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New catalytic strategies for leaving group activation in nucleoside hydrolases

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General acid catalysis is a powerful and widely used strategy in enzymatic nucleophilic displacement reactions. For example, hydrolysis/ phosphorolysis of the N-glycosidic bond in nucleosides and nucleotides commonly involves the protonation of the leaving base concomitant with nucleophilic attack. However, in the nucleoside hydrolase of the parasite *Trypanosoma vivax* the leaving purine of the substrate is bound in an apolar environment and mutagenesis studies of the active site failed to identify a general acid.

The T. vivax nucleoside hydrolase binds the purine base of the substrate between the aromatic side chains of two tryptophans. We showed via quantum chemical calculations, mutagenesis and substrate engineering that this face-to-face stacking interaction can raise the pK_a of the N7 of the heterocyclic base by several units [1]. In addition, the enzyme fixes the 5'OH of the substrate in a gauche,trans orientation about the C4'-C5' bond, enabling the 5'-oxygen to accept an unusual intramolecular hydrogen bond from the C8 of the purine base, hence also increasing the pK_a of the N7 [2]. Steady state kinetics comprising engineered substrates confirm that a considerable fraction of the catalytic 5'-OH effect can be attributed to leaving group activation. Finally, the crystal structure of the enzyme in complex with the transition-state-analogue inhibitor immucillinH reveals a water channel of highly ordered water molecules, leading from the N7 of the nucleobase towards bulk solvent.

Taken together, the face-to-face aromatic stacking interaction between the nucleobase and the tryptophan residue (Trp260) and the unusual intramolecular C8H---O5' hydrogen bond raise the pK_a of the purine by several units. By lowering the acid dissociation constant of the purine, Trp260 and the 5'-OH group catalyze the transfer of a proton from a solvent molecule to the N-7 nitrogen of the leaving group and function as an alternative to a general acid.

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Tailor Made Activation Segment for Autoinhibition

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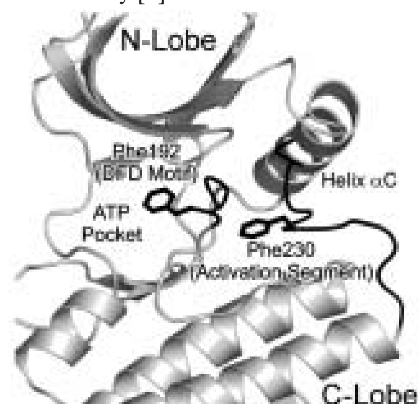
MAP kinase interacting kinases (Mnks) are Ser/Thr kinases that regulate translation initiation events. Mnk activity depends on prior phosphorylation by upstream kinases. Presently, it is unclear how non-phosphorylated Mnks are repressed and how phosphorylation triggers activation. In many other kinases low basal levels of activity are achieved by autoinhibition that can be based on highly diverse molecular mechanisms. Here we show by crystal structure analysis that non-phosphorylated Mnk1 is autoinhibited via an unprecedented strategy that entails conversion of the activation segment into an autoinhibitory module. Repositioning of the Mnk-specific C-terminus of the activation segment alongside the regulatory helix αC leads to (i) detachment of a substrate binding region from its productive site at the C-terminal lobe, (ii) narrowing of the interlobal cleft, (iii) disruption of an essential Lys-Glu ion pair and (iv) locking of the magnesium-binding loop into an ATP-competitive conformation [1]. As a consequence, both ATP binding and substrate positioning are impaired. Structural rearrangements required for the activation of Mnks upon phosphorylation are apparent from the co-crystal structure of a $Mnk2^{D228G}$-staurosporine complex [1] and can be modeled on the basis of Mnk2 crystal packing interactions [2]. Homology modeling and NMR-based nucleotide affinity studies on Mnk1 and on inactive, active and mutant Mnk2 further support a novel regulatory mechanism for the Mnk subfamily [1].



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