

## Rates and Intermediates in Ser26 Mutants of Benzoylformate Decarboxylase

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Benzoylformate decarboxylase (BFDC), a thiamine diphosphate dependent enzyme, catalyzes decarboxylation of benzoylformate to benzaldehyde and CO<sub>2</sub>. The BFDC reaction proceeds through at least four individual chemical steps and, recently, NMR spectroscopy has been used to measure the ratios of intermediates in the overall reaction. This method permits calculation of rate constants for formation of the first intermediate, mandelylThDP ( $k_2$ ) and its subsequent decarboxylation ( $k_3$ ), as well as the combined breakdown of the enamine and product release ( $k_4$ ). As part of a study of the contributions of the active site residues, Ser26, His70 and His281, to the individual catalytic steps several Ser26 variants were expressed and purified. Initially, the variants were characterized using steady-state kinetics. Subsequently, the enzymes were mixed with benzoylformate and the mixture immediately acid quenched to trap intermediates of the reaction. NMR spectroscopy was used to identify and quantitate individual catalytic intermediates. Rate constants for the formation of these intermediates were then determined and compared to those of the wild-type enzyme. Here we report those results and discuss their implications for the role of Ser26 in the BFDC reaction mechanism.