

Active site mutants of *Vibrio* sp. alkaline phosphatase

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Cold-active enzymes are efficiently able to maintain their dynamic movements at temperatures where their mesophilic and thermophilic counterparts are unable to sustain enough flexibility for catalytic reactions. Increased catalytic efficiency at low temperatures as well as increased thermolability are common features of these enzymes and are believed to be a consequence of enhanced structural flexibility. How this is achieved, is not fully understood. Cold active alkaline phosphatase (AP) from a *Vibrio* sp. is very thermolabile. It is still unknown whether these characteristics are originated in the active site locus or in more general overall structural factors. Three metal ions are present in the active site (usually Zn, Zn, Mg). Homology alignment shows that the amino acid residues that bind the two zinc ions are totally conserved in all known APs. However, two residues that bind the Mg ion are different in various APs: Asp153/Lys328 in *E. coli* AP, are His116/Trp274 in *Vibrio* AP, and His/His in mammalian APs. This may explain their different catalytic efficiencies. We have mutated Trp274 in *Vibrio* AP to Lys, His or Ala. These various mutants all displayed increased heat stability of the active conformation together with reduced substrate affinity (K_m) and lower overall reaction rate (k_{cat}). The Lys274 and His274 mutants also displayed an increase in global heat stability. These results are consistent with the general theory that catalytic rate and substrate binding are closely related to structural flexibility. The Ala274 and His274 mutants also had increased stability at lower pH.

Overall the results show that a single amino acid substitution in the active site is sufficient to alter the structural stability as well as kinetic properties of the *Vibrio* AP.