

Structural basis of the substrate specificity of human and bacterial kynureninase
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Kynureninases are key enzymes in the metabolism of L-tryptophan in animals and bacteria. In mammals and some bacteria, this pathway provides a de novo route to NAD(P)⁺. The constitutive mammalian kynureninases preferentially catalyze the hydrolysis of 3-hydroxy-L-kynurenine, while L-kynurenine is the preferred substrate of inducible bacterial kynureninases, giving 3-hydroxyanthranilate and anthranilate, respectively, and L-alanine. Crystals of recombinant human kynureninase that diffracted to 2.0 Å were obtained, and the atomic structure of the PLP-bound holoenzyme was determined and compared with that of *Pseudomonas fluorescens* kynureninase. Docking of 3-hydroxy-L-kynurenine into the human kynureninase active site suggests that Asn-333 and His-102 contact the substrate and are involved in substrate recognition by inducible and constitutive kynureninase. The crystal structure of the complex of human kynureninase with 3-hydroxyhippurate, a competitive inhibitor, confirms the docking results. Site-directed mutagenesis of human kynureninase at these positions converts the human substrate specificity to that of the bacterial enzyme.