Biochemical characterisation of berberine bridge enzyme (BBE), a covalently flavinylated oxidase involved in benzophenanthridine alkaloid biosynthesis

Winkler A., Macheroux P.
Institute of Biochemistry, Graz University of Technology, Petersgasse 12/2, 8010 Graz

Berberine bridge enzyme ((*S*)-reticuline oxidase) is an important enzyme of alkaloid biosynthesis in specific plants, catalysing the oxidative transformation of the N-methyl group of (*S*)-reticuline to the berberine bridge C-8 carbon atom of (*S*)-scoulerine [1]. Due to the unique reaction catalysed by BBE a detailed biochemical characterisation and elucidation of the 3-dimensional structure are of high interest for further structure-function studies. In order to provide sufficient quantities of the enzyme, we heterologously expressed BBE from *Eschscholzia californica* in *Pichia pastoris* enabling the isolation of 110 mg homogenous protein per litre fermentation volume in a two step purification procedure.

Biochemical characterisation primarily focused on the covalently attached flavin cofactor and its spectral properties. Similar to other members of the group of flavin containing oxidases, BBE stabilises a red semiquinone radical intermediate upon anaerobic photoreduction and forms a characteristic sulphite adduct. The identity of the flavin was then further analysed by HPLC separation of tryptically digested enzyme and subsequent mass spectrometric analysis of the flavin containing peptide. Additional experiments addressed the characteristic flurorescence of the flavin cofactor and the N-glycosylation pattern of BBE, which might be important for the overall stability of the enzyme.

Besides the biological importance and the unique mechanism of this reaction, it could also serve as a potential chemical catalyst. For biocatalysis, only the first part of the biological reaction would be exploited and should result in N-demethylation of substrates which lack the structural requirements for completing the full cyclisation reaction. The applicability of BBE for this purpose will be tested on a variety of substrates ranging from small N-methyl containing compounds to larger substrates resembling the core structure of (*S*)-reticuline.

References

[1] T.M. Kutchan, H. Dittrich: Characterization and Mechanism of the Berberine Bridge Enzyme, a Covalently Flavinylated Oxidase of Benzophenanthridine Alkaloid Biosynthesis in Plants, *J Biol Chem*, **1995**, *270*, 24475-24481.