

Restricting dynamic active-site loop-movement by engineered disulfide-bonds increases stability and reduces catalytic activity of a cold-adapted alkaline phosphatase.

Bjarni Ásgeirsson, Guðjón Andri Gylfason, og Björn Viðar Aðalbjörnsson,
Department of Biochemistry, Science Institute, University of Iceland.
bjarni@raunvis.hi.is.

Alkaline phosphatase is an extracellular enzyme that is membrane-bound in eukaryotes but resides in the periplasmic space of Gram-negative bacteria. It normally carries four cysteine residues that form two disulfide bonds, for instance the APs of E. coli and vertebrates. An AP variant from a Vibrio sp. has only one cysteine residue. This cysteine is second next to the nucleophilic serine-102 in the active site. We have individually modified six residues that are on two loops within a 5Å radius. Three of them formed a disulfide-bond to the endogenous cysteine. Thermal stability was monitored by circular dichroism and activity measurements. A significant increase in stability was observed for the disulfide containing variants, together with a reduction in substrate binding affinity (higher K_m) and much reduced catalytic rate (k_{cat}). The results suggest that mobility near the entrance to the active site, and in the helix carrying the endogenous cysteine, is essential for full catalytic efficiency in the cold-adapted AP.