

P001 Evolution and engineering of transketolase substrate specificity
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A phylogenetic analysis of known TPP dependent enzymes compares the evolution of their PP and Pyr domains. We have also constructed the transketolase (TK) phylogeny for 54 protein sequences from extant bacterial, yeast and plant sources. Reconstruction of the TK sequences of ancestral nodes of this tree allows us to examine which of the 52 active-site residues, likely responsible for substrate specificity, have changed during evolution. Ancestral mutants from extant *E. coli* back to the common ancestor were constructed using site-directed mutagenesis. Kinetic characterisation of these mutants with a host of natural and non-natural substrates provides insight into the modulation of substrate specificity during natural evolution. The role of the C-terminal domain in TK function and evolution remains unclear. C-terminal truncation mutants retain TK activity suggesting that the domain may have other roles, perhaps regulation of function *in vivo*, protein stabilisation, or even an unrelated activity.

The substrate specificity of transketolase is also investigated by saturation mutagenesis of the enzyme active site. Mutated residues are chosen using two strategies: a) those changing during natural TK evolution; and b) those closest to the substrate in the enzyme structure. The relative merits of each strategy are presented, along with details of the mutants obtained with altered substrate specificity.