

W0062

A Thiamine Diphosphate-Dependent Enzyme with a Small Product Trapped in the Active Site. Asim K. Bera, Miriam S. Hasson, Dept. of Biological Sciences, Purdue Univ., West Lafayette, IN 47907-2054.

Thiamine diphosphate is used as a cofactor in many key metabolic enzymes in different pathways and catalyzes a broad range of reactions. Benzoylformate decarboxylase (BFD), a ThDP-dependent enzyme, is quite favorable for structural and enzymological studies because of its ability to form crystals that diffract to a high resolution (to 1.0 Å) and its retention of observable intermediates. We solved three structures of wild-type BFD in two different space groups that have trapped the “forgotten product,” carbon dioxide. The aqueous form of carbon dioxide, bicarbonate, appears bound to the active site. This suggests that the carboxylate group may remain after its removal from the substrate, stabilized in part by serine 26. Structural studies have suggested that serine 26 is important only in the decarboxylation step. However, our catalytic experiments have suggested that serine 26 is active throughout the whole reaction. The observation that serine 26 may be important in the movement of the carboxylate group suggests its role throughout the entire reaction. The retention of the smaller product may accelerate the reaction by facilitating the release of the larger product, benzaldehyde.