Carboxyl Methyltransferases: Natural Functions and Potential Applications in Industrial Biotechnology

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The use of methyltransferases (MTs) in industrial biotechnology to replace toxic alkylating agents is of increasing interest. Carboxyl MTs (CMTs) are a subgroup of MTs that methylate the hydroxyl oxygen of carboxylic acids. Research initially focussed on their natural functions in protein regulation and production of volatile methyl esters in plants. In this review we highlight this potentially valuable group of enzymes that show promise for formation of a wide range of structurally diverse methyl esters from the parent acids under aqueous conditions. CMTs have been used to generate intermediates for biofuels, bioplastics and pharmaceuticals. These biocatalysts could also be integrated into cascades with other enzymes such as acetyltransferases that function under aqueous conditions. Recent approaches for regenerating the required cofactor S-adenosylmethionine (SAM) are discussed including in vitro recycling, improvement in in vivo production and the use of more stable analogues. Advances in these areas will further improve the potential to use carboxyl MTs in industrial biotechnology.

1. Introduction

Methylation is a common modification in biological systems which involves the transfer of a methyl group (CH₃) from a donor molecule to a substrate. In nature, this reaction is catalysed by methyltransferase (MT) enzymes, a large and diverse class of enzymes with key roles in cellular function including regulation of gene expression, biosynthesis and signalling.[3] The most common class of MTs are S-adenosyl-L-methionine (SAM)-dependent methyltransferases (SAM-MTs) which employ the ubiquitous cofactor SAM as a methyl donor to methylate a range of structurally distinct substrates such as nucleic acids, proteins and small molecules.[4] SAM (or AdoMet) is the second most used biological cofactor after adenosine triphosphate (ATP) and is generated enzymatically from ATP and methionine.[5] The methyl group of SAM is attached to a positively charged sulfur atom, which makes it readily transferable to N, C, S, O, Se, As or halide atoms of the substrate. The methyl group transfer proceeds through an S₂N₂ displacement reaction and produces S-adenosylhomocysteine (SAH) as the by-product of the reaction (Scheme 1).[1,4]

Although SAM is the most commonly used cofactor, there are other MTs that are non-SAM dependent which employ other methyl donors such as methanol, methyl tetrahydrofmate (THF), vitamin B₁₂ and methanethiol. For example, methionine synthase is a tetrahydrofolate- and B₁₂-dependent methyl transferase.[5]

A large proportion of MT research has been focused on DNA methyltransferases (DNMTs) as they have important functions in epigenetics and development. Mutations of DNMTs are often connected with cancer and other human diseases.[6] There is increasing interest in the use of MT enzymes as biocatalysts in synthetic applications to replace commonly used and toxic alkylating agents. Several reviews summarise recent advances in the use of MT enzymes, their substrate specificity, cofactor requirements and application in biotechnology.[7,8] A notable example is the use of catechol-O-MT (COMT) in engineered E. coli to produce vanillin, a commercially valuable flavouring compound and an intermediate in some pharmaceutical processes.[9]

Carboxyl methyltransferases (CMTs) catalyse methyl group transfer from SAM to the hydroxyl oxygen atom of a carboxylic acid group to form a methyl ester. Carboxyl alkylation was first discovered in 1957 by Sato et al. in which they described the biosynthesis of pectinic acid methyl esters.[10] In 1967, Kauss et al. then demonstrated that carboxyl methylation was enzyme-catalysed, using SAM as a methyl donor.[11] So far, research on carboxyl MTs has been largely focused on protein methylation for regulation and repair. However, there are an emerging number of CMTs that catalyse methylation of small
molecule organic substrates such as salicylic acid, jasmonic acid, gibberellins, loganic acid, perillic acid and fatty acids.

