Antifungal properties of *Canavalia ensiformis* urease and derived peptides

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Ureases (EC 3.5.1.5) are metalloenzymes that hydrolyze urea to produce ammonia and carbon dioxide. These proteins have insecticidal and fungicidal effects not related to their enzymatic activity. The insecticidal activity of urease is mostly dependent on the release of internal peptides after hydrolysis by insect digestive cathepsins. Jaburetox is a recombinant version of one of these peptides, expressed in *E. coli*. Another important property of ureases is their antifungal activity, which occurs in submicromolar doses for certain filamentous fungi, causing damage to the cell membranes, as visualized by scanning electron microscopy. Antifungal molecules from plants represent an alternative strategy to fight the emergence of resistant fungal species. Considering that the antifungal activity of urease is about 3-4 orders of magnitude more potent than most of the antifungal proteins already described, in this study, we evaluated the toxic effect of *C. ensiformis* urease (JBU) on different species of yeast. Additionally, studies aiming to identify antifungal domain(s) of JBU were carried out. The results showed that JBU exerts toxic effects on different yeast species, indicating that antifungal activity is not restricted to filamentous fungi. The effects of JBU in yeast varied according to the genus and species of yeasts, both qualitatively and quantitatively, indicating a species-specific selectivity. The fungitoxic effects consisted in inhibition of proliferation, induction of morphological alterations with formation of pseudohyphae, changes in the transport of H+ and carbohydrate metabolism, and permeabilization of membranes, eventually leading to cell death. Hydrolysis of JBU with papain resulted in fungitoxic fragments with molecular mass ~ 10 kDa. These peptides were analyzed by mass spectrometry, revealing the presence of a fragment containing the N-terminal sequence of the entomotoxic peptide Jaburetox. We tested the recombinant peptide Jaburetox for antifungal effects and observed its fungitoxic activity on yeasts and filamentous fungi. The antifungal activity of Jaburetox requires 2-3 fold larger concentrations than those required for the holoprotein JBU, suggesting the possibility that other protein domains are involved in this activity. The discovery of new antifungal agents is imperative to face the increasing number of cases of invasive mycoses. Plant ureases, such as JBU, and its derived peptides, may represent a new alternative to control medically important mycoses as well as phytopathogenic fungi, especially considering their potent activity in the range of 10⁻⁶ to 10⁻⁷ M.

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