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Introduction

β-Carbonic Anhydrases (β-CAs) are metalloenzymes that are essential to the growth of bacteria, making it a target for antibiotic research in the drug industry. For use in this research, the protein must be purified, and carbonic anhydrases native to a variety of bacterial species can be isolated via immobilized metal affinity chromatography (IMAC) due to the coordination of histidine tags on the protein's surface with the metal ions such as like Ni2+, promoting isolation of the bound protein from others in a sample. This IMAC approach was used to successfully enhance metal ion affinity with the *Haemophilus influenzae* carbonic anhydrase (HICA), by introducing histidine residues on the surface of a HICA protein via site-directed mutagenesis. The carbonic anhydrases isolated were eluted on a column using increasing concentrations of imidazole, which demonstrate the ion affinity on the surface of these metalloenzymes. An SDS-PAGE analysis was completed with all the isolated mutants, and this demonstrated successful overexpression.

Background

E. coli carbonic anhydrase (ECCA) have endogenous nickel affinity, and we used their structure to recreate the affinity in the HICA protein by completing a likely histidine cluster using site-directed mutagenesis. The HICA mutations resulted in mutant proteins that mimicked the endogenous affinity of ECCA to Ni-NTA, without the use of a random coil tag. This work paved the way for a number of mutations to be formed. Many questions remain unanswered however, namely, are there any limitations to how many histidine residues can be introduced and how might the mutations affect the kinetics of the carbonic anhydrase?

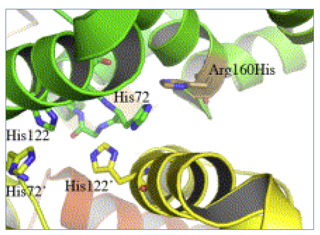


Figure 1. Histidine tags. This is a sit in which a cluster of histidine residues are positioned optimally to be able to bind to nickel, especially considering that this interface between monomers, one shown in green and the other shown in orange of the HICA tetramer are partially exposed to solvent (1).

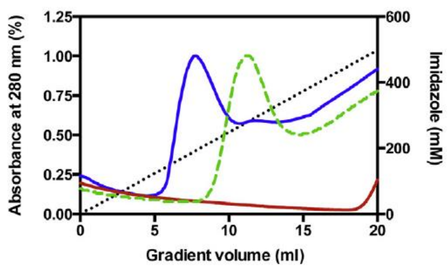


Figure 2. HICA and ECCA elution profile. ECCA (green) has endogenous metal affinity due to the presence of surface-clustered His residues (Fig. 1) and elutes at 278 mM imidazole. Wild-type HICA (red) does not bind to a nickel column. R160H HICA (blue) has a greater affinity for nickel than wild-type HICA and elutes from the column at 190 mM imidazole (1).

Experimental Design

- PyMOL modeling software was used to design His mutant clusters varying in distance and number (2).
- Plasmids expressing the mutant HICA gene were constructed using site-directed mutagenesis.
- Mutant plasmids were introduced into an expression vector and the mutant HICA protein was overexpressed.
- Cell lysate was loaded onto a nickel-NTA column and the concentration of imidazole required to elute the mutant protein was determined (3).
- Protein was quantitated by BCA assay and fractions were analyzed by SDS-PAGE.
- The Cary50 UV-Vis Spectrophotometer was used to begin trials of a kinetic assay of each mutant HICA sample.

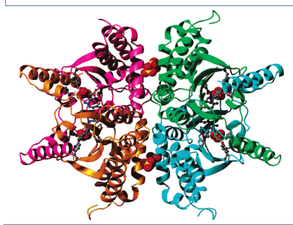


Figure 3. The HICA tetramer. This figure depicts the crystal structure of the HICA protein [PDB: 2A8D]. Each monomer of the tetramer structure is colored differently, in pink, orange, green and blue to indicate the distinct monomers.

Designed HICA mutants

Once all mutants were designed, cloned and overexpressed, the cells were lysed, and this lysate was eluted on a Ni-NTA column with imidazole. The imidazole concentrations required of each mutant for elution demonstrates the strength of their ion affinity. If a greater imidazole concentration is observed, then that demonstrates enhanced ion affinity. The data in Table 1 below indicate the imidazole concentration required to elute each mutant.

