High-resolution Structures of Benzoylformate Decarboxylase and Its Cofactor, Thiamin Diphosphate

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Introduction

Benzoylformate decarboxylase (BFD), an enzyme dependent on thiamin diphosphate, has provided unexpected results in both structural and enzymological studies, improving our understanding of members of this important enzyme family. The structure of BFD was first determined to 1.6 Å [1]. The APS Bio Consortium for Advanced Radiation Sources (BioCARS) beamline 14-BM-C has allowed the collection of data to 1 Å, providing a more detailed understanding of both the protein and bound molecules. Inspection of the structures of enzyme-substrate, enzyme-inhibitor, and covalent intermediate complexes in several crystal forms has helped us understand unexpected aspects of the mechanism of this thiamin-diphosphate-dependent enzyme.

Methods and Materials

BFD was crystallized by the hanging drop vapor diffusion method. Diffraction data were collected at BioCARS beamline 14-BM-C. For collection of data at high resolution (to 1 Å), the detector was offset to a 2θ angle of 30°. Data were processed by using the programs Denzo and Scalepack.

Results and Discussion

Several of our recent structures have shown that one of the products of decarboxylation, carbonate or bicarbonate, is bound tightly in the active site in one of the crystal forms. Without the high-resolution data collected at BioCARS, the interpretation of these maps would have been difficult, if not impossible. The position of the Ser 26 in the structures suggests that it would help in the removal of the carboxyl group, possibly through formation of a covalent bond. These results suggest the true power of residues, such as this serine, as provided by their position in the active site. Together, these results may help us understand not just BFD but also other members of the family of enzymes dependent on thiamin diphosphate.

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Reference

[1] M. S. Hasson, A. Muscate, M. J. McLeish, T. K. Harris, J. A. Gerlt, G. Kenyon, G. A. Petsko, and D. Ringe, “Crystal structure of benzoylformate decarboxylase at 1.6 Å resolution: Diversity of catalytic residues in thiamine pyrophosphate-dependent enzymes,” Biochemistry **37**, 9918-9930 (1998).