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Exploring the binding mechanisms of diaminopimelic acid analogs to *meso*-diaminopimelate dehydrogenase by molecular modeling

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ABSTRACT

Meso-Diaminopimelic acid (*meso*-2,6-diamino-heptanedioic acid, DAP) is an important component of the cell wall of many bacteria. *Meso*-diaminopimelate dehydrogenase (m-Ddh) is a critical enzyme in the process of converting tetrahydrodipicolinate to DAP. Here, we are proposing that DAP analogs targeting m-Ddh may be considered as potential antibiotics. Four DAP analogs without significant structural change from DAP have been obtained and their inhibitory potencies against m-Ddh from the *P. gingivalis* strain W83 show significant differences from that of DAP. However, their inhibitory mechanisms as for how simple structural change influences the inhibitory potency remain unknown. Therefore, we employed molecular modeling methods to obtain insight into the inhibitory mechanisms of DAP and analogs with m-Ddh. The predicted binding mode of DAP was highly consistent with the experimental structural data and disclosed the important roles played by the binding pocket residues. According to our predictions, the isoxazoline ring of compounds **1** and **2** and the double bonds in compounds **3** and **4** had distinct influences on these compounds' binding to m-Ddh. This enriched understanding of the inhibitory mechanisms of DAP and these four analogs to m-Ddh has provided new and relevant information for future rational development of potent inhibitors targeting m-Ddh.

1. Introduction

Since the 1940s, antibiotics have contributed greatly to a significant reduction of illness and death from infectious diseases. However, with the widespread use of antibiotic therapy, bacterial resistance to antibiotics is now commonly occurring. According to the Centers for Disease Control and Prevention, at least 2 million people are infected with bacteria each year in the United States, among which 23,000 people die because of antibiotic resistance [1]. The increase in bacterial resistance to current antibiotics and the mounting incidence of bacterial infection have resulted in researchers paying more attention to identifying novel druggable targets from which to develop more effective antibiotics without resistance potential [2,3].

Meso-Diaminopimelic acid (*meso*-2,6-diamino-heptanedioic acid, DAP, Fig. 1) plays an important role in the peptidoglycan layer of the cell wall of many bacteria [4,5]. It is the key immediate biosynthetic precursor of L-lysine and can be synthesized within the lysine biosynthetic pathway. Notably, mammals lack the diaminopimelic acid biosynthetic pathway. Hence adopting the enzymes in this pathway as a druggable target to develop potent and specific inhibitors should not produce drugs inherently toxic to humans. As described in previous studies, DAP can be generated from two molecules: L-tetrahydrodipicolinate and L, L-DAP [6,7] (Fig. 1). The synthesis of DAP from L-tetrahydrodipicolinate (THDPA) utilizes *meso*-diaminopimelate dehydrogenase (m-Ddh) to catalyze the conversion of THPDA to m-DAP. While the formation of DAP from L, L-DAP is catalyzed by DAP epimerase [8,9]. Thus, m-Ddh and DAP epimerase are considered to be promising targets for the development of novel antimicrobial agents. Additionally, since DAP is the substrate of m-Ddh and DAP epimerase [10,11] and the binding sites of DAP in m-Ddh and DAP epimerase are the same as that of other m-Ddh and DAP epimerase inhibitors [8], we believe that design and synthesis of DAP analogues targeting m-Ddh and/or DAP epimerase is a promising strategy for antibacterial drug discovery.

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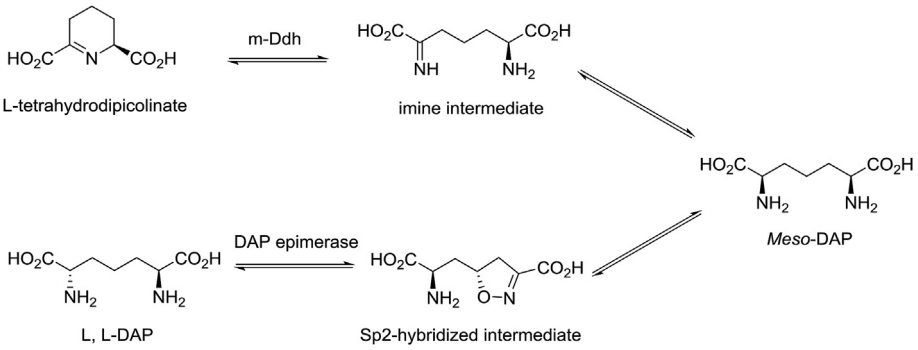

Fig. 1. Two biosynthetic routes to generate DAP.

