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Diversification of function in the haloacid dehalogenase enzyme superfamily: The role of the cap domain in hydrolytic phosphorus—carbon bond cleavage ☆,☆☆

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Abstract

Phosphonatase functions in the 2-aminoethylphosphonate (AEP) degradation pathway of bacteria, catalyzing the hydrolysis of the C—P bond in phosphonoacetaldehyde (Pald) via formation of a bi-covalent Lys53ethylenamine/Asp12 aspartylphosphate intermediate. Because phosphonatase is a member of the haloacid dehalogenase superfamily, a family predominantly comprised of phosphatases, the question arises as to how this new catalytic activity evolved. The source of general acid-base catalysis for Schiff-base formation and aspartylphosphate hydrolysis was probed using pH-rate profile analysis of active-site mutants and X-ray crystallographic analysis of modified forms of the enzyme. The 2.9 Å X-ray crystal structure of the mutant Lys53Arg complexed with Mg^{2+} and phosphate shows that the equilibrium between the open and the closed conformation is disrupted, favoring the open conformation. Thus, proton dissociation from the cap domain Lys53 is required for cap domain–core domain closure. The likely recipient of the Lys53 proton is a water-His56 pair that serves to relay the proton to the carbonyl oxygen of the phosphonoacetaldehyde (Pald) substrate upon addition of the Lys53. The pH-rate profile analysis of active-site mutants was carried out to

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☆☆ The coordinates of the refined structures have been deposited with the Protein Data Bank, accession codes 2IOH and 2IOF for K53R and $NaBH_4$ reduced phosphonatase, respectively.
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test this proposal. The proximal core domain residues Cys22 and Tyr128 were ruled out, and the role of cap domain His56 was supported by the results. The X-ray crystallographic structure of wild-type phosphonatase reduced with $NaBH_4$ in the presence of Pald was determined at 2.4 Å resolution to reveal *N*ε-ethyl-Lys53 juxtaposed with a sulfate ligand bound in the phosphate site. The position of the C(2) of the *N*-ethyl group in this structure is consistent with the hypothesis that the cap domain *N*ε-ethylenamine-Lys53 functions as a general base in the hydrolysis of the aspartylphosphate bi-covalent enzyme intermediate. Because the enzyme residues proposed to play a key role in P—C bond cleavage are localized on the cap domain, this domain appears to have evolved to support the diversification of the HAD phosphatase core domain for catalysis of hydrolytic P—C bond cleavage.

Keywords: Schiff-base; Phosphoryl transfer; Phosphoenzyme; Phosphoaspartate; Structural enzymology; HAD superfamily; General acid catalysis; General base catalysis; Enamine; Phosphate ester hydrolysis; Phosphonatase; Phosphonate; Cap domain; Core domain; Electrophilic catalysis

1. Introduction

Phosphonoacetaldehyde hydrolase (phosphonatase) catalyzes the hydrolysis of phosphonoacetaldehyde (Pald) to acetaldehyde and orthophosphate (Fig. 1) [1]. Phosphonatase functions in the 2-aminoethylphosphonate (AEP) degradation pathway operative in strains of bacteria adapted for the use of the natural phosphonate AEP as a source of carbon, phosphorus, and nitrogen [2–4]. Phosphonates, which contain a P—C bond, differ in chemical stability from the more common phosphate monoesters, which possess a P—O—C bond linkage [5–7]. The catalytic pathways for their hydrolysis also differ [8,9]. We were thus intrigued by the discovery that phosphonatase is a member of the haloacid dehalogenase superfamily (HADSF) [10], a family predominantly comprised of phosphatases.

The vast majority of the enzymes in the HAD family are phosphatases[11,12], followed by ATPases [13,14] and phosphomutases [15]. The HAD phosphotransferases conserve a core catalytic domain that positions an Asp residue to function in nucleophilic catalysis[15,16], and a second Asp residue to function in general acid/base catalysis. The second Asp protonates the leaving group oxygen in the first half of the reaction and deprotonates the water or alcohol nucleophile for reaction with the phospho-enzyme intermediate in the second half of the reaction. In addition, the catalytic domain provides a Lys/Arg residue to bind the substrate phosphoryl group and position the Asp nucleophile, and two carboxylate groups to assist in the binding of the Mg^{2+} cofactor (Fig. 2). The Mg^{2+} cofactor forms a coordinate with the Asp nucleophile and the substrate phosphoryl group, thereby orienting and shielding the negative charges in the ground state and transition state [17].

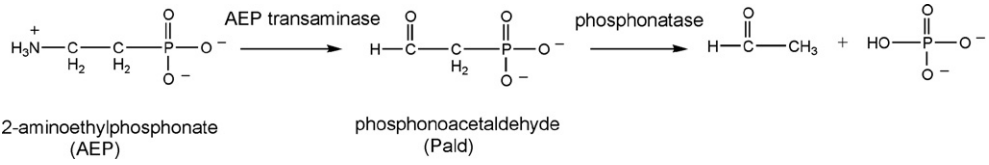

Fig. 1. The bacterial AEP degradation pathway.