

P012 D-amino acid oxidase: molecular and process-related determinants of enzyme stability

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An outstanding example for the implementation of an oxidizing enzyme in an industrial multi-ton-per-year process is the use of immobilized D-amino acid oxidase from the yeast *Trigonopsis variabilis* (TvDAO) for the conversion of cephalosporin C. TvDAO is a FAD-dependent enzyme that catalyzes the strictly stereospecific oxidative deamination of α -D-amino acids to the corresponding α -keto acids with the concomitant release of ammonia and H_2O_2 . The enzyme displays absolute enantioselectivity and broad side chain specificity as a combination of properties that is much desired for the chiral synthesis of fine chemicals. Although TvDAO is considered to be a comparably robust O_2 -dependent biocatalyst, its operational stability is not completely satisfactory from an economical point of view.

We have therefore performed a detailed kinetic analysis of the thermal inactivation of TvDAO and - using evidence from experiments and mathematical modeling - we have identified and characterized three major pathways of inactivation. This study on TvDAO stability was complemented by experiments in a miniaturized reactor system under process-near conditions. The identification of molecular and process-related determinants of TvDAO stability could provide a mechanistic tool for rational stabilization of this industrially important enzyme for improved process performance.