The identification and development of enzymes that can methylate carboxylic acids under aqueous conditions would enable their integration into multistep enzyme cascades. For example, acyl transferases can catalyse efficient acyl transfer from methyl esters to alcohols or amines under aqueous conditions, where there is a kinetic preference for acyl transfer over hydrolysis.\textsuperscript{[12–16]} The ability to methylate carboxylic acids \textit{in situ} would therefore enable one-pot conversion of acids into esters or amides (Scheme 2). This avoids the need to isolate acids prior to chemical activation by methylation (MeOH/H\textsuperscript{+}), acid chloride/anhydride formation under dry conditions or use of coupling agents for amide formation. Carnell \textit{et al.} have shown that carboxylic acids can be generated from alcohols in a one-pot, two step biooxidation using the galactose oxidase mutant GOase M\textsubscript{15} and periplasmic aldehyde oxidase (PaoABC) from E. coli.\textsuperscript{[17]} The same enzymes were used to convert 5-hydroxymethylfurfural (HMF) into 2,5-furandicarboxylic acid (FDCA) under aqueous conditions.\textsuperscript{[18]} Availability of CMT enzymes for methylation of carboxylic acids would thus provide a way to enzymatically activate them \textit{in situ} (Scheme 2).

In this review we will explore the substrate specificities of the main types of CMTs and the prospects and current challenges for using these enzymes as biocatalysts in industrial biotechnology. We will also discuss promising applications in biotechnology such as use of CMTs for biomethylation of non-plant derived, microbially produced fatty acids to fatty acid methyl esters (FAMES) as a ‘methanol-free’ approach for the production of biodiesel.

2. The SABATH family of Methyltransferases

In plant secondary metabolism, methylation is one of the most common modifications catalysed by enzymes.\textsuperscript{[19]} Methyl esters constitute a large family of plant compounds that make up components of plant scent and have various roles including attracting pollinators, defence responses and resistance to disease.\textsuperscript{[20]} In the late 1990s, the SABATH family of methyltransferases was discovered in plants, named after the first few enzymes to be identified (Salicylic Acid MT, Benzoic Acid MT and Theobromine MT).\textsuperscript{[21]} These have no significant sequence similarity to any other groups of methyltransferase enzymes and are composed of both carboxyl MTs as well as a number of nitrogen-MTs.\textsuperscript{[22,23]} This ability for enzymes in one MT family to transfer to both oxygen and nitrogen is an interesting feature of the SABATH MTs, as most plant MT families function to transfer methyl groups to one specific type of nucleophile.\textsuperscript{[22]} The first SABATH family was discovered in \textit{Arabidopsis thaliana}, which contains 24 SABATH genes.\textsuperscript{[22]} Six of these have been identified as carboxyl methyltransferases catalysing methylation of salicylic acid (SAMT), benzoic acid (BAMT), jasmonic acid (JMT), indole-3-acetic acid (IAMT), farnesic acid (FAMT) and gibberellic acid (GAMT) (Table 1).\textsuperscript{[22,24–27]} Following this, SABATH gene families have also been identified in other plant species including rice (\textit{Oryza sativa}) and \textit{Populus trichocarpa} which contain 41 and 28 SABATH genes, respectively.\textsuperscript{[28,29]} Often these species have multiple genes which appear to encode the same type of methyltransferase. Some of these genes are pseudogenes and others cannot be detected at a transcriptional level indicating that the protein is not produced.\textsuperscript{[30]}

The first SABATH MT identified was salicylic acid MT (SAMT) from \textit{Clarkia breweri} which catalyses the formation of methylsalicylate, a volatile ester with a wintergreen scent that acts as a chemoattractant in addition to roles in pathogen resistance.\textsuperscript{[21,31]} The enzyme is well characterised, including a crystal structure which shows a homodimeric structure with a distinct dimerisation interface that differs significantly from other plant natural product MTs as it involves only a small percentage of the enzyme surface area, a common feature of other SABATH MTs.\textsuperscript{[22,30]} Crystal structures for two other SABATH CMTs have also been solved (Table 1); LAMT from \textit{Catharanthus roseus} and IAMT from \textit{Arabidopsis thaliana} which also both exist
Scheme 2. Proposed cascade for multistep conversion of alcohols to esters and amides using known enzymes for alcohol to acid, followed by combination of a CMT and acyl transferase.

