Crystallographic analysis of tryptophan halogenases AbeH and BorH

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Flavin-dependent halogenases represent potential green catalysts for generating specific regioisomers of aryl halides for use in cross-coupling reactions and other synthetic applications [1]. They use $FADH_2$ (provided by a separate flavin reductase component), O_2, and Cl^- to generate HOCl, which transfers Cl^+ to an aromatic substrate in an electrophilic aromatic substitution reaction involving conserved Lys and Glu residues [2,3]. Structural analysis of several tryptophan halogenases suggests regioselectivity is dictated by the orientation of substrate in the active site, overriding any electronic effects [4].

AbeH and BorH were annotated as putative flavin-dependent tryptophan halogenases encoded by soil bacteria biosynthetic gene clusters producing the bisindole alkaloid natural products BE54017 [5] and borregomycin A [6] respectively. We have cloned, expressed, and purified AbeH and BorH, and experimentally verified that both proteins have halogenase activity against L-Trp. BorH chlorinates L-Trp with k_{cat} = 4.4 min^{-1} and K_M = 9.8 μM. NMR analysis of Cl-Trp produced by BorH confirmed regioselectivity for C6. BorH also can brominate L-Trp at C6.

Both AbeH and BorH have been crystallized. The AbeH-FAD complex crystallized in space group $P2_12_12_1$, and the structure has been solved using molecular replacement and refined to $R_{working}$ = 19 % and R_{free} = 21.7 % at 1.65 Å resolution (Fig. 1). BorH has crystallized in two different crystal forms (orthorhombic and hexagonal) and structure determination is in progress. Structural studies of both enzymes with bound L-Trp are ongoing with the goal of improving our understanding of the structural basis for regioselectivity in flavin-dependent halogenases.

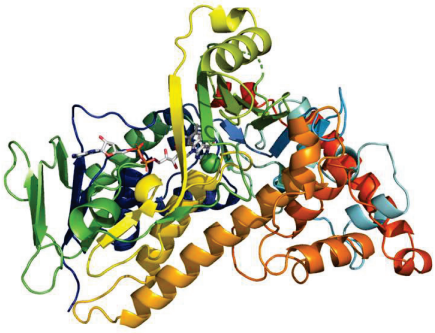

Fig. 1. AbeH:FAD complex

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