lethal because it is crucial for cell growth and proliferation. There are no biosynthetic pathways involving DapE in mammals, so inhibitors that target DapE exhibit selective toxicity against bacteria and have little effect on humans. This makes DapE a promising target of antibiotics and effective method in treating bacterial infections.

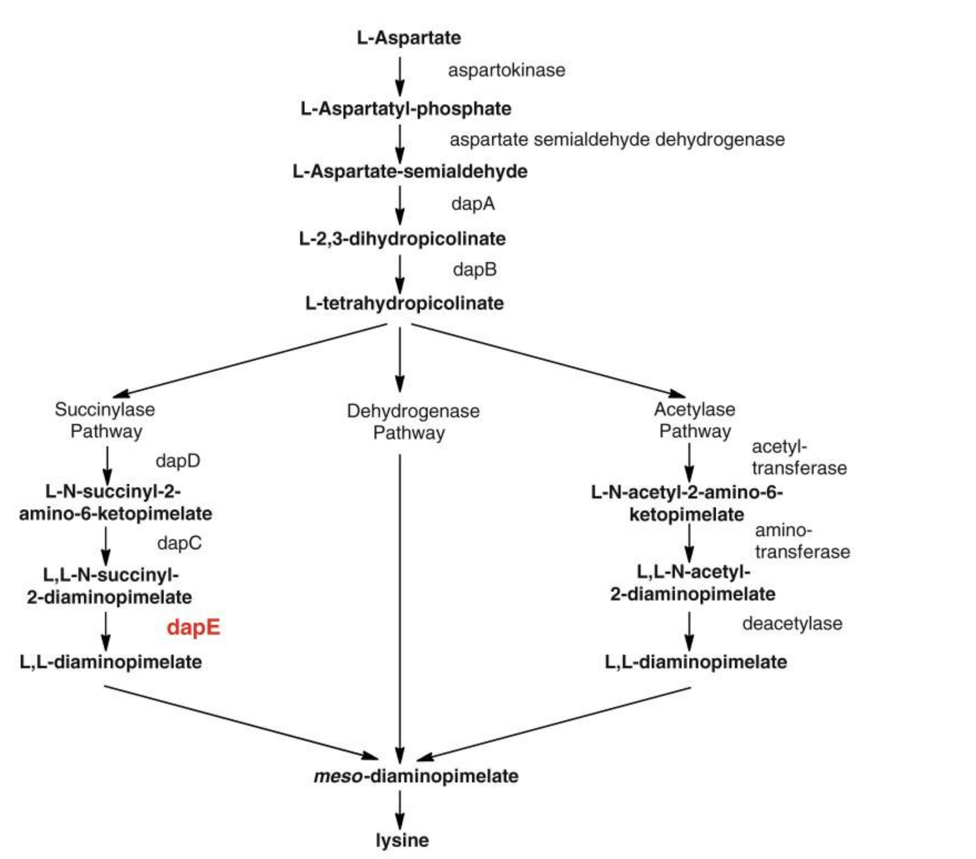

Figure 2 – Lysine Biosynthesis Pathways: Bacteria use the pathway on the left but mammals do not.

Molecular Dynamics (MD)

The focus of this project is to analyze and design particular molecules that could bind to DapE using molecular dynamic (MD) simulations. These simulations help us visualize the different sites of the DapE molecule and also helps us choose potential inhibitors that could bind to the binding sites. Molecular Dynamics (MD) can be a very useful tool in understanding interactions and calculating the energies of interactions. It is also useful because it calculates the electrostatic, Van der Waals and total forces of these interactions. These processes are run on mini-supercomputers in the computational lab and simulates how the ligand interacts with the binding site of DapE to minimize the binding energy.

Sulfate Ions Bound to DapE

A survey of DapE X-ray structures displayed in the table below shows that DapE often binds sulfate ions. These ions are bound in positions that correspond to the binding sites for the products of the reaction as seen in Figure 3.

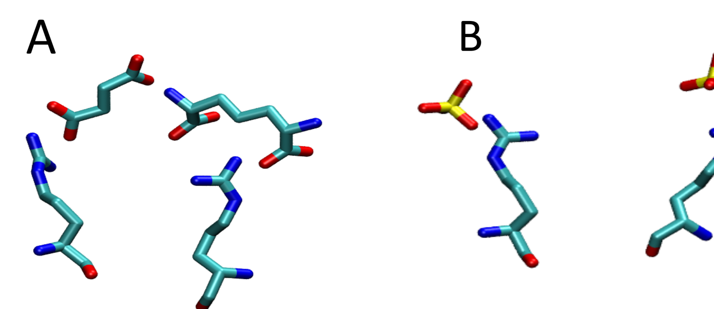

Figure 3 – A. Products of DapE interacting with 2 Arg residues in structure 5VO3. B. Sulfate ions bound to the same Arg residues in structure 5UEJ.

