Phosphoethanolamine methyltransferases add three methyl groups successively to their substrate to produce phosphocholine, an important precursor for phospholipid biosynthesis in diverse organisms. New work from Lee and Jez reveals critical domain movements that explain how multiple methylation reactions are uniquely coordinated by plant methyltransferases and provides insights into the evolution of this class of enzymes. As opposed to closely related family members, the intermediates in this pathway are likely shunted between two tethered domains to ensure complete methylation.

The phosphobase methylation pathway, in which phosphoethanolamine (pEA)\(^2\) is converted to phosphocholine (pCho) in three successive methylation steps, is the primary route of pCho biosynthesis in plants, nematodes, and apicomplexan parasites (1–5). pCho generated through this pathway feeds into the Kennedy pathway to produce the membrane phospholipid, phosphatidylcholine. pCho is essential for normal growth and development, and its biosynthetic pathway is a potential target for therapeutic intervention against nematode and Plasmodium infections (4). In plants, pCho also serves as a precursor for the osmoprotectant glycine betaine, and increased pCho production via the phosphobase methylation pathway confers resistance to drought and salinity (6).

Phosphoethanolamine methyltransferases (PMTs) are responsible for the S'-adenosylmethionine (SAM)-dependent conversion of pEA to phosphomonomethylethanolamine (pMME), phosphodimethylethanolamine (pDME), and finally pCho. These enzymes have been grouped into three distinct classes (Fig. 1). Type I PMTs, found in plants, contain two catalytic methyltransferase domains: The N-terminal domain (MT1) catalyzes the initial conversion of pEA to pMME, and the C-terminal domain (MT2) converts pMME to pDME and then to pCho (3, 5, 7). Type II PMTs, found in apicomplexans, have a single MT2-like domain capable of all three methyl group transfer reactions (1, 8). Type III PMTs, found in nematodes, are similar to type I PMTs in that they have two distinct domains, but only one of the methyltransferase domains is functional in a given protein and the other is no longer catalytically active (9). As such, the pMME produced by the MT1 domain of the first enzyme in the pathway must be released and then bound by the MT2 domain of the second enzyme of the phosphobase methylation pathway. These observations raise intriguing questions about the evolution of this pathway, its differential regulation in organisms, and additional potential fates for the released intermediates of this pathway.

Previous structural and biochemical characterizations of type II and type III PMTs have provided insights into their substrate specificity and catalysis. Despite considerable sequence divergence, the catalytic domains of these enzymes share a similar overall fold and canonical SAM-binding sites. However, they use distinct catalytic and substrate recognition mechanisms that coincide with their domain architecture. The single-domain type II enzymes evolved an active site that can accommodate all three potential substrates. The di-domain type III enzymes have a single catalytically competent domain that has specificity for pEA alone or pMME and pDME. In contrast, molecular studies of type I PMT have been limited, and our understanding of this PMT class has been based primarily on comparisons with the type II and III enzymes.

In the current study by Lee and Jez (10), the authors describe the in vitro and in vivo characterizations of three closely related isoforms of the type I Arabidopsis thaliana PMT (AtPMT1–3). Previous work on AtPMTs suggested that AtPMT1 was involved in root development and stress responses (5, 7), but detailed kinetic and structural characterizations were not performed. AtPMT2 was initially characterized as a phosphomethylethanolamine N-methyltransferase, unable to use pEA as a substrate (3), and AtPMT3 had not been previously studied. To clarify the functions of these enzymes, Lee and Jez created knockout lines for each of the three isoforms and demonstrated that atpmt1 expression is required for optimal root development and salt tolerance, presumably through increased pCho production. In contrast, atpmt2 or atpmt3 disruption did not result in an overt phenotype, suggesting potential functional redundancy. To investigate this further, the kinetic parameters for purified recombinant AtPMT1, AtPMT2, and AtPMT3 were obtained and revealed distinct yet comparable kinetic profiles that are unlikely to account for the differences in phenotypes. Strikingly, AtPMT2 was shown to use pEA as a substrate, suggesting that the previous characterizations used an N-terminal deletion variant that may not be physiologically relevant. On the mRNA level, only atpmt1 expression shows a significant
increase under salt stress, whereas atpmt3 expression is mildly reduced and atpmt2 expression remains unchanged, suggesting different functional roles for these three closely related enzymes for processes not yet defined. Interestingly, tissue-specific gene expression profiling reveals that atpmt2 and atpmt3 are most highly expressed in leaf tissue, suggesting they may play a role later in development.

Using X-ray crystallography, the authors were able to define the overall domain structure of AtPMT1 and AtPMT2 and identify local active site rearrangements and global conformational changes that accompany catalysis (10). As described above, the MT1 domain of type I PMT catalyzes the initial methylation in the conversion of pEA to pCho, and the MT2 domain performs the subsequent methylations. The structures indicate that, although SAM binding is similar, MT1 uses a histidine–tyrosine catalytic dyad to activate the substrate. What is particularly captivating in the current study is that comparison of the AtPMT1 and AtPMT2 structures suggests how large-scale conformational changes are required for the conversion of pEA to pCho. Central to this transition is a deformation of the extended α9-helix that bridges the MT1 and MT2 domains into two smaller helices (highlighted in yellow in Fig. 1), thus shifting the MT1 domain closer to the MT2 domain and positioning a critical activity–site tyrosine appropriately for the second methyl transfer reaction. These choreographed motions are observed only in the type I PMT and illustrate how domain movements coordinate the two chemical transformations of this bifunctional enzyme.

This work highlights the power of combining structural, enzymatic, and functional in vivo characterizations to systematically investigate isoform function, particularly since the structural similarity examined here suggests any isoform could functionally compensate for another but expression data show that location is everything. In addition, the high resolution crystallographic studies of AtPMT1 and AtPMT2 provide unprecedented molecular details of representative type I PMT and highlight the contributions of conformational dynamics to enzyme catalysis. Comparisons with type II and type III PMT structural and biochemical data suggest potential evolutionary pathways for this class of enzymes and establish this system as a viable platform to examine engineering of bifunctional enzymes. These studies may help in the design of biosynthetic “assembly line” enzymatic systems.

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