학술상1

STRUCTURE AND FUNTION OF MULTIMER ASSEMBLY OF AVENACOSIDASE

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Avenacosidase (oat β-glucosidase) hydrolyzes avenacosides to C26-desgluco- avenacosides which have anti-fungal activity. The enzyme exists in two isomeric forms of type I and type II in oat plastid. It has a unique quaternary protein structure of a three dimensionally radiated assembly of long fibrillae. Type I isozyme is a homomultimer of As-Glu 1 subunit and type II is a heteromultimer of As-Glu 1 and As-Glu 2 subunits. The cDNAs of As-Glu 2 encodes a plastid-directing transit peptide of 57 amino acid residues and mature proteins of 521 amino acid residues, and the amino acid sequences of both As-Glu 1 and As-Glu 2 subunits are highly homologous each other. When expressed into an active enzyme, the As-Glu 1 subunit plays a crucial role for the formation the multimer assemblies of both type I and type II isozymes. Type I multimer is more stable than type II multimer but lower in catalytic activity.The assembly of long fibrillar structure of type I enzyme has been elucidated by cryo-electron microscopy: Type I avenacosidase is assembled by a linearstacking of hollow trimeric units and the resulting fibril has a long central tunnel connecting to the outer medium via regularly distributed side fenestrations. The enzyme kinetics and chemical modification of type I indicate that the enzyme active sites are localized within the central tunnel of the long fibrillar assembly. This unique multimer assembly increased enzyme affinity to the *in vivo* substrate, avenacosides, and the side fenestrations are likely to have a regulatory role of the substrate entry to the active sites which may function to discriminate avenacosides from many other kinds of β-glucosides in oat seedlings. Molecular swapping and site-directed mutagenesis indicate that the crucial binding sites for the multimer assembly are located in M479-N498 of As-Glu 1 subunit. These results together indicate that the long fibrillar multimer of avenacosidase is a novel quaternary protein structure that may increase the specificity of enzyme for avenacosides.

References

1. Y.-W Kim, P.-S. Song and I.-S. Kim (1996) Purification and characterization of isoenzymes of β-glucosidase from etiolated oat seedlings. Mol. Cells, 6, 773-779