PDB ID	Bacterial Species	Crystallization Conditions	Number of Zinc's in active sites	Number of Sulfates in Active Site	Distance from Sulfates to Closest Zinc/ Relevant Residues (Å)
1VGY	Neisseria Meningitidis	Not listed in the article or the PDB file.	Zero	Zero	N/A
3IC1	Haemophilus Influenzae	1 M ammonium sulfate, 0.2 M NaCl, and 0.1 M sodium acetate, pH 4.4	Two	One in Chain A, Three in Chain B	Chain A: 3.1 to Arg179, about 3 to Arg258, about 5.2 to Arg178. 14 to Zn Chain B: Sulfate 1 is 3 to Zn, but no other residues within 7 Sulfate 2 is 13 to Zn, 2.5 to Arg178 and 4.5 to Arg179 Sulfate 3: 9.2 to Zn and 3 to both Arg329 and Arg258
3ISZ	Haemophilus Influenzae	Same as 3IC1	One	One in Chain A, One in Chain B	Chain A: 2.7 to Arg178, 2.9 to Arg179, 6.5 to both Arg258 and Arg329. 13 to Zn Chain B: 2.9 to Arg178, 4.5 to Arg329, 3.7 to Arg258 and 2.8 to Arg179. 13.5 to Zn
4O23	Neisseria Meningitidis	[20% (w/v) PEG 3350 and 100 mM HEPES (pH 7.5)] The PDB file also lists 0.1 M succinic acid	One in Chain A, Two in Chain B	One in Chain A, One in Chain B	Chain A: 3.25 to Arg259, 3.8 to His195, 11.5 to Zn Chain B: 2.9 to Arg259, 2.6 to His195, 7.8 to Zn
4PPZ	Neisseria Meningitidis	[15% (w/v) PEG 3350 and 100 mM succinic acid (pH 7.0)] The PDB file also lists 0.2M Li_2SO_4 and 0.1M HEPES	Two in Chain A	One in Chain A	Chain A: 2.8 to Arg259, 8.8 to Zn
4PQA	Neisseria Meningitidis	0.2 M ammonium acetate, 0.1 M TRIS (pH 8.5), and 25% (w/v) polyethylene glycol 3350]	Two in Chain A	One in Chain A, Also Captopril Is bound	Chain A: 2.9 to Arg259, 8.65 to Zn
4ONW	Vibrio Cholerea	(20% (v/v) 1,4-butanediol, 0.1 M sodium acetate pH 4.5)	Zero	Zero	Only the Catalytic Domain is crystallized in this structure
4OP4	Vibrio Cholerea	(20% (v/v) 1,4-butanediol, 0.1 M sodium acetate pH 4.5)	Two in Chain A, Two in Chain B	Zero	Only the Catalytic Domain is crystallized in this structure
4H2K	Haemophilus Influenzae	(0.2 M ammonium acetate, 0.1 M BIS-TRIS pH 5.5, 25% (w/v) polyethylene glycol 3350)	Two in Chain A, Two in Chain B	Zero	Only the Catalytic Domain is crystallized in this structure
5UEJ	Neisseria Meningitidis	From PDB File: 0.2M Li_2SO_4, 0.1M Tris:HCl, 1.26M $(NH_4)_2SO_4$, 0.05M DMSO, PH 8.5	Two in Chain A, Two in Chain B	Two in Chain A, Two in Chain B	Chain A: Sulfate 1 is 3.5 to Arg179, 8.4 to Zn Sulfate 2 is 4 to Arg259, 12.65 to Zn Chain B: Sulfate 1 is 3 to Arg179, 9 to Zn Sulfate 2 is 3.
5VO3	Haemophilus Influenzae	0.05 M HEPES (pH 7.3), 10.7% (w/v) PEG MME 2000, and 8.6% (w/v) PEG 2000 PDB File Lists 0.15M HEPES and 0.06M Sodium Potassium Tartrate	Two in Chain A, Two in Chain B	None	This was a products-bound closed structure

Results

The initial inhibitor is a bis-sulfonate with eight methylene groups separating the sulfonates (Figure 4), which were placed in the positions of the sulfate groups in Figure 3B. An adjustment of the number of methylene groups can increase the binding affinity, however eight methylene groups was chosen as the baseline. A replacement of the sulfonates with neutral hydrogen-bonding groups will take place to allow it to pass the membrane. Also a replacement of some of the methylene groups with hydrogen bonding groups will increase the affinity for the substrate binding site. Using the sulfate binding sites seen in the X-ray structure of DapE as guides to where the sulfonates would bind, we have completed our initial simulation. The results show that the compound with 8 methylene groups is not long enough for the most effective binding.

Results

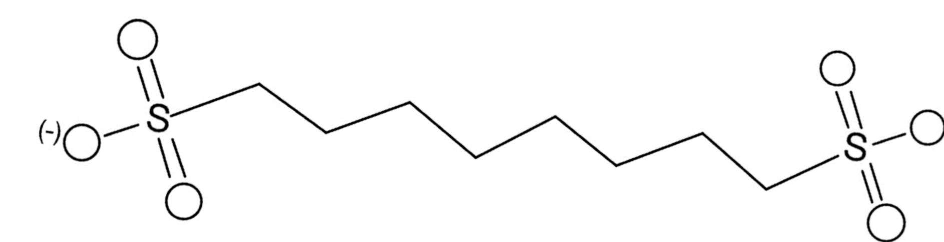

Figure 4. Shown above is initial inhibitor tested on the enzyme DapE called octane-1,8-disulfonate.

