Biosynthesis of the Fungal Wall – Mechanisms and Implications

The First Fleming Lecture

By G. W. GOODAY

Department of Microbiology, University of Aberdeen

*(Delivered at the General Meeting of the Society for General Microbiology
on 16 September 1976)*

‘The blue and white and several kinds of hairy mouldy spots... are all of them nothing else but several kinds of small and variously figur’d Mushrooms,... which will not be unworthy of our serious speculation and examination, as I shall by and by shew.’ Robert Hooke, *Micrographia*, 1665.

‘...Consumption 129...Feaver 332...Plague 6544...Thrush 6...’ *The Diseases and Casualties this Week, London, From the 12 of September to the 19, 1665.*

In 1665, at the same time as Robert Hooke gives us the first clear account of microscopic fungi, the official record lists thrush as a fatal disease. We now ascribe this to the fungus *Candida albicans*. Perhaps it is surprising that it was not overlooked altogether during the ravages of the Great Plague, but today we recognize it as one of a number of medical mycoses that are becoming increasingly important.

Fungi have long been overshadowed as agents of human disease by the more virulent viruses, bacteria and protozoa. However, they are now more prevalent – in part because of the success of Alexander Fleming and his contemporaries in their battle to cure and prevent viral and bacterial disease. Fleming (1929), in his original paper describing penicillin, emphasized its selective action in allowing ready growth of the resistant *Haemophilus influenzae* by preventing growth of its competitors. In the same way the use of antibacterial antibiotics will selectively encourage the growth of fungi. This is probably too simplistic an explanation in the complexities of human disease, but nevertheless it is clear that sophisticated therapies using antibacterial antibiotics, and steroid and immunosuppressive drugs, can lead to a much greater incidence of fungal pathogens.

The fungi are eukaryotic, and so from the point of view of antibiotic therapy they are more insidious pathogens than the bacteria. There are fewer differences in essential metabolism between them and us for antibiotics to exploit, and so it is no surprise that there are far fewer useful antifungal antibiotics. Those currently in therapeutic use include griseofulvin which appears to exploit a difference in mitotic spindle construction to specifically disrupt fungal mitosis (Gull & Trinci, 1973), and the polyene antibiotics, such as nystatin and amphotericin, which appear to exploit a difference in cell membrane construction to specifically disrupt the composition of the fungal cytosol (Gale, 1974; Kerridge, Koh & Johnson, 1976). But if we are to extend the rational antifungal chemotherapy we must learn more about how fungi grow and develop.

The fungal spore germinates by producing a hypha whose branches grow out and fill in the spaces between each other to give the familiar expanding circular colony (Bull & Trinci, 1977; Carlile, 1977). Such a mode of growth is well suited to penetration through a substrate, be it dead wood, a living apple, or human tissue. That the resultant mycelial growth is known to a very wide audience is not only because of Fleming’s illustration of his con-

taminated culture plate, but also because of his own obvious interest in its form (Fleming, 1945; Fleming & Smith, 1944). The origin and subsequent growth of his *Penicillium* culture have themselves been the subject of much speculation, and more recently of direct experimentation (Hare, 1970).

The fungal wall

The most distinctive part of the fungal cell is its wall. Its protoplasm behaves much like any other eukaryotic protoplasm, and can be likened to an amoeba in a tube. The wall is the physical manifestation of the fungus, directly giving us the structures that we recognize as hyphae, spores and fruiting bodies.

The walls of fungal hyphae and yeast cells are all similar in that polysaccharides are the major constituents, but there is a considerable range of qualitative and quantitative composition. Bartnicki-Garcia (1970) divides the fungi into eight groups on the basis of their wall chemistry, and shows how this classification fits in well with others based on a range of criteria of phylogenetic significance. Except for the Oomycetes, which have cellulosic walls, the most characteristic component of fungal walls is chitin. All human pathogenic fungi have chitin in their walls (for references, see Bartnicki-Garcia, 1973).

Chitin, a (1→4)- β homopolymer of *N*-acetylglucosamine, is almost totally restricted to fungi and invertebrates, and in both it has a skeletal role. It is a strong, inert macromolecule forming very stable crystalline structures. Chemically it is analogous to the glycan backbone of peptidoglycan, the (1→4)- β alternate *N*-acetylglucosamine and *N*-acetylmuramic acid which is the characteristic component of prokaryotic walls. In the fungal wall it forms microfibrils, which can be visualized in the electron microscope by chemically or enzymically removing overlying wall components such as glucans and proteins (Hunsley & Burnett, 1970). In the growing hyphal apex these microfibrils are randomly oriented and extend right over the tip (Hunsley & Kay, 1976) and, presumably, are largely responsible for the structural integrity of the apex. In some cases, the microfibrils are predominantly oriented in a particular direction, more reminiscent of the orientations of cellulose microfibrils in the walls of higher plants and some algae. An example is seen in the stipe cells of the toadstool *Coprinus cinereus*, where the chitin microfibrils are predominantly transverse (Gooday, 1975). This must contribute greatly to the lateral strength of the wall, while still allowing elongation of the cell by intussusception of new microfibrils. The chitin is associated with other polymers in the walls, such as glucans and proteins, and so the wall has been likened to carbon-fibre reinforced resin (Winterburn, 1974).