Table 1. Overview of structures, substrate specificities and homology of the first identified SABATH carboxyl methyltransferases from various sources.

| Enzyme name and abbreviation | First identified source | Substrate specificity (relative activity) | Structure PDB code | % similarity to C5SAMT | References |
|-----------------------------|-------------------------|------------------------------------------|--------------------|------------------------|------------|
| Salicylic acid methyltransferase (SAMT) | Clarkia breweri | • Salicylic acid (100%)  
• Benzoic acid (48%)  
• 3-Hydroxybenzoic acid (13%)  
• Vanillic acid (5.1%) | 1 M6E | 100% | [21] [32] |
| Benzoic acid methyltransferase (BAMT) | Antirrhinum majus | • Benzoic acid (100%) | N/A | 41.2% | [20] |
| Jasmonic acid methyltransferase (JMT) | Arabidopsis thaliana | • Jasmonic acid (100%)  
• Dihydrojasmonic acid (18%) | N/A | 41.9% | [24] |
| Indole-3-acetic acid methyltransferase (IAMT) | Arabidopsis thaliana | • Dichlorophenoxyacetic acid (30%)  
• Indole-3-butyric acid (12%) | 3BSI | 30.9% | [32] [25] [28] |
| Farnesoic acid methyltransferase (FAMT) | Arabidopsis thaliana | • Farnesoic acid (100%)  
• Geranic acid (28%)  
• Lauric acid (18%) | N/A | 29.2% | [26] |
| Gibberellic acid methyltransferase (GAMT1/2) | Arabidopsis thaliana | • GA9 (GAMT1 100%)  
• GA4 (GAMT2: 100%)  
There are 136 identified GAs but GAMT1 and GAMT2 have different substrate specificities | N/A | 30.6% | [27] |
| Loganic acid methyltransferase (LAMT) | Catharanthus roseus | • Loganic acid (100%)  
• Secologanic acid (10%) | 6 C8S 6 C8R | 32.1% [39] [40] |
| Anthranilic acid methyltransferase (AAMT1/2/3) | Zea mays (maize) | • Anthranilic acid (100%)  
• Benzoic acid (AAMT1 3%, AAMT2 3%, AAMT3 26%)  
• Salicylic acid (AAMT3 2%)  
• Indole-3-acetic acid (AAMT3 2%)  
• Jasmonic acid (AAMT3 1%) | N/A | 36.8% | [30] |
| Cinnamate/p-coumarate methyltransferase (CCMT1/2/3) | Ocimum basilicum | • Cinnamic acid (100%)  
• p-Coumaric acid (CCMT1 29.6%, CCMT2 83.5%, CCMT3 23.5%)  
• Hydrocinnamic acid (CCMT1 21.4%, CCMT2 6.8%, CCMT3 39.3%)  
• 4-Hydroxyhydrocinnamic acid (CCMT1 22.4%, CCMT2 25.4%, CCMT3 23.5%)  
• m-Coumaric acid (CCMT1 6.8%)  
• Benzoic acid (CCMT1 7.8%, CCMT2 10.1%, CCMT3 6.2%) | N/A | 37.9% | [41] |

as homodimers.\cite{28,33} In homodimeric plant small molecule OMTs that are not part of the SABATH family, dimerisation is critical to enzyme activity as the dimer interface contributes to the substrate binding site.\cite{34} In contrast, dimerisation of SABATH MTs is not required for enzyme activity as the residues from one monomer do not contribute to the catalytic site of the related monomer.\cite{32,35} Additionally, the SAM binding pocket
is highly conserved among SABATH carboxyl MTs whereas substrate binding sites exhibit more variability.[36]

