

P019 An internal reaction chamber in dimethylglycine oxidase provides efficient protection from exposure to toxic formaldehyde: Part I

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The methylated amines dimethylglycine and sarcosine are intermediates of choline catabolism. The catabolism of dimethylglycine through oxidative demethylation potentially liberates toxic formaldehyde, a problem common to many amine oxidase and dehydrogenases. We now report on a synthetic biology approach to demonstrate substrate channeling in the bifunctional flavoprotein dimethylglycine oxidase (DMGO).

Using a novel synthetic *in vivo* reporter system for cellular formaldehyde, we reveal that the oxidation of dimethylglycine is coupled to the synthesis of 5,10 methylene tetrahydrofolate through an unusual substrate channeling mechanism. We also show that uncoupling of the active sites is achieved by mutagenesis or deletion of the 5,10 methylene-THF synthase site and that this leads to accumulation of intracellular formaldehyde. Channeling occurs by non-biased diffusion of the labile intermediate through a large solvent cavity connecting both active sites. Computer simulations indicate the channeled intermediate to be a lactone. The central “reaction chamber” of DMGO is created by a modular protein architecture that appears primitive when compared to the sophisticated design of other substrate channeling enzymes. This work demonstrates the utility of synthetic biology approaches to study technically challenging enzyme mechanisms *in vivo* and points to novel channeling mechanisms that protect the cell *milieu* from toxic reaction products.