X-ray diffraction patterns of fungal chitin indicate that it is in the form of α -chitin (Rudall, 1969). This is the most stable form and consists of chains of *N*-acetylglucosamine residues linked in piles by CO— —NH hydrogen bonds. Each chain is a helix, with two residues per turn. In each pile all of the chains are in the same direction, but adjacent piles are anti-parallel, so that the structure has alternating piles of chains running in opposite directions (Rudall & Kenchington, 1973).

Site of chitin deposition

In the growing vegetative hypha, the major localization of chitin deposition is at the hyphal apex, which is the site of invasion of substrate or tissue. This can be visualized by the use of light microscopic autoradiography (Gooday, 1971). For example, after 1 min incubation of hyphae of *Neurospora crassa* with *N*-[^3H]acetylglucosamine, the silver grain density over the apical 1 μm of the hyphal wall was more than 50 times higher than that from 50 to 75 μm behind the apex. The sub-apical deposition of chitin in vegetative hyphae, evidenced by their low and relatively uniform silver grain density on these autoradiographs, must

represent the process leading to the increases in the size of the chitin microfibrils and in the thickness of the chitin layer that are observed in electron micrographs (Hunsley & Burnett, 1968; Hunsley & Kay, 1976). Non-apical deposition of chitin also occurs during septum formation, during formation of side branches, where apices must arise *de novo* in the lateral hyphal walls, and during the rare intercalary elongation of cells in such specialized structures as the stipes of Agaric fruit bodies (Gooday, 1977).

The importance of chitin synthesis during tissue invasion is indicated by the finding of three times as much chitin in the walls of the invasive mycelial form of *C. albicans* as in the walls of the yeast form (Chattaway, Holmes & Barlow, 1968). Lehmann, White & Ride (1974) show how assaying for chitin gives a measure of the sites and extent of systemic infections of animals with *Aspergillus fumigatus*.

Chitin biosynthesis

Although chitin is structurally analogous to the backbone of bacterial peptidoglycan, its biosynthesis is quite distinct, as it is accomplished by just one enzyme activity. Curiously, lysozyme, discussed later from the point of view of its lytic action on peptidoglycan, can make a chitin-like polymer under some conditions (Kravchenko, 1967), but this process is totally unrelated to the synthesis of fungal chitin. Rather it is an extreme manifestation of the transferase activity that lysozyme shows with oligomers of chitin (Rupley & Gates, 1967).

Chitin synthase activity was first described by Glaser & Brown in 1957. It has since been reported from a wide range of fungi, and its properties appear to be very similar from all of these sources (Table 1). It would seem that the constraints put on the enzyme so that it makes a crystalline homopolymer in the right place at the right time have not allowed any significant variation in its properties.

The toadstool *Coprinus cinereus* was chosen as a source of the enzyme for the work described here with the reasoning, which has proved valid, that the dramatic elongation of the stipe during development would involve massive wall synthesis and a concomitant high activity of the enzyme. Apart from being a weed in some mushroom cultivations, and a tool for geneticists, the fungus has no economic significance to man, except that recently it became a fatal opportunist pathogen by growing on and occluding a heart valve following heart surgery (Speller & MacIver, 1971).

The enzyme preparations extracted from the stipe of *C. cinereus* can be related to biosynthesis in the living cell with some faith as they have an activity *in vitro* that is sufficient to account for the rate of chitin synthesis observed *in vivo*. For example, the observed increase in chitin content in elongating stipes is about $30 \mu\text{g mm}^{-1}$, which, at an elongation rate of about 10 mm h^{-1} , requires an enzyme activity of 24 nmol min^{-1} . Stipe homogenates show an enzyme activity approaching 30 nmol min^{-1} under optimum assay conditions.