Interestingly, comparisons of SABATH MT binding sites and engineering studies suggest that their substrate specificity may be controlled by single amino acid residues in the binding site of the enzyme. This has been suggested to play an important role in controlling substrate activity with structurally similar compounds.[36] There have been a number of examples in which engineering of SABATH MTs to change an amino acid residue has altered substrate activity. Barkman et al. investigated SAMT from Datatura wrightii which demonstrates substrate activity with salicylic acid (SA) and to a lesser extent, benzoic acid (BA) (10 fold more active with SA than BA).[37] A methionine residue at position 156 within the binding site was found to create a small pocket that allows the planar conformation of internally H-bonded salicylic acid but does not effectively accommodate benzoic acid, which has rotation around the aryl-carboxylate bond.[32,38] When this methionine residue is replaced with histidine, the mutated enzyme exhibits little difference in substrate preference between SA and BA, resulting in the production of similar amounts of methyl salicylate and methyl benzoate.[33] This is in keeping with the general observation that most SAMT-like enzymes have a methionine at this position and BAMT-like enzymes have a histidine.[39] A similar pattern was also shown by Han et al. who demonstrated that a single amino acid change in SABATH carboxyl MTs in Populus trichocarpa can result in a substrate switch between SA and BA.[29] This suggests that substrate specificity of SABATH MT enzymes could be readily modulated by semi-rational targeted site saturation mutagenesis approaches.

3. Protein Carboxyl Methyltransferases

Early research on carboxyl MTs was centred around protein carboxyl MTs, found in almost all organisms. Methylation of protein carboxyl groups is a post-translational modification important in protein function, regulation and repair.[38] Protein methylation is often irreversible, but protein carboxyl methylation is a reversible modification, facilitated by esterases.[3,38] The first structurally characterised example was a carboxyl MT known as CheR, a bacterial chemotaxis receptor methyltransferase.[35] Response to chemical stimuli is controlled by methylation levels of chemotaxis receptors at specific ω-carboxyl group on glutamate residues. A methylesterase (CheB), catalyses the hydrolysis of the glutamyl methyl ester, which is increased in response to negative stimuli.[42,43] Carboxyl methylation is also important for the regulation of protein phosphatase 2A (PP2A), which plays a key role in cell cycle arrest in all eukaryotes. In mammals, the enzyme leucine carboxyl methyltransferase 1 (LCMT1) catalyses methylation of a specific leucine carboxyl group in the C subunit of PP2A and aids in the assembly of the holoenzyme. LCMT1 activity is linked to the development of Alzheimer’s Disease (AD) as PP2A assembly is essential for dephosphorylation of tau, a key protein involved in the pathology of AD.[44,45]

Protein L-isoaspartyl methyltransferase (PIMT) recognises atypical protein structures and plays a role in their repair.[46] PIMT is widely expressed and a number of crystal structures have been determined including the human enzyme.[46] In proteins, L-isoaspartyl (L-Asp) and L-isoasparaginyl (L-Asn) residues are spontaneously isomerised to L-aspartyl (L-Asp) and dL-isoaspartyl (dL-isoAsp) residues. This occurs most often in age-damaged proteins and these atypical residues can adversely affect protein activity.[47] The function of PIMT is to repair the proteins by catalysing the conversion of these abnormal residues to γ-Asp residues (Scheme 3).[46] PIMT catalyses the transfer of the methyl group from SAM to either the α-carboxyl group of L-isoAsp or β-carboxyl group of γ-Asp to form a methyl ester. The methyl ester then spontaneously cyclises to a succinimide intermediate which spontaneously racemises. Non-enzymatic hydrolysis regenerates some repaired γ-Asp residues, which is not a substrate for PIMT, allowing accumulation of the repaired residue. Hydrolysis also forms γ-Asp and L-isoAsp residues that can re-enter the repair cycle. L-isoAsp is also formed but is not known to be a substrate for PIMT.[46]

