enzymes has been proposed, details of the reaction process are still not clear. This paper presents findings from a study that is aimed at understanding the mechanism of action in amidases, particularly the contribution of each of the catalytic residues in the reaction process.

The four catalytic residues (C165, E61, E139 and K131) have been mutated individually in the *Nesterenkonia* sp. amidase (NitN). In each case, the mutant was reacted with a range of amide substrates and the results analyzed by mass spectroscopy and X-ray crystallography. Mutation of the catalytic cysteine has allowed visualization of non-covalently bound substrates in the active site. However, unexpected reactions resulting in unusual adducts at the reactive cysteine have been observed with the mutants of the two glutamates and the lysine.

Mutation of the two active site glutamates and the lysine resulted in unstable mutants that lacked biochemical activity. While the reaction of E61Q/L mutants with some amide substrates resulted in thioester acyl-enzyme intermediates, 'abnormal' reactions have been observed with fluoroacetamide (FAE) and acrylamide (ACR). These include an S_N2 reaction which displaces the fluorine of FAE and a Michael addition of ACR at the catalytic cysteine, both of which have been visualized in the E61L/Q mutants. E139Q mutant does not exhibit any reactions with most of the amide substrates except with ACR where a Michael addition is observed. A cacodylate adduct at the catalytic cysteine has been visualized in the E139Q active site. The K131Q/H mutants showed no reactions, adducts or intermediates with the tested amide substrates, but a crystal structure of K131Q has revealed an adipamide reaction intermediate covalently attached to the catalytic cysteine. The trapped intermediate is likely to be of an *E.coli* metabolite that reacted with the mutants during protein expression.

The occurrence of unusual reactions with the NitN mutants highlights the importance of the catalytic residues (particularly the glutamates) in ensuring optimal positioning of the substrates in the active site and has also given insights into the critical balance of forces necessary for enzymatic catalysis in amidases.

Keywords: catalysis, mechanism, adduct

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Structural investigation of *Pseudomonas aeruginosa* glyoxalase i enzymes

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Methylglyoxal is a naturally produced cytotoxic electrophile. The toxicity of methylglyoxal is thought to be due to attack of nucleophilic centres of macromolecules including DNA, RNA and proteins. The glyoxalase enzyme system serves to detoxify methylglyoxal via a two-step pathway dependent on cellular glutathione. The first step is the conversion of the non-enzymatically formed glutathione-methylglyoxal hemithioacetal to the corresponding thioester by the metalloenzyme Glyoxalase I. Glyoxalase II cleaves the thioester to produce D-lactate and regenerate glutathione.

Pseudomonas aeruginosa is a multi-host, opportunistic pathogen. It is implicated in the pathology of pneumonia, septic shock, and other types of infection. *P. aeruginosa* uniquely possesses three separate enzymes with glyoxalase I activity, GloA1, GloA2 and GloA3. Other organisms only contain a single glyoxalase I enzyme. Interestingly, GloA1 and GloA2 contain a Ni^{2+} catalytic centre and are inactive when bound to Zn^{2+} unlike GloA3, which is active when bound to Zn^{2+} [1]. Having more than one enzyme with different metal centres may equip the bacteria with greater chemical flexibility allowing the microbe to inhabit more environments, i.e. areas that are deficient in Zn^{2+} but not Ni^{2+}. The presence of three different glyoxalase I proteins within the same organism provides the unique opportunity to compare both metal ion activation classes of this enzyme. X-ray crystallographic studies can provide detailed structural information about how the protein binds to the metal ion as well as metal ion-substrate interactions.

We report the crystal structures of GloA2 with Ni^{2+} and with Zn^{2+} bound at the active site. Anomalous data were collected to confirm the identity of the metal ion. The coordination geometries of the Zn^{2+} and Ni^{2+} centered *P. aeruginosa* GloA2 enzyme are almost identical as seen in the *E. coli* glyoxalase I enzyme [2], which is also only active with an octahedrally coordinated metal group [3].

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Structural basis for increased thermal stability of adenylate kinase variants

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Engineering proteins for higher thermal stability is an important and difficult challenge. We describe a comprehensive approach to mutate proteins to be more stable, and report structural analysis of the redesigned protein variants.

First, we identified mutations for thermal stabilization of our model, adenylate kinase based on a variety of experimental and computational techniques. Then, we designed variants by combining the individual stabilizing mutations together. In the experiment using differential scanning calorimetry, the adenylate kinase variants displayed considerable increases in their thermal stabilities.

We determined crystal structures of the variants to confirm the structural basis for their thermal stabilization. The structures showed that the resulting variants have mutation(s) for extra electrostatic interactions by newly added ion pairs, additional hydrophobic interaction and optimized local structural entropy suggesting their contribution to thermal stabilization of the variants.

Keywords: thermostable, mutagenesis, enzyme

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Structure of the β-galactosidase from *Kluyveromyces lactis*

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β-galactosidase (EC 3.2.1.23) is the enzyme responsible of the hydrolysis of the disaccharide lactose into glucose and galactose. It is