Probing covalent flavinylation in the flavocytochrome *p*-cresol methylhydroxylase

L.M. Cunane[†], Z-W. Chen[†], F.S. Mathews[†], and W.S. McIntire[‡,Δ]
[†]*Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110 USA*
[‡]*Molecular Biology Division, Department of Veterans Affairs Medical Center, San Francisco, CA 94121 USA*
[Δ]*Department of Biochemistry & Biophysics, University of California, San Francisco, CA 94143 USA*

Introduction

p-Cresol methylhydroxylase (PCMH) is a flavocytochrome c found in the periplasm of certain pseudomonads responsible for the degradation of *p*-cresol and related phenols. It is an $\alpha_2\beta_2$ heterotetramer of 136 kDa that can be resolved into a flavoprotein dimer and two c-type cytochrome subunits. The structures of PCMH from *Pseudomonas putida* at 2.5 Å resolution and that of its enzyme-substrate complex at 2.75 Å are known [1].

The PCMH cofactor FAD is covalently tethered to the enzyme by a tyrosine residue. The reason for covalent flavinylation in flavoproteins in unknown, although in PCMH it has been suggested as a means of facilitating electron transfer between the flavoprotein and cytochrome subunits [2]. It has also been shown that in PCMH, the presence of the bound cytochrome subunit is necessary for the process of covalent flavinylation to occur [2].

The expression of the flavoprotein subunit in *E. coli* [3] has allowed the flavoprotein alone to be studied as distinct from the intact flavocytochrome. Detailed structural studies of the flavoprotein and comparison with the whole enzyme will be used to investigate the mechanism of covalent flavinylation and other catalytic properties of PCMH that depend on the presence of cytochrome [3].

Methods and Materials

A two-pass data set to 1.3 Å resolution was collected from a crystal of the flavoprotein subunit and another set to 1.65 Å from a crystal of the flavoprotein with the cofactor FAD non-covalently bound. Data for the flavoproteins were collected at the Structural Biology Center CAT beamline 19-ID-D.

Results

The structures were solved by molecular replacement with the native PCMH structure as the search model. They have been partially refined, giving Rfree = 0.173, R = 0.153 for the flavoprotein with covalently-bound FAD and Rfree = 0.229, R = 0.199 for the flavoprotein with noncovalently bound FAD.

Acknowledgments

Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38.

References

[1] L.M. Cunane, Z.-W. Chen, N. Shamala, F.S. Mathews, C.N. Cronin, and W.S. McIntire, *J. Mol. Biol.* **295**, 357–374 (2000).
[2] J. Kim, J.H. Fuller, V. Kuusk, L. Cunane, Z.-W. Chen, F.S. Mathews, and W.S. McIntire, *J. Biol. Chem.* **270**, 31202–31209 (1995).
[3] S. Engst, V. Kuusk, I. Efimov, C.N. Cronin, and W.S. McIntire, *Biochemistry* **38**, 16620–16628 (1999).