4. Potential Applications of Carboxyl Methyltransferases

4.1. Pharmaceuticals

As previously mentioned, loganic acid MT (LAMT) is a SABATH carboxyl methyltransferase. This enzyme was first isolated from Catharanthus roseus (previously known as Vinca rosea), a flowering plant species that is the source of two anti-cancer drugs: vincristine and vinblastine.[48,49] C. roseus produces two monoterpoid indole alkaloids (MIAs), catharanthine and vindoline, that are precursors to these chemotherapy drugs (Scheme 4).[40]
LAMT catalyses the methylation of loganic acid to produce loganin. Vinblastine/vincristine is formed from two molecules of loganin, through a pathway that diverges at stemmadenade and then ultimately re-converges through the combination of catharanthine and vindoline to form 3,4′-anhydrovinblastine.[61] Extensive studies of LAMT have been carried out including a crystal structure of the C. roseus enzyme (Table 1) and LAMT demonstrates high substrate specificity and strict stereospecificity.[33-40] As well as being part of the biosynthetic pathway of MIAs, loganin is also bioactive including the lowering of blood glucose levels and neuroprotective effects.[35] As such, LAMT and related enzymes have potential for application in the design and synthesis of novel classes of MIAs for pharmaceutical purposes.[35]

### 4.2. Biofuels

Carboxyl group methylation of fatty acids using MT enzymes is a route currently being explored for production of biodiesel.[56] Biodiesel is composed of fatty acid methyl esters (FAMEs) and is a sustainable alternative to petroleum fuels.[58] The standard approach to produce FAMEs is through transesterification of triglycerides (from vegetable oil) with methanol, using an alkaline catalyst.[59,60] However, a more direct approach is by MT-catalysed methylation of free fatty acids. Recent research has focused on microbial engineering to generate the requisite fatty acid precursors, thus reducing competition with food supply for feedstock oils.[60] In cells, expression of fatty acid thioesterases leads to production of free fatty acids. These can then be directly methylated to form FAMEs with a suitable CMT, or they can be CoA thioesterifed prior to coupling with ethanol to form the corresponding ethyl esters (FAEES) (Scheme 5). Nawabi et al. reported using a fatty acid MT from Mycobacterium marinum to directly synthesise FAMEs in engineered E. coli with an enriched medium-chain free fatty acid pool. However the level of FAMEs produced was relatively low (16 mg/L), most likely because the MT used was found to have preferential activity for 3-hydroxy fatty acids over medium-chain free fatty acids.[56] Sherkhanov et al. investigated a similar route to intracellularly produce FAMEs in E. coli but used an alternative carboxyl MT enzyme, juvenile hormone (JH) acid O-methyltransferase from Drosophila melanogaster (DmJHAMT).[59] DmJHAMT naturally functions to catalyse the carboxyl methylisation of JH acids, including farnesoid acid, to produce active JHs.[61] This enzyme has a broad substrate range and was found to methylate a spectrum of free fatty acids producing medium-chain FAMEs in E. coli at a significantly higher level than previously reported (560 mg/L).[62]

In a related approach, the cyanobacterium, Synechocystis sp. 6803 was engineered to convert CO₂ into excreted FAMEs.[63] Further improvements are needed but there is significant potential for carboxyl MTs to be employed in biodiesel production.

### 4.3. Bioplastics

Terephthalic acid (TA) is a synthetic precursor of the plastic polyethylene terephthalate (PET) and is currently sourced from petrochemicals for PET production.[59] Methyl perillate (MePA) is a naturally occurring monoterpene in some plant species and can be employed as a renewable starting material for the synthesis of TA, for the production of bio-based materials (Scheme 6).[64] A perlic acid (PA) methyltransferase (SdPAOMT) was identified that is able to catalyse the methylation of PA as part of the MePA biosynthetic pathway in Salvia dorisiana.[65] Jongedijk et al. elucidated the biosynthesis pathway of MePA and reconstituted it in Nicotiana benthamiana (a more suitable expression system), including the expression of SdPAOMT.[66,67] Chemical oxidation of MePA was readily carried out in two steps to give TA.[68] Future expression of the pathway in E. coli or another suitable host would allow large scale production of

