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X-ray crystallographic studies and quantum mechanical modeling of the amidase reaction mechanism

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<p>The amidases catalyse the conversion of amides to the corresponding carboxylic acid and ammonia. We have visualized the structure of the amidase (MW= 30 kDa) from a bacterium, Nesterenkonia sp. at atomic resolution by X-ray crystallography. The primary components of the active site are two negatively charged carboxylates (E1 and E2) arising from two conserved glutamate side chains, a positively charged amino group (K) arising from a conserved lysine, a sulphydryl arising from a conserved cysteine and a water molecule. Mutation of the active site residues has enabled us to visualize the unreacted substrate and a number of artificial intermediate states which have led to our insights. We propose a reaction mechanism which passes through a thioester adduct to the cysteine. Although this in itself is not controversial, our studies demonstrate for the first time how exquisitely accurate geometrical placement of the various groups leads to accurate stereoelectronic alignment which allows the transition states to form. The configuration of E1, E2 and K ensures that K remains positively charged throughout the reaction thus enabling it to act as an acid catalyst and provide an oxyanion hole to stabilize the tetrahedral transition states. The configuration also accurately positions the substrate for nucleophilic attack by the sulphydryl by hydrogen bonding to the amide, positions E1 as a base catalyst and positions the water with the correct stereoelectronic alignment for a nucleophilic attack on the thioester. The transition state energies, electron densities and hydrogen placements calculated using Gaussian09 demonstrate the plausibility of our proposal.

Level (Hons, MSc,
   PhD, other)?

PhD

Consider for a student
   award (Yes / No)?

No

Would you like to <br> submit a short paper <br> for the Conference
 Proceedings (Yes / No)?

Possibly

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