

# Structural and kinetic analysis of a highly catalytically promiscuous enzyme with seven activities

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Catalytic promiscuity, i.e. the ability of enzymes to catalyse several chemically distinct reactions has implications for our understanding of enzyme function and evolution.

Recent publications have shown that promiscuous side activities of enzymes could be the starting point for the evolution of new enzyme functions specialised for the former side activities. Hence, the low-level side functions found in promiscuous enzymes could be the remaining traces of their evolutionary past and could thus serve as an additional criterion for the definition of enzyme superfamilies.

Apart from the evolutionary implications, catalytic promiscuity is also interesting under an enzymological point of view. It is generally assumed that enzymes are only able to bring about remarkable rate enhancements of up to  $10^7$  to  $10^{19}$  if their active sites are finely tuned to differentiate efficiently between the substrate in the ground state and the altered structure in the transition state. Even minor changes in bond lengths, angles and charges would have to be specifically recognized by the active site. Nevertheless, it seems that some highly reactive catalytic features in enzymes, e.g. metal-activated nucleophiles, confer a catalytic flexibility which is paid for with comparably small decreases of catalytic efficiencies.

We present the 1.42 Å crystal structure of a phosphonate monoester hydrolase (PMH) from *Rhizobium leguminosarum* that establishes PMH as a new member of the alkaline phosphatase superfamily. PMH shows remarkable catalytic promiscuity with seven different activities of high rate accelerations ( $k_{\text{cat}}/k_{\text{uncat}}$ ) ranging from  $10^{12}$  to  $10^5$ . Apart from phosphonate monoesters it hydrolyses sulphate monoesters, phosphate monoesters, -diesters, -triesters, sulphonates and glucosides. This means that PMH can not only accommodate a wide range of substrate charge, size and reaction centres but is also able to catalyse reactions that show dissociative and associative anionic as well as cationic transition states in solution. PMH therefore combines a large number of the activities present in other members of the alkaline phosphatase superfamily.

Its structure shows homology to sulphatases and *E. coli* alkaline phosphatase, which underlines the evolutionary relationship between these two enzyme families, not only catalytically but also structurally. The metal-activated nucleophile in the active site is a formylglycine, which originates from post-translational modification of a cysteine and has so far only been found in sulphatases. Kinetic analysis of active site mutants shows that PMH employs a mechanism to hydrolyse phosphonates which is analogous to arylsulphatases hydrolysing sulphate monoesters, sharing a very similar active site composition. Hence, small changes in the catalytic machinery during the evolution of sulphatases and phosphatases could have facilitated the switch in selectivity towards a phosphate or sulphate substrate.

Thus, our structural and catalytic evidence provides further support for the theory that promiscuous activities could be the starting point for the evolution of new enzyme functions.