Structural and kinetic studies of a novel nerol dehydrogenase from Persicaria minor, a nerol-specific enzyme for citral biosynthesis

ABSTRACT

Geraniol degradation pathway has long been elucidated in microorganisms through bioconversion studies, yet weakly characterised in plants; enzyme with specific nerol-oxidising activity has not been reported. A novel cDNA encodes nerol dehydrogenase (PmNeDH) was isolated from Persicaria minor. The recombinant PmNeDH (rPmNeDH) is a homodimeric enzyme that belongs to MDR (medium-chain dehydrogenases/reductases) superfamily that catalyses the first oxidative step of geraniol degradation pathway in citral biosynthesis. Kinetic analysis revealed that rPmNeDH has a high specificity for allylic primary alcohols with backbone ≤10 carbons. rPmNeDH has ~3 fold higher affinity towards nerol (cis-3,7-dimethyl-2,6-octadien-1-ol) than its trans-isomer, geraniol. To our knowledge, this is the first alcohol dehydrogenase with higher preference towards nerol, suggesting that nerol can be effective substrate for citral biosynthesis in P. minor. The rPmNeDH crystal structure (1.54 Å) showed high similarity with enzyme structures from MDR superfamily. Structure guided mutation was conducted to describe the relationships between substrate specificity and residue substitutions in the active site. Kinetics analyses of wild-type rPmNeDH and several active site mutants demonstrated that the substrate specificity of rPmNeDH can be altered by changing any selected active site residues (Asp280, Leu294 and Ala303). Interestingly, the L294F, A303F and A303G mutants were able to revamp the substrate preference towards geraniol. Furthermore, mutant that exhibited a broader substrate range was also obtained. This study demonstrates that P. minor may have evolved to contain enzyme that optimally recognise cis-configured nerol as substrate. rPmNeDH structure provides new insights into the substrate specificity and active site plasticity in MDR superfamily.

Keyword: Nerol dehydrogenase; Persicaria minor; Citral biosynthesis; Crystallography; Mutagenesis