Structure Analyses of Betaine-homocysteine Methyltransferase and Methionine Synthase

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Introduction, Methods, and Materials

Our recent studies of enzyme structure and function have focused on reactions in the pathways of one-carbon metabolism. Two of the catalysts in these pathways, betaine-homocysteine methyltransferase (BHMT) and B_{12}-dependent methionine synthase (MetH), convert homocysteine to methionine in reactions that require zinc ion as a cofactor. MetH is a large modular protein (138 kD) with distinct regions that bind homocysteine, methyltetrahydrofolate, B_{12}, and adenosylmethionine (AdoMet). BHMT, a smaller polypeptide (45 kD), is encoded by a sequence very similar to the N-terminal module of methionine synthase, but it uses glycine betaine rather than methylcobalamin as a methyl donor. In both enzymes, the essential zinc binds to three cysteine residues and ligates the sulfur of the homocysteine substrate, as demonstrated by extended x-ray absorption fine structure (EXAFS) measurements by Penner-Hahn and colleagues. These enzymes, with their distinctive metal sites, are members of a family of proteins that catalyzes alkylation of thiol- or selenol compounds.

The structure analyses will also provide information about conformation changes that are linked to enzyme catalysis. MetH adopts several different conformations in order to present substrates to the B_{12} cofactor and is a prototype for other enzymes that undergo domain rearrangements during catalysis. BHMT is expected to undergo local structural rearrangements upon binding of substrates.

Studies of methionine synthase and BHMT have implications for medical research as well as fundamental enzymology. Because these are the two enzymes that convert homocysteine to methionine, they control plasma levels of homocysteine (Hcy). Within the last decade, elevated homocysteine has been recognized as an important independent risk factor for development of cardiovascular disease, and very recently, high Hcy levels have been correlated with risk for Alzheimer's disease.

Results and Discussion

With single- and multi-wavelength anomalous diffraction data from samarium derivatives collected at the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) and DuPont-Northwestern-Dow CAT (DND-CAT) sectors at APS, we were able to determine the structure of an oxidized form of BHMT. In this structure, the zinc binding site is housed in a distorted $(\beta\alpha)_8$ barrel, but the metal has dissociated, and two of the cysteines that ligate zinc are cross-linked by a disulfide bond. Reduction in the presence of zinc and a bisubstrate analog subsequently allowed us to determine the structure of the bisubstrate complex by molecular replacement. Data for this latter study were collected at the Bio Consortium for Advanced Radiation Sources (BioCARS) at APS. The substrate sulfur is bound to zinc as expected, and the structure reveals the features that determine substrate binding and substrate specificity. These results have been submitted for publication.

BHMT will furnish a structural model for our ongoing x-ray analyses of methionine synthase. At DND-CAT and IMCA-CAT, initial data sets have been collected from crystals of the N-terminal fragments of methionine synthases.

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