

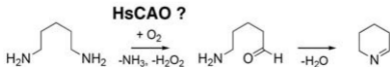
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Huperzia serrata 由来新規銅含有アミノオキシダーゼの探索

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【Objective】 *Huperzia serrata* is a traditional Chinese medicinal plant that contains Lycopodium alkaloids including Huperzine A (HupA), a potent, highly specific and reversible inhibitor of acetylcholinesterase. However, despite medicinal importance of the product, the biosynthetic pathway of the alkaloids remains poorly understood. To elucidate the biosynthetic pathway of Lycopodium alkaloids, we carried out the molecular cloning and characterization of a copper amine oxidases (CAO) gene from the Chinese club moss.

【Method & Results】 A full-length cDNA encoding 681 amino acids was cloned from roots of *H. serrata* and subcloned into pET22b (+) vector. The recombinant HsCAO was thus expressed in *E. coli* BLR as a his-tag fusion protein at C-terminus, and tested for enzyme activities using aromatic and aliphatic primary amines as substrates. The amino acid sequence showed 44-56% identity to other CAOs, and a consensus sequence Asn-Tyr-Asp/Glu of CAO. Interestingly, LC-MS analyses revealed that HsCAO accepts cadaverine to produce piperieine, which is the putative precursor of Lycopodium alkaloids. Furthermore, steady-state kinetics analyses of HsCAO, with respect to a H₂O₂ formation activity, also demonstrated that HsCAO shows a better catalytic efficiency for cadaverine, than the other tested amine substrates. The findings thus provided important information to shed light on the understanding of the biosynthetic pathway of the Lycopodium alkaloids.



【Reference】 Sun, Morita, Chen, Noguchi, and Abe, Manuscript in preparation (2011).