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*Scheme 4.* LAMT catalyses carboxyl group methylation to give loganin which is a biosynthetic precursor to both anticancer compounds vinblastine and vincristine.[40]

*Scheme 5.* Whole cell approaches for the production of medium chain fatty acid esters (biodiesel). Full arrows show approach using DmJHAMT; dotted arrows show approach involving CoA ester formation followed by coupling with ethanol.[54,56]
MePA by fermentation and therefore access to TA from renewable sources. The study also demonstrated that SdPAOMT has high similarity to SAMT from the SABATH family of MTs and has substrate activity with SA and BA when produced recombinantly in E. coli.[61] As such, this enzyme appears to show relaxed substrate specificity and accepts both aromatic and monoterpenic carboxylic acid substrates, making it of interest for further study.

5. Prospects and Future Challenges

Although there is significant potential for application of carboxyl methyltransferases in the biotechnology industry, there are still significant challenges in using SAM-dependent methyltransferases as biocatalysts. SAM is expensive to synthesise and is relatively unstable. Production synthesises a mixture of two diastereomers (S,S– and R,S–), with only the S,S– isomer being biologically active.[60] In nature, SAM is produced by the action of SAM synthetase (SAMS) which catalyses adenosyl group transfer from ATP to the sulphur atom of methionine.[60] This forms part of the SAM regeneration cycle (Scheme 7). Following a methyl transfer reaction, the S-adenosyl homocysteine (SAH) by-product is hydrolysed by SAH hydrolase (SAHH) generating adenosine and homocysteine. Methionine can then be regenerated from homocysteine catalysed by methionine synthase (MS) using N5-methyltetrahydrofolate as a methyl donor.[65] However, similar to chemical synthesis of SAM, enzymatic synthesis of SAM using SAMS on a large scale is not suitable due to the high cost of ATP as a substrate. To overcome these limitations, recombinant microbial systems for SAM synthesis have been explored. This approach is relatively low cost and has lower energy consumption.[66] Nan et al. employed a method of using a coupled E. coli and S. cerevisiae expression system in which SAMS was expressed recombinantly in E. coli and ATP was provided by S. cerevisiae. This system successfully produced SAM at 1.7 g/L where most of the SAM produced was secreted into the culture medium allowing for a simple purification process.[63] At present, these microbial systems are the most effective sources of SAM.[66]

SAM upregulation in bacterial strains has also been used to improve the yield of target methyl ester products. As discussed above, Sherkhanov et al. synthesised FAMEs in engineered E. coli by introducing a carboxyl MT (DmJHAMT), able to methylate fatty acids. As part of this work, SAM was identified to be a limiting factor for in vivo FAME production. Methionine synthase from rat liver (Mat1A) was introduced on a plasmid to increase intracellular SAM levels. Initially, the SAM pool was increased by 8.5-fold over 48 h but this resulted in a decrease of FAME production, suggested to be a result of increased metabolic demand and competition for resources. A single copy of the Mat1A gene was then introduced into the host E. coli genome under the control of a T7 promoter to allow regulation of enzyme activity, which resulted in a 3-fold increase of SAM levels over the same time period. This reduced the negative demands of uncontrolled SAM production, and led to a 19% increase in FAME production.[55]

