Chasing a Substrate Complex of NIS Synthetase DesD

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Introduction

Streptomyces coelicolor is a soil bacterium that produces two NIS siderophores, Desferroxiamine B and E, through a process called the NIS pathway[1]. The key enzyme in this process, Desferroxiamine D (DesD), belongs to a new group of enzymes called NIS Synthetases and is used as a standard model for studying them. Although *Streptomyces coelicolor* is not pathogenic to humans, DesD is a good target for structure-based drug design because it is like enzymes found in dangerous bacteria like MRSA and anthrax[2]. NIS synthetases are potential drug targets due to their unique structure, chemistry, and their role in virulence in bacteria[3].

The biochemical characteristics of DesD and its structure are limited to ATP[4]. However, an acyl-adenylate analog effectively binds to the active site of DesD, suggesting its potential as a structural basis for developing inhibitors of this iterative siderophore synthetase[2]. To fully understand the structural interactions or the binding sites, we need the structural complex to fully define the inhibitor space we need to fill. The proposed two-step hypothesis is shown below in **Figure 1** for a single turnover with the presence of the adenylate intermediate.

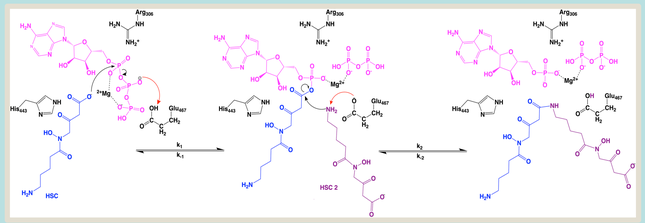

Figure 1: Proposed Mechanism for DesD. In the first step, a covalent intermediate (shown in magenta at the center) is formed from ATP (magenta, left) and HSC (blue, left), along with the generation of pyrophosphate (black, center). During the second step, the adenylate intermediate is resolved, resulting in the formation of a peptide bond between the HSC molecules (blue, right).

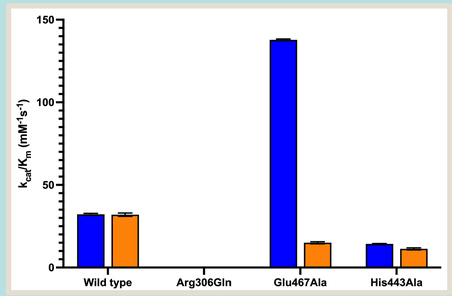

Figure 2: Catalytic Efficiency Comparison of Wild Type and Protein Variants. This bar graph shows the catalytic efficiency (k_{cat}/K_m) for the wild type and three variants: Arg306Gln, Glu467Ala, and His443Ala.

Glu467Ala exhibits a much higher catalytic efficiency, peaking around 130 $mM^{-1}s^{-1}$, while the wild type and other variants remain below 50 $mM^{-1}s^{-1}$.

Results

We used search models, AlphaFold predictions, and GOLDD to refine partial structures and optimize crystallography along with CCP4. Although we didn't obtain complete structures, the process revealed some interesting findings. As shown in **Figure 2**, our kinetic data demonstrated unexpectedly high turnover rates for the Glu467Ala variant, especially under ATP-limited conditions.

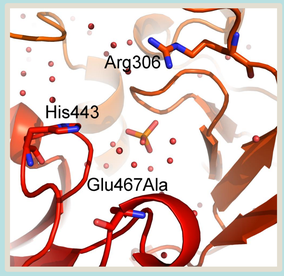

Figure 3: Absence of ATP and Intermediates in the Active Site of Glu467Ala Variant. The crystal structure highlights the active site of the Glu467Ala variant, showing no ATP or adenylate intermediates bound.

Further analysis of the crystal structure in **Figure 3** showed no evidence of ATP or any nucleotide intermediate trapped in the active site. This was an unexpected result, as previous models predicted the presence of ATP or AMP in the binding pocket[1]. The absence of these intermediates, combined with the high turnover observed in kinetic assays, supports the hypothesis that ATP autolysis might occur under the conditions tested, leading to the release of inorganic phosphate without intermediate formation.

