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Structural Studies on Hydrophobins from *Neurospora*

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ABSTRACT.

Background:

Hydrophobins are a group of low molecular weight, cysteine-rich, fungal cell-wall proteins with unique biophysical properties. Principal among these is the ability of hydrophobin monomers to self-assemble into insoluble, chemically resistant amphipathic films at the interface between hydrophobic and hydrophilic surfaces. This enables fungi to coat their hyphae and fruiting bodies with a hydrophobic layer that prevents these structures from becoming waterlogged. Proposed industrial and medical applications have sought to exploit these protein's polymeric hydrophobins to reverse the wettability of a surface upon binding.

The hydrophobin protein EAS (product of the gene *eas*) coats macroconidia produced by the model ascomycete *Neurospora crassa*, making this species an ideal subject for structural studies on hydrophobins.

Results:

(1) Genes homologous to *eas* were detected in each of the *Neurospora* species examined. EAS proteins isolated from each of the conidiating species proved to be identical to that known in *N. crassa*. The aconidiate homothallic *Neurospora* species also possess copies of *eas,* essentially identical to that from *N. crassa*, but transcription studies implied that the gene is inactive in these species.

(2) I attempted to express EAS in its native form and I succeeded in generating recombinant *Pichia pastoris* and *Escherichia coli* as isolates. However, I did not detect the expression of EAS in any of these isolates. This was despite the fact that the *Pichia* isolates were actively transcribing the recombinant gene.

(3) EAS was chemically digested according to Wu and Watson (1997). Mass spectrometric analysis of these digests revealed that the four intramolecular disulfide bridges in EAS exist between Cys_9-Cys_{60}, Cys_{18}-Cys_{54}, Cys_{19}-Cys_{45}, and Cys_{61}-Cys_{80}. This arrangement is identical to that recently determined for the class II hydrophobin HFB2.

(4) Atomic force microscopic analysis of rodlet films deposited on hydrophilic mica and hydrophobic graphite revealed the presence of a central cleft in the hydrophobic and hydrophilic sides of individual rodlets. This cleft is believed to be the boundary between the long protofilaments that are bundled together to form polymeric rodlets. Also seen were shorter oval structures, consistent with short protofilaments detected during real-time analysis of amyloid polymerisation.

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