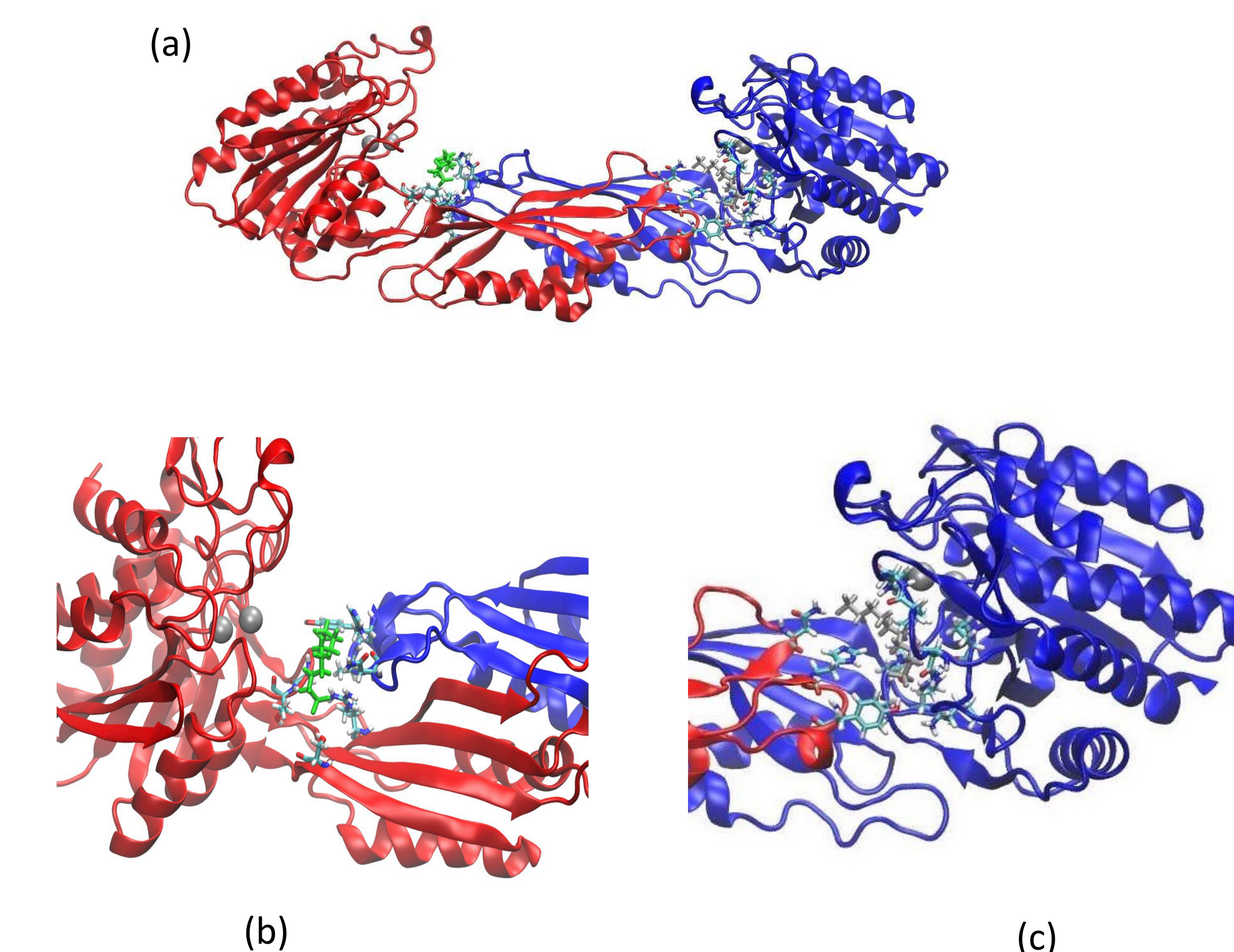

Figure 5. (a) Dimer of DapE binding initial inhibitor to both chains. (b) Closeup of subunit A binding site with inhibitor bound. (c) Closeup of subunit B binding site with inhibitor bound.
DapE is in red and blue; Zn ions in silver; inhibitor in green or silver; amino acid residues interacting with the inhibitor in atomic colors

The interaction energies shown in the table indicate that the best binding of a bis-sulfonate would occur when the chain had 16 methylene groups. This somewhat surprising result indicates that it needs flexibility to link the two sulfate binding sites and that the methylene chain also contributes to the binding.

Interactions Energies

# of CH_2	Elec	VDW	Total
4	-288	-16	-294
6	-207	-12	-219
10	-195	-35	-230
12	-209	-36	-245
14	-254	-35	-289
16	-315	-42	-357
18	-230	-33	-263

*Energies are in kcal/mpe.

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Acknowledgements

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