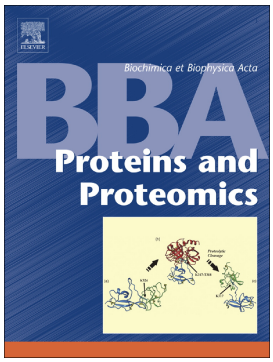

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Calorimetric study of substrate binding in individual active sites of bifunctional human ATIC

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Abstract

Aminoimidazolecarboxamide ribonucleotide formyl transferase (AICARFT): Inosine monophosphate cyclohydrolase (IMPCH, collectively called ATIC) is a bifunctional enzyme that catalyses the penultimate and final steps in the purine *de novo* biosynthesis pathway. The bifunctional protein is dimeric and each monomer contains two different active sites both of which are capable of binding nucleotide substrates, this means to a potential total of four distinct binding events might be observed. Within this work we used a combination of site-directed and truncation mutants of ATIC to independently investigate the binding at these two sites using calorimetry. A single S10W mutation is sufficient to block the IMPCH active site allowing investigation of the effects of mutation on ligand binding in the AICARFT active site. The majority of nucleotide ligands bind selectively at one of the two active sites with the exception of xanthosine monophosphate, XMP, which, in addition to binding in both AICARFT and IMPCH active sites, shows evidence for cooperative binding with communication between symmetrically-related active sites in the two IMPCH domains. The AICARFT site is capable of