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Identification of the Amine Substrate Specificity Domain of Pepper Tyramine and Serotonin *N*-Hydroxycinnamoyltransferases by Chimeric Constructs

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Objectives

In spite of considerable progress in the study of *N*-hydroxycinnamoyltransferases, at both the molecular and substrate specificity analysis levels, a more detailed study of the residues and domains required for substrate binding and enzyme activity has not been conducted. In this study, we attempted to determine the regions of *N*-hydroxycinnamoyltransferase that are required for amine binding and enzyme activity, using pepper *N*-hydroxycinnamoyltransferases.

Materials and Methods

We first isolated from pepper a tyramine *N*-hydroxycinnamoyltransferase (THT) that is not able to use serotonin as an acyl acceptor. A functional analysis using chimeric proteins constructed from the serotonin *N*-hydroxycinnamoyltransferase (SHT) and THT sequences was performed to identify a domain responsible for the amine-binding specificity, as well as key amino acids in the pepper SHT and THT proteins.

Results and Discussion

Pepper SHT catalyzes the synthesis of *N*- hydroxycinnamic acid amides (HCAAs) of serotonin, including feruloylserotonin and *p*-coumaroylserotonin. To elucidate the domain that determines the amine substrate specificity, we isolated a tyramine *N*-hydroxycinnamoyltransferase (THT) gene from *Capsicum annuum*. Purified recombinant THT protein catalyzed the synthesis of HCAAs of tyramine, including feruloyltyramine and *p*-coumaroyltyramine, but did not accept serotonin as a substrate. Both the SHT and THT mRNAs were found to be expressed constitutively in all pepper organs. Pepper SHT and THT, which have primary sequences that are 75% identical, were used as models to investigate the structural determinants responsible for their distinct substrate specificities and other enzymatic properties. A series of chimeric genes was constructed by reciprocal exchange of DNA segments between the SHT and THT cDNAs. Functional characterization of the recombinant chimeric proteins revealed that the amino acid residues 129-165 of SHT and the corresponding residues in THT, 125-160, are critical structural determinants for the amine substrate specificity. Several amino acids, in particular Gly-158, are strongly implicated in the determination of the amine substrate specificity. The results from the chimeras and the kinetic measurements provide a framework for three-dimensional structural analysis; they will also direct the creation of additional novel *N*-hydroxycinnamoyltransferases from the various *N*-hydroxycinnamoyltransferases found in nature.

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