The equation for the reaction (EC. 2.4.1.16) is:

The substrate, uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), activates the enzyme. This has the appearance of allosteric activation, as plots of velocity against substrate give sigmoidal curves. Hill plots give lines with slopes corresponding to a Hill number close to 4 at low substrate concentrations (below 0.1 mM) and close to 2 at higher concentrations (Figs 1 and 2; de Rousset-Hall & Gooday, 1975). This suggests that the enzyme molecule has four binding sites for UDP-GlcNAc, but there is reduced interaction

Table 1. *Comparison of chitin synthase preparations from different fungi*

Class	Chytridio- mycetes	Zygomycetes	Hemi- ascomycetes	Ascomycetes	Basidio- mycetes
Cell wall category*	V. Chitin- glucan	IV. Chitin- chitosan	VI. Mannan- glucan	V. Chitin- glucan	V. Chitin- glucan
Species	<i>Blastocladiella emersonii</i>	<i>Mucor rouxii</i>	<i>Saccharomyces cerevisiae</i>	<i>Neurospora crassa</i>	<i>Coprinus cinereus</i>
' K_m ' or $[S]_{0.5}$ for UDP-GlcNAc (mM)	1.8-4.1	0.5-1.5	0.6-0.9	1-2	0.9
' K_m ' for GlcNAc activation (mM)	3-4	12.5	4.7	4.5	0.6
Optimum Mg^{2+} concn (mM)	6-20	30	10	—	30
Inhibition by 0.5 mM-UDP (%)	—	53	62	—	44
' K_i ' for polyoxin inhibition (μM)	—	0.6	0.5	1.4	3
Inhibitory concn of polyoxin <i>in vivo</i> (μM)	50	19	2000	190	0.5
References†	(1)	(2)	(3)	(4)	(5)

* From Bartnicki-Garcia (1970).

† (1) Camargo *et al.* (1967); Matsumae & Cantino (1971). (2) McMurrough & Bartnicki-Garcia (1971); Bartnicki-Garcia & Lippman (1972). (3) Keller & Cabib (1971); Bowers, Levin & Cabib (1974). (4) Glaser & Brown (1957); Endo, Kakiki & Misato (1970). (5) Gooday & de Rousset-Hall (1975); Gooday, de Rousset-Hall & Hunsley (1976).

between these at high UDP-GlcNAc concentrations. Whatever the mechanism, the result will be that in the absence of other effectors, the enzyme is only appreciably active when the substrate is present in sufficient concentration to give the long-chain macromolecular product.

The co-substrate in the reaction, the acceptor or 'primer', poses a problem in a consideration of the enzyme from *C. cinereus*, as the purified preparations are active without any primer, and the only glycan product observed is macromolecular crystalline chitin. Once a chitin chain has started, this will act as acceptor, but how does it start? *In vivo* there is, presumably, no shortage of ends of chitin chains to act as primers, but in the purified enzyme the molecules presumably carry tightly-bound primer molecules along with them. Attempts to remove these putative chitin oligomers have been unsuccessful.

The magnesium cation is essential for activity, as it is with other enzymes utilizing nucleotide sugars as substrates. If the enzyme is prepared in Mg^{2+} -free buffer, there is no activity in the absence of added Mg^{2+} .

One enzyme product is UDP. This is strongly inhibitory to chitin synthase (Table 1, Fig. 1) with an apparent K_i value of 0.6 mM. The *Coprinus* enzyme preparations contain a diphosphatase that yields UMP, which is not nearly so inhibitory.

The glycan product has been identified as chitin by every criterion so far tried. Acid hydrolysis gives glucosamine; enzymic hydrolysis gives *N*-acetylglucosamine. Its infrared spectrum is nearly identical to that of purified lobster chitin; certainly all of the characteristic bands are present. It is birefringent in polarized light, and so it has an ordered crystalline structure. Ruiz-Herrera *et al.* (1975) have shown that the product of the enzyme preparation from *Mucor rouxii* is in the form of microfibrils that can be visualized in the electron microscope, and has an X-ray diagram characteristic of crystalline chitin. Again, these results give confidence that the activities of these enzyme preparations can be related to the enzyme activity in the living cell.

Chitin synthase, in common with other allosteric enzymes, is activated and inhibited by a range of effectors. The monomer and dimer of chitin, *N*-acetylglucosamine and diacetylchitobiose, both activate the enzyme considerably at very low substrate concentrations

Fig. 1. (a) Rate of reaction of chitin synthase from *C. cinereus* at different concentrations of UDP-GlcNAc without added inhibitor (●), and in the presence of 0.5 μM -polyoxin D (○) or 0.5 mM-UDP (■). The preparation and assay of the solubilized enzyme were as described by Gooday & de Rousset-Hall (1975). A linear rate of reaction during the time of the assay had previously been established for concentrations of 1000 and 50 μM -UDP-GlcNAc. Values for each inhibitor are averages of three enzyme preparations, and are all normalized to control values at 1000 μM -UDP-GlcNAc [individual values ranged from 290 to 470 nmol GlcNAc incorporated min^{-1} (mg protein) $^{-1}$]. Previously unpublished results of Gooday & de Rousset-Hall. (b) The same results expressed as Hill plots. The slopes are close to 2, but increase with decreasing substrate concentration.

