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Crystal Structure of YecO from *Haemophilus influenzae* (HI0319) Reveals a Methyltransferase Fold and a Bound S-Adenosylhomocysteine

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Beamline(s): X12C

Introduction: HI0319 from *H. influenzae* and its closest gene sequence relatives (some labeled YecO in the SwissProt database) are bacterial proteins that have been all annotated 'hypothetical'. PSI-BLAST sequence analysis identified homology to some methyltransferases only at the second iteration cycle. The goal of the crystallographic work was to test whether the structure provides clues about the function of the protein.

Methods and Materials: HI0319 has been cloned and expressed in *E. coli*, and purified as a seleno-methionine protein. Crystals have been obtained and characterized on the home x-ray facility, and conditions for flash-cooling established. MAD data have been collected on APS iMCA-CAT beamline and on the NSLS X12C beamline. The structure has been determined and refined at 2.2Å resolution.

Results: The structure reveals a fold typical of S-adenosyl-L-methionine- dependent (AdoMet) methyltransferase enzymes. Moreover, a processed cofactor, S-adenosyl-L-homocysteine (AdoHcy), is bound to the enzyme, further confirming the biochemical function of HI0319 and its sequence family members. An active site arginine, shielded from bulk solvent, interacts with an anion, possibly a chloride ion, which in turn interacts with the sulfur atom of AdoHcy. The AdoHcy and nearby protein residues delineate a small solvent-excluded substrate binding cavity of 162 Å³ in volume. The environment surrounding the cavity indicates that the substrate molecule contains a hydrophobic moiety and an anionic group. Many of the residues that define the cavity are invariant in the HI0319 sequence family but are not conserved in other methyltransferases.

Conclusions: The structure analysis indicates that the substrate specificity of YecO enzymes is unique and differs from the substrate specificity of all other methyltransferases that have been sequenced to date. Examination of the Enzyme Commission list of methyltransferases prompted a manual inspection of 10 possible substrates using computer graphics, and suggested that the ortho-substituted benzoic acids fit best in the active site.

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