

A “Moonlighting” Dinuclear Hydrolase: Activities toward Phosphoester Hydrolysis and Catechol Oxidation

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The aminopeptidase from *Streptomyces griseus* (SgAP) is a hydrophobic-specific dinuclear metalloenzyme. Recently, it has been found to perform interesting alternative catalyses, in which a few di-metal metal derivatives of this enzyme were found to hydrolyze the phosphodiester bis(*p*-nitrophenyl) phosphate (BNPP) and the phosphonateester *p*-nitrophenylphenyl phosphonate with enormous catalytic proficiencies in terms of the first-order rate constant ($k_{\text{cat}}/k_0 = 0.94\text{--}67$ billion and $0.043\text{--}0.29$ million, respectively). Since tetrahedral phospho-centers often serve as transition-state analogues in peptide hydrolysis, an effective hydrolysis of a phosphoester by a peptidase is not practical and must take place through a unique pathway. Study of the alternative catalyses of SgAP may provide further insight into its mechanism that is otherwise not obtainable by the use of the regular peptide substrates. The versatile hydrolytic activities of SgAP and its di-metal derivatives suggest that they can serve as novel dinuclear “*natural* model systems” (as opposed to “*synthetic* model systems”) to provide further insight into dinuclear hydrolysis/catalysis in chemical and biological systems. To demonstrated this concept further, we have investigated the di-Cu(II) derivative of this enzyme toward the oxidation of a prototypical catechol oxidase substrate, 3,5-di-*t*-butyl-catechol. Significant activity was observed with rate constants of $k_{\text{cat}} = 1.45 \text{ s}^{-1}$, $K_m = 0.44 \text{ mM}$, and $k_{\text{cat}}/K_m = 3,300 \text{ M}^{-1} \text{ s}^{-1}$.