BIOTRANS
LA ROCHELLE 2023

N°1061 / PC
TOPIC(s) : Biocatalytic cascade reactions

Chemoselective Methyltransferases – Distinguishing between N- and O-Methylation

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PURPOSE OF THE ABSTRACT
Methylation reactions are of great interest for pharmaceutical industry. The small carbon fragment can change the pharmacological functions of drugs in various ways. This phenomenon is described as the 'magic methyl effect'. In industry, reagents such as highly toxic methyl iodide or dimethyl sulfate are used. As an alternative to traditional chemical methods, biocatalytic approaches present a sustainable route for the transfer of methyl groups.[1]
Methyltransferases (MTs) catalyse methylations in different biosynthetic pathways. They transfer the methyl group from the cofactor S-adenosyl-L-methionine (SAM) onto different atoms chemo- and regioselectively. Regarding the (hetero) atom that receives the methyl group, MTs are divided into O-, N-, S- and C-MTs. The enzymatic reaction is based on an SN2 mechanism,[2] involving different enzyme residues to increase the nucleophilic character of the species attacking the electrophilic methyl group.
There are several classification systems for MTs available. Joshi et al. introduced a system for O-MTs. One key difference between class I and II O-MTs is their dependance on metal ions. Class II enzymes do not require divalent cations and are often found in plant organisms catalysing reactions involved in secondary metabolism.[3] The classes are not strictly limited to O-MTs but also include N-MTs with related amino acid sequences. Hence, the anthranilic acid N-MT (ANMT) from Ruta graveolens (Rg) is considered as a class II member, just like its closest relatives caffeic acid O-MTs (CaOMTs).[4]
Zubieta et al. identified a histidine in position 269 to be the catalytic base in CaOMT from Medicago sativa (Ms) deprotonating the hydroxyl group of the natural substrate.[5] RgANMT contains a histidine residue in the same position. Nevertheless, both enzymes are highly chemoselective and methylate different functional groups (Figure 1 b/c).[4] This indicates that the mechanisms of the enzymes or the involved residues must differ in some ways.[5] Here, we present an extended substrate scope for RgANMT and a CaOMT from Prunus persica (Pp). The substrates analysed contained both, an amino- and hydroxyl group and were methylated chemoselectively leading to a broad range of pure products. This offers a good starting point for upscaling biocatalytic reactions methylating more complex molecules with different nucleophiles.[6, 7]

FIGURES

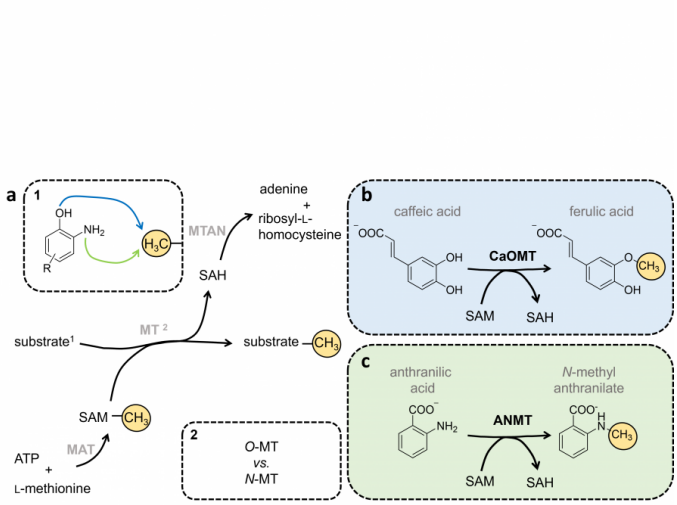

FIGURE 1

Three-enzyme cascade for methylation reactions

a: Three-enzyme cascade used for methylation reaction. b: Natural methylation reaction of the caffeic acid O-MT (CaOMT) forming ferulic acid. c: Natural reaction of anthranilic acid N-MT (ANMT) methylating anthranilic acid in N-position.

FIGURE 2

KEYWORDS

chemoselective methyltransferases | S-adenosyl-l-methionine | enzyme cascade

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