Table 1: HICA mutants

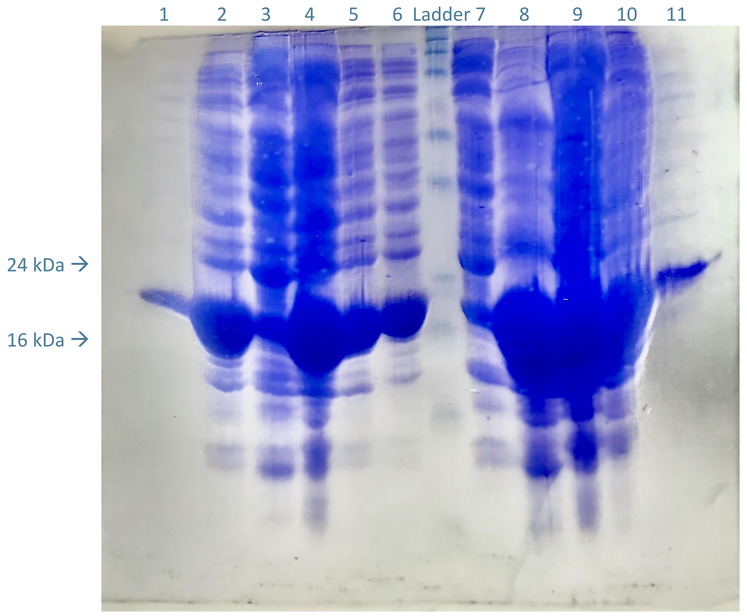
Mutation	[Imidazole] needed to elute (mM)
S13H	50
T34H	50-100
Y37H	25-50
E56+E59H	150-200
D125H	25
I126H	25
K129H	25
L133H	25
I126H+K129H	100
K129+L133	50-100
K136H	50-100
K136H+S138H+R142H	100-150
D144	25
I194	25
M194+T196	100-150
T200H	25-100
M194+T196+T200H	150
S204H	50
S175H+T200H+S204H	100
M194+T196+D216H+E218H	100-200

Results

We designed a kinetic assay in which the HICA protein solution reacted with a substrate, 4-nitrophenyl acetate (4-NPA), in a PBS buffer, and as the 4-NPA was converted into the product, 4-nitrophenol, there was a color change from a clear and colorless solution, to a clear yellow solution. To eliminate as many variables as possible, we controlled for temperature, enzyme concentration, and substrate concentration. Using the Cary50 UV-Vis Spectrophotometer, we quantified the rate of reaction in terms of the change in absorbance over time.

Our results for the kinetic assay are inconclusive at this time, however we were able to successfully confirm the presence of the overexpressed HICA within the cell lysate, assuring that the reaction that is occurring is catalyzed by the HICA enzyme. In figure 3, we show that thick bands, indicative of overexpression, formed from 11 mutant enzymes. The most distinct band in each of these samples is seen at a molecular weight of 19.0 ± 0.3 kDa, which is consistent with monomers of the HICA tetramer.

Figure 3. SDS-PAGE gel. This gel contains 11 mutant HICA samples, and it identifies the presence of HICA in each lane at approximately 19.0 kDa and identifies the overexpression.



Further Research

- The Cary50 UV-Vis Spectrophotometer will be used in future trials of a new kinetic assay protocol.
- Based on the information we learned, it will be important that future assays are conducted in a neutral solvent, because any level of basicity can lead to base catalyzed hydrolysis of the substrate, which introduces error into the kinetic data.
- Additionally, the Cornely lab may pursue the use of HICA's physiological substrate, carbon dioxide, for the assay to maximize the substrate specificity and ensure more accurate kinetic results.

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- To the CHM 310L students at Providence College and the Chem 443L students at Gonzaga University for piloting the research and designing the various mutants.

References:

1. Hoffmann, K., Wood, K. M., Labrum, A., Lee, D. K., Bolinger, I. M., Konis, M. E., Blount, A. G., Prussia, G. A., Schroll, M. M. and Watson, J. M. (2014) Surface histidine mutations for the metal affinity purification of a β-carbonic anhydrase *Anal. Biochem.* **458**, 66-68.
2. DeLano, W. L. *The PyMOL Molecular Graphics System* (2002) DeLano Scientific, Palo Alto, CA USA.
3. Block, H., Maertens, B., Spriestersbach, A., Brinker, N., Kubicek, J., Fabis, R., Labahn, J., and Schäfer, F. Chromatography (IMAC): A Review, *Methods in Enzymology,* **463**, 439-473.