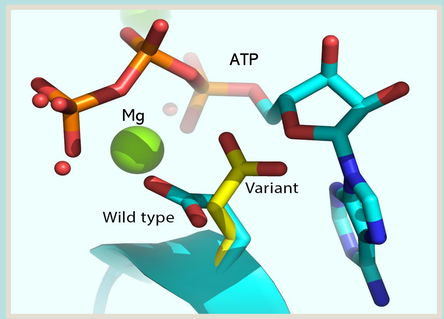

Figure 4: Comparison of Wild Type and Glu445 Variant Structures. This figure compares the wild type (left) and Glu445 variant (right) structures. The Glu445 variant represents the modified structure from this study. Both structures are depicted without bound ligands, showing coordination with the magnesium ion (Mg) which is in turn coordinating the ATP.

In the absence of ATP, Glu445 is observed to be flipped outward, away from the active site, due to the lack of interactions stabilizing it. The wild type structure may coordinate better with ATP than the variant. In many interactions, the wild type is optimized for stabilizing substrates like ATP, whereas mutations (like the variant in this case) may disrupt or alter key interactions that stabilize the ATP binding.

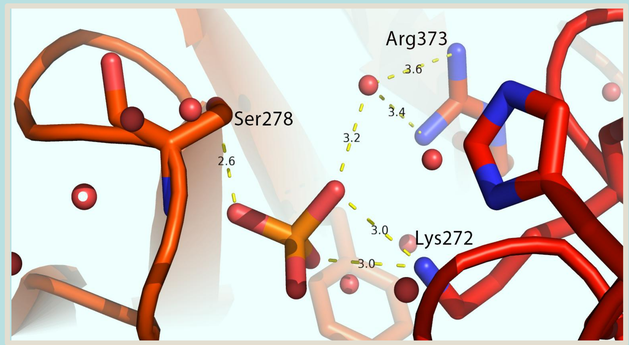

Figure 5: The Phosphate Pocket Coordination. The binding pocket shows involving key residues Ser278, Lys272, and Arg373. The yellow dashed lines represent the distance measured in angstroms (Å) between each interaction within the pocket.

The phosphate group in **Figure 5** is stabilized by direct interactions with Ser278 and Lys272, while a bridging water molecule coordinates with Arg373. This combination of basic and polar residues secures the phosphate within the active site, likely enhancing the enzyme's catalytic efficiency.

Conclusions and Future Work

As illustrated in **Figure 4**, if ATP was present, it would induce a significant conformational change by Glu445 moving inward to interact with ATP, which would trigger a **coil-to-helix transition** in the nearby region. This structural rearrangement results in the formation of **one turn of an alpha helix.** However, due to the absence of ATP, the region around Glu445 does not undergo the **coil-to-helix transition** that would typically occur when ATP is bound. In an apo state, we conclude that Glu445 likely adopts a more relaxed or extended conformation, which prevents the formation of the alpha helix and leaves the active site in a more open or less structured state, reducing the enzyme's catalytic efficiency.

The higher turnover rate shown in **Figure 2** possibly indicates ATP autolysis, where ATP hydrolyzes without transferring its phosphate group, preventing adenylate intermediate formation. Rather than disrupting the mechanism at the second step, as shown in **Figure 1**, the reaction is cut off at the first step before the intermediate can form.

If the side chain of Glu467 were removed, it would block the second step of the reaction, thereby trapping the adenylate intermediate. However, the reaction was blocked earlier than expected, preventing the intermediate from forming in the first place. This suggests that instead of successfully trapping a transition state intermediate, we targeted the right residue but in the wrong variant. The alanine mutation used was too drastic, so we plan to attempt a more conservative mutation. Therefore, future works would propose changing the Glu467Ala mutation to a more conservative Glu467Gln mutation, which preserves the size and polarity of the residue while avoiding the drastic change in acidity.

References and Acknowledgements

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