

Abstract

The evolution of new catalytic activities and specificities within an enzyme superfamily requires the exploration of sequence space for adaptation to a new substrate with retention of those elements required to stabilize key intermediates/transition states. Phylogenetic analysis, mechanistic information, and structure determination is used to reveal novel ways in which the catalytic scaffold of a mechanistically diverse superfamily is tailored to new biochemical functions. We propose that core residues in the large enzyme family, the haloalkanoic acid dehalogenase enzyme superfamily (HADSf) form a “mold” in which the trigonal bipyramidal transition-states (TBPST) formed during phosphoryl transfer are stabilized by electrostatic forces. As a test for the operation of the trigonal bipyramidal phosphorane mold, X-ray crystal structure determination has been performed on a phosphatase complex that contains a ligand that is stabilized in an otherwise high-energy (distorted) coordination state- tungstate. The complex is compared to that of vanadate, as an example of a ligand that can more easily expand its coordination geometry. A composite TBPST derived from the analysis of 12 liganded HADSf structures, reveals absolutely conserved elements which serve to stabilize the axial and equatorial atoms of the phosphoryl group.