Efficient methods for recycling SAM in vitro would also improve the prospects of using SAM-MTs as isolated or immobilised enzymes in industrial processes. The by-product of methyl transfer reactions, SAH, is a potent non-selective feedback inhibitor for most SAM-MTs which can also lead to complications for using carboxyl MTs on a large scale. SAH competes for the SAM binding site of SAM-MTs but not all SAM-MTs that are inhibited by SAH demonstrate equal sensitivity.[66] As previously mentioned, SAH is hydrolysed by SAHH in the natural cycle of SAM, which is a highly conserved enzyme that acts to prevent the inhibition of SAM-dependent methylation in cells.[66] Another hydrolase enzyme, SAH-nucleosidase (SAH-nuc), catalyses the cleavage of SAH to 5'-ribosylhydr-
mocysteine and adenine. This enzyme is often used in MT activity assays where additional enzymes use the products of this reaction as substrates leading to a measurable output. Recombinant SAH-nuc or SAHH are often added to reactions performed with SAM-MTs to break down SAH and prevent feedback inhibition. For large scale reactions with carboxyl MTs, inhibition by SAH would need to be prevented if the MT used is sensitive to SAH as an inhibitor. This could be achieved by the addition of SAHH or SAH-nuc in the reaction. However, recycling the SAH by-product back to SAM in vitro would also prevent inhibition by SAH. In 2019, Seebeck et al. reported a simple system for SAM recycling using another MT enzyme, halide methyltransferase (HMT), to directly catalyse the remethylation of SAH back to SAM under physiological conditions (Scheme 8). However, this reaction requires methyl iodide as the stoichiometric methyl donor, which has significant safety issues for use on a large scale. As such, further approaches are required to identify less hazardous methyl donors for preparative processes using SAM-dependent MTs, including carboxy-MTs.

Another recent advance in MT research is the use of SAM analogues as synthetic cofactors to transfer alternative alkyl groups. There are a number of approaches to producing these analogues, including chemoenzymatic synthesis using halogenases and SAM synthetases. There is also evidence of naturally occurring SAM analogues such as carboxy-SAM, produced by the E. coli enzyme CmoA. It has been suggested that some MTs may have been incorrectly classed as SAM-MTs and actually use carboxy-SAM in carboxymethyl transfer reactions. Some SAM derivatives also have the advantage of increased stability in comparison to SAM itself. Huber et al. described the synthesis of SAM isosteres as competent cofactors for DnrK, a prototypical Class I MT involved in the biosynthesis of daunomycin. These SAM isosteres are reported to display resistance to typical SAM degradation mechanisms. For example, 13°AdoMet (Figure 1) is fully resistant to depurination and intramolecular cyclisation degradation mechanisms. When studied, 13°AdoMet demonstrated no degradation activity when measured over 55 hrs. Currently, there are no examples of the use of SAM analogues with carboxyl MTs. However future research in this area could increase the versatility and applications of this class of MTs through transfer of alternative alkyl groups and potentially overcoming the stability limitations of SAM.

6. Summary

Carboxyl MTs are a potentially valuable subgroup of MT enzymes that could be exploited in biotechnology for the in situ synthesis of methyl esters under aqueous conditions. This would enable incorporation of a methyl ester formation into synthetic biology approaches to chemical synthesis for a wide range of products from biodiesel to pharmaceuticals. The enzymes could be used in vitro or as part of a whole cell system for multistep synthesis. Most carboxyl MT research has focused on the natural substrates of protein carboxyl MTs and the SABATH family of MTs in plants. However, these enzymes represent a potential source of biocatalysts for applications in industrial biotechnology, with some promising conversions already demonstrated. Large scale application of carboxyl MTs as well as other families of SAM-dependent MTs is currently limited by the requirement of SAM as a cofactor. However, there is ongoing research to overcome these challenges, including upregulation of SAM production and new methods for cofactor recycling. Currently, upregulation and enzymatic recycling of SAM within cells maybe the best approach for industrial biotechnology applications, given its sensitivity and cost. Use of SAM analogues has potential to impact future SAM-MT research in terms of extending the range of product esters and providing more stable cofactors. These approaches require further improvement, but they demonstrate the potential of SAM-MTs to be applied more widely and on an industrial scale.

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Conflict of Interest

The authors declare no conflict of interest.

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