The Nodulation Factor Hydrolase of Medicago truncatula : Characterization of an Enzyme Specifically Cleaving Rhizobial Nodulation Signals

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Nodule formation induced by nitrogen-fixing rhizobia depends on bacterial nodulation factors (NFs), modified chitin oligosaccharides with a fatty acid moiety. Certain NF scan be cleaved and inactivated by plant chitinases. However, the most abundant NF of Sinorhizobium meliloti, an O-acetylated and sulfated tetramer, is resistant to hydrolysis by all plant chitinases tested so far. Nevertheless, this NF is rapidly degraded in the host rhizosphere. Here, we identify and characterize MtNFH1 (for Medicago truncatula Nod factor hydrolase 1), a legume enzyme structurally related to defense-related class V chitinases (glycoside hydrolase family 18). MtNFH1 lacks chitinase activity but efficiently hydrolyzes all tested NFs of S. meliloti. The enzyme shows a high cleavage preference, releasing exclusively lipodisaccharides from NFs. Substrate specificity and kinetic properties of MtNFH1 were compared with those of class V chitinases from Arabidopsis (Arabidopsis thaliana) and tobacco (Nicotiana tabacum), which cannot hydrolyze tetrameric NFs of S. meliloti. The Michaelis-Menten constants of MtNFH1 for NFs are in the micromolar concentration range, whereas nonmodified chitin oligosaccharides represent neither substrates nor inhibitors for MtNFH1. The three-dimensional structure of MtNFH1 was modeled on the basis of the known structure of class V chitinases. Docking simulation of NFs to MtNFH1 predicted a distinct binding cleft for the fatty acid moiety, which is absent in the class V chitinases. Point mutation analysis confirmed the modeled NF-MtNFH1 interaction. Silencing of MtNFH1 by RNA interference resulted in reduced NF degradation in the rhizosphere of M. truncatula. In conclusion, we have found a novel legume hydrolase that specifically inactivates NFs.
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