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Chitinases and Peptide Mimotopes

Inhibition of glycosylhydrolases by peptides mimicking carbohydrates opens a new field in antifungal research.

The manuscript of Rao et al. [1] is a detailed study of the interactions between two cyclopentapeptides, argifin and argafin, and a "bacterial" chitinase secreted by the fungus *Aspergillus fumigatus*.

Chitin is one of the major structural components of the cell wall of fungi and the exoskeleton of animals. The presence of chitin in the extracellular matrix of fungi is in total agreement with the animal origin of fungi. Over time, enzymes associated with the synthesis and remodeling of this polysaccharide have evolved in number and complexity. Recent surveys of fungal genomes have shown that *A. fumigatus*, whose genome is one of the most recently defined among the filamentous fungi, has 8 chitin synthase and 18 chitinase genes whereas only 3 and 2 respectively have been found in yeasts.

At some point during their complex life cycle, such as cytokinesis in yeast and conidial germination or hyphal branching in molds, all pathogenic fungi have to hydrolyze chitin. Accordingly, chitinase was thought for a long time to be an attractive antifungal target. Chitinase inhibitors could have potential against pathogenic fungi, but also against insects and parasites, since these organisms have homologous chitinases. All putative inhibitors will be totally harmless to humans since chitinases have no essential role for the human organism [2]. New antifungals targeted to cell wall are especially needed for fungi like *A. fumigatus*, that is to date the most threatening air-borne pathogen in industrialized countries and is responsible for numerous deaths among immunocompromised patients. High mortality in the hospital setting is due to lack of efficient antifungals available to the clinician.

Chitinases are universal enzymes found in fungi, bacteria, parasites, plants, and insects. Two families of chitinases (plant and bacteria) are distinguished based on sequence homologies. These two families have the same conserved block of amino acids containing invariant Asp and Glu residues (DXE motif) implicated in the catalytic mechanism. In filamentous fungi, such as *Aspergillus* and *Coccidioides* species, "bacterial" chitinases have deduced molecular masses of approximately 46–48 kDa, are secreted, and do not have potential *0*-glycosylation sites [3]. They are responsible for the digestion and utilization of exogenous chitin as a source of organic nutrients for energy and biosynthesis [4]. Accordingly, disruption of the *A. fumigatus* ChiB1, encoding the chitinase, analyzed in the study of Rao et al. [1], had no effect on growth or morphogenesis of the organism. The "plant" chitinases of *Aspergillus* and *Coccidioides* species have an apparent MW higher than enzymes of the bacterial class (deduced molecular mass 83–97 kDa). Further, the plant chitinase family contains a serine-threonine-rich domain, suggesting potential sites of *0*-mannosylation as shown in *S. cerevisiae* [3]. This domain is followed by a cysteine-rich, high-affinity chitin binding region [4]. Five homologs of the plant chitinases have been identified in *A. fumigatus*. Only one member of this family has a putative GPI anchor and associated cleavage site (http://www.tigr.org/tdb/e2k1/afu1/), suggesting that this enzyme may function in morphogenesis since most of the GPI anchored proteins of *A. fumigatus* have a putative role in cell wall construction [5]. Disruption of the ortholog of this gene in *A. nidulans* leads indeed to a decrease in the frequency of spore germination and a lower rate of hyphal formation [6]. In *A. fumigatus*, the role of the four other plant chitinases will await the disruption of their encoding genes [4]. Although analyzing the interactions of inhibitor peptides with a chitinase that have a role in morphogenesis would have been more appropriate in the study of Rao et al., the results obtained with ChiB1 should be easily compared with plant chitinases. The 3D structure has been determined for various chitinases [7]; data of all families substantiate the strict conservation of the catalytic machinery, show the cleft structure in the active site, and show that substrate degradation occurs with retention of the configuration at the anomeric carbon. Sequence similarities are also confirmed biologically since anti-human chitinase antibodies have been shown by immunofluorescence studies to label the tip of *A. fumigatus* conidial germ tubes (J.-P. Latgé, unpublished data).

The two cyclopentapeptides described by Rao et al. [1] not only mimic the carbohydrate substrate of the glycosylhydrolase but also inhibit the activity because of their binding capacity to the catalytic site. The concept of using peptides that mimic carbohydrates to fight microorganisms emerged in the 1990s. While carbohydrates are critical molecules in the interaction between microbes and host cells, they are not readily studied molecularly using genetic vectors and are poor immunogens. Therefore, the use of peptides (easy to produce, characterize, and manipulate) as substitutes for natural polysaccharides has been developed. To date, most studies in this area are focused on vaccination and immunodiagnosis. For example, in *Mycobacterium tuberculosis*, peptides which mimic the oligomannosidic motifs shared by the bacterial cell envelope are now exploited in the generation of immune responses against *Mycobacterium*, broadening the options for vaccine development against tuberculosis [8]. Similarly, in the fungus *Cryptococcus neoformans*, a peptide mimetic of the capsular polysaccharide glucuronoxylomannan induces a protective response against this fungus [9]. In the MethA tumor, a peptide mimetic of the *0*-b linked N-acetylglucosamine present on the tumor surface-expressed glycoproteins stimulates the regression of the tumor via the activation of specific antitumor cellular responses [10].

Besides argifin and argafin cyclopentapeptides, only two other reports exist in the literature on the role of peptides that block protein activity because they are recognized by the carbohydrate binding site of this protein. The first study concerns peptides mimicking the β-galactose N-terminal moiety of glycosphingolipids which have important roles in cell recognition by viruses and microorganisms [11]. This peptide, potentially interesting for vaccination, has also a physiological activity since it has a potent inhibitory effect on β-galactosi-

dase, thus indicating that it may act specifically on the enzymes. The other study comes from the research group of van Aalten, which investigated the inhibition of another bacterial chitinase by a cyclic dipeptide called CI-4 [12]. However, until now, the mechanism of interaction between the peptide-mimicking carbohydrates and the protein, and its binding site, was unknown; the structure of the complexes between chitinase and the cyclic peptide presented by Rao et al. [1] give several hints on how to increase the affinity of the peptides for the family 18 chitinase and how to modify, for example, the side chains to increase the efficiency of the inhibitor. Concurrent analysis of the data presented by Rao et al., and another prominent study of a peptide binding to the a mannopyranoside site of ConA, showed related types of hydrogen bonds and hydrophobic interactions during the replacement of a carbohydrate by a peptide [13].

In conclusion, a new development of carbohydrate minotopes is seen through this study. Screening of natural peptides or peptide libraries, chemically synthesized or obtained by phage display, could lead to the identification of new, simple inhibitors with known interactions. The manuscript of Rao et al. is a landmark in this area. It provides not only a way to analyze interactions and modify chemically the cyclopeptide inhibitors to obtain species-specific inhibitor but also opens new research avenues for nonsugar derivatives to inhibit glycosylhydrolases. The results also suggest a potential for peptides to inhibit other cell wall enzymes in addition to the chitinases. For example, echinocandins are cyclohexapeptides that inhibit fungal β1,3 glucan synthases. Based on the results of Rao et al., the use of peptides that mimic carbohydrates to inhibit glycosylhydrolases should become prominent in the near future.

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Selected Reading

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