THE COMPLEX CHITINOLYTIC SYSTEM OF ASPERGILLUS FUMIGATUS

THE COMPLEX CHITINOLYTIC SYSTEM OF *ASPERGILLUS FUMIGATUS*

by

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Abstract

Aspergillus fumigatus is one of the most important fungal pathogens of humans and there is an urgent need for new drugs to counter infections caused by *A. fumigatus* and other pathogenic species. Enzymes of chitin metabolism, and their regulators present novel targets for antifungal agents. During the work described here, the patterns of expression of the *chiA1* and *chiB1* chitinase genes of *A. fumigatus* during batch culture were investigated using real-time, reverse-transcription PCR. The *chiA1* gene, encoding the fungal/plant chitinase ChiA1, was expressed at significant levels throughout the six days of culture. However, the level of expression of *chiB1*, encoding the fungal/bacterial chitinase ChiB1, was only just detectable on day one but had been induced 1280-fold, to a level similar to that detected for *chiA1* expression, by day 6. The results suggest markedly different roles for these enzymes. The gene encoding the transcription factor CreA was cloned and expressed, as a glutathione S-transferase (GST) fusion protein, in *Escherichia coli*. In electrophoretic mobility shift assays purified GST-CreA, or an *A. fumigatus* cell extract, bound specifically to putative CreA binding sites upstream of the *chiB1* gene. CreA may therefore have a role in the regulation of chitinase activity in *A. fumigatus*. The effects of a range of compounds on *A. fumigatus* chitinase activity were determined. The cyclopentapeptides, argadin and argifin (each at 0.6 μM), were potent inhibitors of enzyme activity. The cyclic dipeptides, D-Leu-D-Pro, cyclo-(D-Leu-D-Pro) and cyclo-(L-His-L-Pro) (each at 300 μM), did not inhibit chitinase activity, while the methylxanthines, pentoxyfylline and theophylline, caused significant inhibition at concentrations of 75 μM and 300 μM, respectively. In preliminary expression studies, ChiA1 was fused with GST or maltose-binding protein (MBP) and expressed in *E. coli*. In addition, ChiA1-His_6 peptide was expressed in *Pichia pastoris*. These constructs will be used in future work which will further explore the complex chitinolytic system of *A. fumigatus* and which may lead to the exploitation of this system as a target for antifungals.

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Abbreviations

The following abbreviations are used:

ATCC	American Type Culture Collection
ddH_2O	Double distilled water
DIG	Digoxygenin
DMSO	Dimethylsulfoxide
DTT	Dithiothreitol
EDTA	Diaminoethane tetraacetic acid, disodium salt
EMSA	Electrophoretic Mobility Shift Assay
GlcNAc	N-acetylglucosamine
GST	Glutathione S-transferase
HEPES	N-[2-Hydroxyethyl]piperazine-N'-(2-ethylsulfonic acid)
IPTG	Isopropyl β-D thiogalactoside
LB	Luria-Bertani
MBP	Maltose-binding protein
MMS	Minimal Medium with 1 M sucrose
MOPS	3-(N-morpholino)propanesulfonic acid
MU	Methylumbelliferone
NBT/BCIP	Nitro blue tetrazolium chloride / 5-Bromo-4-chloro-3-indoyl phosphate
NCCLS	The U.S National Committee of Clinical Lab Standard
NTP	Nucleoside Triphosphates
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PEG	Polyethylene glycol

PMSF	Phenylmethylsulphonylfluoride
rpm	Revolutions per minute
SDS	Sodium dodecyl sulphate
TBS	Tris-buffered saline
Tris	Tris(hydroxymethyl)aminoethane
TTBS	Tween Tris-buffered saline
YNB	Yeast Nitrogen Base with ammonium sulfate without amino acids
YPD	Yeast Peptone Dextrose

Chapter 1

General Introduction

1.1 Human Mycoses

Fungi can cause a variety of diseases of humans and other animals ranging from minor superficial skin and mucous membrane infections to life-threatening, systemic involvement of the internal organs. There are three major types of disease caused by fungi: allergies, poisonings and fungal infections (Schaechter *et al.*, 1998). Allergic reactions to fungi are caused by sensitivity to fungal proteins, such as those present in inhaled fungal spores, while poisonings result from the ingestion of fungal toxins in contaminated food or poisonous mushrooms. Fungal allergies and poisonings are important concerns in agriculture and other industries where fungal contamination is common. Fungal infections, or mycoses, result from the invasion of living tissue by a fungus and they represent the most common form of fungal disease (http://www.merckshарpedohme.com).

There are more than 100,000 recognised species of fungi, but only about 400 are known to infect humans (de Hoog & Guarro, 1995). Some species of fungi, called primary pathogens, cause disease regardless of the individual's state of health. Other species that infect individuals with a weakened immune system are described as opportunistic pathogens. However, among patients with severe immunosuppression, almost any type of fungus can exhibit pathogenic potential which may have devastating consequences for the host (Dunn, 2000). The most common fungal infections in immunocompromised hosts are candidiasis, aspergillosis, cryptococcosis, mucormycoses (zygomycoses) and *Pneumocystis carinii* pneumonia (http://merckmedicus.com).