ROLES OF CHITINASES IN Fungal Growth

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INTRODUCTION

There are three roles for chitinases in fungi:

(a) Most spectacularly, they are involved in the gross autolysis associated with the release of spores in some basidiomycete fruit bodies. Examples include the maturation of puffballs, Lycoperdon species, and the autodigestion of gill tissue following spore release in the ink-caps, Coprinus species.

(b) They have a nutritional role. In the case of soil saprophytes such as Aspergillus species and Trichoderma species, chitinase enables them to utilise chitinous debris from dead invertebrates and fungi as food sources. In the case of pathogens of crustacea, insects and fungi, chitinases also enable them to penetrate their hosts. Examples include the crayfish pathogen Aphanomyces astaci, insect pathogens such as Beauvaria and Cordyceps species. There is however no convincing evidence that there is any appreciable re-cycling of the chitin in cell walls even though chitinase accumulates to a marked extent in old cultures, i.e. there does not seem to be any appreciable autophagic utilisation of chitin in starving cultures.

(c) They have a morphogenetic role in the growth and differentiation of all chitin-containing fungi. This, the most fundamental of the three roles, has however proved the most difficult to obtain evidence for. Indeed some authors question whether chitinase and other lytic enzymes are involved in hyphal apical growth. Burnett suggests that "teleologically speaking, the apex would seem to be a most dangerous location for a lytic entity!", and Wessels proposes a model for hyphal growth that does not require wall lytic enzymes. The unitary model for hyphal growth proposed by Bartnicki-Garcia does however see the control of apical wall growth as being the result of a "delicate balance between wall synthesis and wall lysis", with some of the vesicles being transported to the apex containing lytic enzymes, to keep the wall at the apex in a plastic and extensible condition. Certainly in every case investigated chitinase activities can be detected in actively growing chitinous fungi. Examples include Mucor and Phycomyces species, Neurospora crassa, Aspergillus nidulans, Saccharomyces cerevisiae and Candida albicans.
The rest of this article asks the question "is chitinase involved in the vegetative growth of hyphae?"

CHITINASE SYSTEMS IN MUCOR MUCEDO

When Mucor mucedo is grown on agar, three sites of chitinase activity can be distinguished:

(i) High-speed centrifugation of cell homogenates yields a supernatant activity\textsuperscript{17-19}. From 18 hour cultures this is the major activity. It has not yet been purified, and may be heterogeneous.

(ii) This centrifugation also yields a microsomal activity. Quantitatively this is only a minor activity, but it has important properties: it is membrane-bound, showing a bi-phasic Arrhenius plot and requiring phospholipids; it can be solubilised by mild detergents such as Triton X-100 or Zwittergent-14; it is partially zymogenic, being activated by trypsin; and as we shall show below, it appears to be physically associated with chitin synthase.

(iii) There is extracellular activity. This has not been characterised further.

We have no further information on the sub-cellular localisations of the supernatant or microsomal activities, or on any relationships between the enzymes in these three sites. In Saccharomyces cerevisiae about half of the chitinase was in intracellular vesicles, and the other half was in the periplasm, clearly suggesting a process of storage and secretion\textsuperscript{24}.

Cellular chitinase during growth

Using methods described before\textsuperscript{17-19}, the microsomal and supernatant chitinase activities were assayed during the growth of Mucor mucedo strain Z46 (+) on agar (Table 1). Under these conditions, germ tubes formed at about 12 hours, the first branches at about 15 hours, exponential growth ceased at about 30 hours, and sporulation occurred at about 48 hours. The sporangiospores had detectable chitinase activities. The ratio of microsomal:supernatant chitinase was much higher in spores and in early germinating spores than in the resultant mycelial cultures. The ratio of native microsomal chitinase activity to trypsin-treated activity increased dramatically during the first 5 minutes of germination. The specific activity of the supernatant chitinase showed little change from spores and then throughout mycelial growth and differentiation.

Association between microsomal chitinase and chitin synthase

In these cultures of Mucor mucedo, the microsomal chitinase and chitin synthase have been shown to have the following properties in common: (i) they are both membrane-bound, requiring phospholipids for activity\textsuperscript{17-19}, and (ii) they are both partially zymogenic, being activatable by endogenous or exogenous proteases\textsuperscript{17-19}. From the results in Table 1 it is seen that the activities of the two enzymes follow very similar trends during growth of a culture. Both have high specific activities in spores, and both show a very marked increase in ratio of native to trypsin-activated activity after 5 minutes germination.

Evidence for a physical association between the two enzymes comes from two types of experiment; assays for net chitin synthesis \textit{in vitro} and \textit{in situ}.