Currently, a number of compounds have been designed and synthesized based on the skeleton of DAP, and their inhibitory mechanisms towards m-Ddh and/or DAP epimerase also have been explored [9–11]. For example, the fluorinated analogs of DAP were synthesized for the inhibition of DAP epimerase. From the interactions between DAP epimerase and the fluorinated compounds, Michael et al. discovered that the conformational orientations of the fluorinated compounds interacted with DAP epimerase have significant influences on their inhibitory potencies [9]. In the study by Russell et al. [11], the L-glutamic acid γ-hydrazide and its methylated derivative were chosen from eight DAP analogs as potential m-Ddh and DAP epimerase inhibitors owing to the two compounds had a planar amide nitrogen at the position corresponding to the α-carbon of DAP which made them be potential transition state analogs for m-Ddh and DAP epimerase. Among the reported inhibitors, it is worth to pay special attention to the inhibitor that mimics the sp^2-hybridized intermediate involved in the process of L, L-DAP changing to DAP and the imine intermediate involved in the process of L-tetrahydrodipicolinate converting to DAP [8,12].

In the studies by Scapin and Caplan, they described that antibiotics that mimic the sp^2-hybridized and imine intermediates would be expected to have inhibitory potency against m-Ddh [8,12]. As shown in Fig. 2, compounds **1** and **2** are two diastereomers carrying the DAP backbone and possessing a conformationally restricted isoxazoline ring. Compound **1** showed pH-dependent inhibition against m-Ddh (14.9 μM), which was 10 fold weaker than that of DAP, while on the other hand, compound **2** showed no observable activity (>1000 μM) [13,14]. Compounds **3** and **4** are two unsaturated compounds, which mimic the imine intermediate but lack one of the characteristic amino groups of DAP. Both of these compounds showed interesting inhibitory potencies: the IC_{50} values of compounds **3** and **4** against m-Ddh were 226 and 34 μM, respectively [12].

Based on the above discussion, it is clear that these insignificant and relatively simple structural changes did lead to significantly different inhibitory potencies. To the best of our knowledge, detailed inhibitory mechanisms of DAP and compounds **1**-**4** to m-Ddh have not yet been extensively studied. While we have defined compound **1** as the most promising compound for further our structure-activity relationship characterization and lead optimization [13], it is imperative to understand its mechanism action at an atomic level. Thus, we applied an extensive suite of computational analyses - molecular docking, molecular dynamics (MD) simulations, molecular mechanics/Poisson-Boltzmann surface area (MM/PBSA) free energy calculations [16–26], and molecular mechanics/generalized Born surface area (MM/GBSA) free energy decomposition analysis [27–30] – to explore the binding modes of DAP and these four most closely related analogs with m-Ddh and further disclose the inhibitory mechanisms as how simple structural change influences the inhibitory potency of DAP and compounds **1**-**4**. We believe that these results will help us understand the properties of the m-Ddh binding site, and further provide valuable information to the design of more potent antibiotics based on our defined lead.

2. Materials and methods

2.1. Preparing the structure of complexes

The crystal structures of m-Ddh from *P. gingivalis* strain W83 in complex with the ligand EPE (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (PDB ID: 3BIO) and m-Ddh from *C. glutamicum* in complex with the ligand DAP (PDB ID: 2DAP) were downloaded from the Protein Data Bank at http://www.rcsb.org [8,31], and used in the present work. To build the initial structure of m-Ddh from *P. gingivalis* strain W83 in complex with DAP (m-Ddh/DAP), we used Sybyl-X 2.0 with the following two steps: 1) aligned the structures of 3BIO and 2DAP; 2) merged the DAP ligand into the binding site of 3BIO. After that, hydrogen atoms were added to the 3BIO model (now including DAP) and the model was optimized with 10,000 energy minimization iterations while holding all heavy atoms as a fixed aggregate Gasteiger-Hückel charges were assigned under the Tripos forcefield (TFF). Compounds **1**-**4** were sketched in Sybyl-X 2.0, assigned Gasteiger-Hückel charges and energy minimized to a gradient of 0.05. Then, each of the five ligands was immersed in a cubic box of water molecules and these solvated systems were energy minimized (20,000 iterations), again with the Tripos forcefield.

2.2. Docking studies

The initial structures of m-Ddh binding with compounds **1**-**4** (m-Ddh/compound **1**, m-Ddh/compound **2**, m-Ddh/compound **3**, and m-Ddh/compound **4**) were obtained through molecular docking studies by GOLD 5.4 (an automated genetic algorithm based docking program) with standard default settings [32]. The binding site was defined to include all atoms within 10 Å of DAP in the m-Ddh/DAP complex. After taking into consideration of the GOLD fitness scores and the binding orientation of each ligand within the binding pocket, the highest scored solutions were chosen for further studies.

2.3. MD simulations

After obtaining the initial five structures of m-Ddh/ligand complexes, the missing atoms of m-Ddh were added using the *tleap* program in AMBER15.0[33] The geometries of the ligands were