Structural studies of the recombinant flavin-binding domain of bakers yeast flavocytochrome b2

L.M. Cunane[a], J.D. Barton[a], Z.-w. Chen[a], F.S. Mathews[a], F.E. Welsh[b], S.K. Chapman[b], and G.A. Reid[c]
[a] *Dept. of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110 USA*
[b] *Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JJ, Scotland, UK*
[c] *Institute of Cell and Molecular Biology, University of Edinburgh, Mayfield Road, Edinburgh, EH9 3JR, Scotland, UK*

Introduction

The flavin-binding domain (FBD) of flavocytochrome b2 (FCB2), expressed independently in *E. coli*, is an efficient lactate dehydrogenase when ferricyanide is the electron acceptor but is a poor reductant for cytochrome c, its exogenous oxidant [1]. Electron transfer from FBD to the separately expressed cytochrome b2 domain is also undetectable. Furthermore, the FBD has very poor oxidase activity with molecular oxygen. The structure analysis of FBD was undertaken to investigate why flavin reoxidation was so limited and whether a disordered loop (297–315) in FCB2 near the active site and the interaction site for the cytochrome domain was stabilized. It might also serve as a basis for constructing a cytochrome c binding site on the FBD by protein engineering that would be competent for electron transfer [2].

Methods and Materials

Crystals of FBD were grown by the hanging-drop method. Three μL protein solution (at 10 mg/ml in 0.1 M Tris buffer, pH = 7.5) were mixed with 3 μL reservoir solution (26% PEG 4K, 0.17 M sodium citrate, pH = 5.6, 3% ethylene glycol) and allowed to equilibrate at 4 ºC. Data were recorded to 2.5 Å resolution from a very small (0.05 x 0.05 x 0.2 mm) frozen FBD crystal (soaked one minute in 30% PEG 4K, 0.17 M citrate, pH = 5.6, 3% ethylene glycol) at the Structural Biology Center beamline of the Advanced Photon Source, Argonne, Illinois. The crystal was orthorhombic (P21212) with a = 110.7 Å, b = 147.6 Å, c = 64.8 Å and contains two subunits in the asymmetric unit; the data scaled with Rmerge = 7.6%.

Results and Discussion

A clear solution to the structure was obtained by molecular replacement using the flavin domain of FCB2 as the search model. The structure refinement with CNS [3] was virtually complete at 2.50 Å resolution with R = 0.156 (Rfree = 0.213) and 357 water molecules included.

As expected, the catalytic site for lactate oxidation in FBD is virtually unchanged with respect to FCB2. However, Arg289 (recently demonstrated to be important in catalysis in FCB2 [4]) exists in two alternate conformations as it does in recombinant FCB2 [5], but not in FCB2 purified from *Saccahromyces cerevisiae*. The region of the FBD that forms the interface with the cytochrome domain of FCB2 is generally closer in conformation to subunit 2 of FCB2, where the cytochrome domain is disordered, than to subunit 1 where the cytochrome domain is ordered. The 297–315 loop in the FBD remains disordered as it is in FCB2.

Acknowledgments

This work was supported by National Institutes of Health Grant No. GM-20530 (FSM) by the Royal Society joint Program "Alliance" (SKC), by the BBSRC and the Leverhulme Trust (SKC), and by The Wellcome Trust (GAR).

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] A. Balme, C.E. Brunt, R.L. Pallister, S.K. Chapman, and G.A. Reid, *Biochem. J.* **309**, 601–605 (1995).
[2] F.E. Welsh, L. Kohler, S.L. Rivers, G.A. Reid, and S.K. Chapman, *Flavins and Flavoproteins 1996*, (University of Calgary Press, Calgary, Canada, 1997) 587–590.
[3] A.T. Brünger, P.D. Adams, G.M. Clore, W.L. DeLano, P. Gros, R.W. Grosse-Kunstleve, J.S. Jiang, J. Kuszewski, M. Nilges, N.S. Pannu, R.J. Read, L.M. Rice, T. Simonson, and G.L. Warren, *Acta Cryst. D* **54**, 905-921 (1998).
[4] A.D. Pike, S.K. Chapman, F.D.C. Manson, G.A. Reid, M. Gondry, and F. Lederer, *Flavins and Flavoproteins 1996*, (University of Calgary Press, Calgary, Canada, 1997) 571–574.
[5] M. Tegoni and C. Cambillau, *Protein Sci.* **3**, 303–313 (1994).