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 FADH₂ (provided by a separate flavin reductase component), O₂, 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 $k_{cat} = 4.4 \text{ min}^{-1}$ and $K_M = 9.8 \mu\text{M}$. 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 P2₁2₁2₁, 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

References: