

Kex2 protease converts the endoplasmic reticulum alpha1,2-mannosidase of *Candida albicans* into a soluble cytosolic form.

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Abstract

Cytosolic alpha-mannosidases are glycosyl hydrolases that participate in the catabolism of cytosolic free N-oligosaccharides. Two soluble alpha-mannosidases (E-I and E-II) belonging to glycosyl hydrolases family 47 have been described in *Candida albicans*. We demonstrate that addition of pepstatin A during the preparation of cell homogenates enriched alpha-mannosidase E-I at the expense of E-II, indicating that the latter is generated by proteolysis during cell disruption. E-I corresponded to a polypeptide of 52 kDa that was associated with mannosidase activity and was recognized by an anti-alpha1,2-mannosidase antibody. The N-mannan core trimming properties of the purified enzyme E-I were consistent with its classification as a family 47 alpha1,2-mannosidase. Differential density-gradient centrifugation of homogenates revealed that alpha1,2-mannosidase E-I was localized to the cytosolic fraction and Golgi-derived vesicles, and that a 65 kDa membrane-bound alpha1,2-mannosidase was present in endoplasmic reticulum and

Golgi-derived vesicles. Distribution of alpha-mannosidase activity in a kex2Delta null mutant or in wild-type protoplasts treated with monensin demonstrated that the membrane-bound alpha1,2-mannosidase is processed by Kex2 protease into E-I, recognizing an atypical cleavage site of the precursor. Analysis of cytosolic free N-oligosaccharides revealed that cytosolic alpha1,2-mannosidase E-I trims free Man8GlcNAc2 isomer B into Man7GlcNAc2 isomer B. This is believed to be the first report demonstrating the presence of soluble alpha1,2-mannosidase from the glycosyl hydrolases family 47 in a cytosolic compartment of the cell.

Involved units

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