Biocatalysis of fumarate derivatives by Flavocytochrome c_3

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Flavocytochrome c_3 is a 63.8 kDa soluble fumarate reductase expressed by the bacterium *Shewanella igidimarina* when grown under anaerobic conditions [1]. Electrons are supplied to the active site via four c-type hemes and a non-covalently bound FAD molecule. Fcc_3 catalyses the two-electron reduction of fumarate to succinate via hydride transfer from N5 of the flavin to C2 of fumarate and proton transfer from arginine 402 to C3 of fumarate. As the kinetics of fumarate reduction have been previously established (k_{cat} = 509.s^{-1} ± 15, K_m = 25μM ±2 at pH 7.2), the aim of this work is to investigate the ability of fcc_3 to reduce alternative substrates, particularly those that may result in the production of chiral molecules. Recent experiments have shown that fcc_3 is able to reduce mesaconate (2-methylfumarate) to methylsuccinate *in vitro* (k_{cat} = 8.97.s^{-1} ± 0.43, K_m = 31.7 μM ± 8.5 at pH7.2) and Circular Dichroism spectroscopy revealed that reduction is stereospecific, producing S-methylsuccinate (see fig.). It was also discovered that fcc_3 is able to reduce the ester dimethylfumarate (k_{cat} = 1072.s^{-1} ± 108, K_m = 130μM ± 38 at pH 7.2). These results suggest that fcc_3 may be able to catalyze the reduction of several different substrates. Future work will focus on alteration of the active site at residues involved in binding and orienting substrate molecules, by means of site-directed mutagenesis. This will enable us to find out if it might be possible to engineer fcc_3 to reduce derivatives of fumarate that the wild-type enzyme does not, as well as explore its potential as a biocatalyst in chiral synthesis.

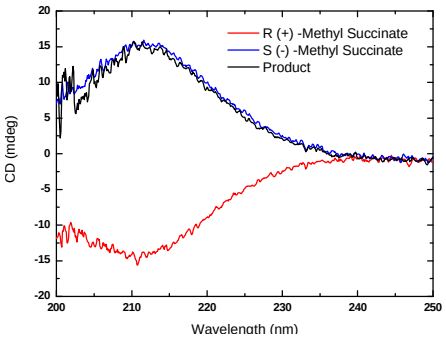

Figure showing CD traces of R and S methylsuccinate and product of mesaconate reduction by fcc_3. The trace for product matches that for S-methylsuccinate.

1. P. Taylor, S. L. Pealing, G.A. Reid, S. K. Chapman, M. D. Walkinshaw, Nature Structural Biology 6, 1108-1112, (1999)