Fig. 2. (a) Rate of reaction of chitin synthase from *C. cinereus* at low concentrations of UDP-GlcNAc without added effector (●), and in the presence of 2 mM-*N*-acetylglucosamine (▲) or 2 mM-diacetylchitobiose (□). Preparations, conditions and controls as for Fig. 1. Values are from three enzyme preparations, normalized in each case to a control value at 100 μM UDP-GlcNAc [individual values were 10.1, 10.6 and 23.2 nmol GlcNAc incorporated min^{-1} (mg protein) $^{-1}$]. Previously unpublished results of Gooday & de Rousset-Hall. (b) The same results expressed as Hill plots. In the absence of added amino sugars the slope increases to close to 4 at the lowest substrate concentrations, but in their presence it is maintained close to 2.

(Fig. 2). These might appear to be simply laboratory observations, of no significance in the living cell, as these sugars are not found as free intracellular components of fungi. However, they will be produced anywhere that chitinase and diacetylchitobiase are acting on pre-existing chitin, and this is precisely where new chitin synthesis is required during morphogenesis, and so these activations may be important in the temporal and spatial control of the enzyme activity. The lytic enzymes will loosen the wall structure so that the synthetic enzyme can insert new material for wall growth. This process must occur at the hyphal tip, where the chitin microfibrils pass right over the apex as a coherent stable structure; it was foreseen in part in 1888 by Marshall-Ward: 'the continuous forward growth of the apex of any hypha takes place by the ferment-substance keeping the cellulose of the hypha at that place in a soft, extensible condition, and the pressure from behind stretches it and drives the tip forwards'. This role for chitinase and other wall lytic enzymes is analogous to that of the bacterial autolysins. The resultant *N*-acetylglucosamine, which it is suggested activates the chitin synthase very locally, does not accumulate, but presumably is phosphorylated rapidly by *N*-acetylglucosamine kinase to be re-used for chitin synthesis. This enzyme is widely distributed in fungi and, although its activity can be increased by feeding *N*-acetylglucosamine in the medium (e.g. in *Candida albicans*; Bhattacharya, Puri & Datta, 1974), nevertheless it does have constitutive activity, and experiments such as the autoradiography of chitin biosynthesis depend on its action.

Activity of chitin synthase

As described above, chitin is deposited in the apical wall during vegetative hyphal growth. All evidence from fungal systems points to the cell membrane itself as the site of polymerization of the chitin. For example, electron microscopic autoradiographs of the incorporation of tritiated *N*-acetylglucosamine into macromolecules in hyphae of *Neurospora crassa* show 92 % of the silver grains associated with the wall or membrane after 10 min incubation (Hunsley & Gooday, unpublished). There is no evidence for any intracellular pre-polymerization and subsequent outward movement of macromolecular products, as has been elucidated for wall glycoprotein synthesis in *Saccharomyces*. This process in yeast involves lipid intermediates such as polyprenol pyrophosphate *N*-acetylglucosamine and polyprenol pyrophosphate diacetylchitobiose (Lehle & Tanner, 1975). There is no evidence for a 'lipid intermediate' involved in chitin biosynthesis.

More direct evidence for the site of action of chitin synthase comes from *Saccharomyces* where Durán, Bowers & Cabib (1975) have shown that cell membrane ghosts contain nearly all of the enzyme activity recoverable from the cells.

In homogenates of stipe tissue of *C. cinereus* most of the chitin synthase activity is associated with the microsomal fraction (Table 2). There is some activity associated with the wall fraction, but this is probably bound to membranes sedimenting with wall debris, as it can be solubilized by treatment with digitonin solution (Table 3). Digitonin disrupts eukaryotic membranes partly by a detergent action and partly by complexing with their sterols. Digitonin treatment yields a very stable active chitin synthase preparation from the microsomal fraction (de Rousset-Hall & Gooday, 1975).

This solubilized enzyme can then be further purified by standard biochemical techniques. For example, with a buffer of 50 mM-Tris/HCl, the enzyme activity was eluted in the void volume of a Sepharose 6B molecular exclusion column (60×1.5 cm), indicating a molecular weight of several million. However, the same enzyme preparation on the same column with the same buffer, but in the presence of 200 mM-NaCl, eluted at a position consistent with a molecular weight of 150000.

Table 2. *Distribution of chitin synthase activity in fractions of homogenized stipe of Coprinus cinereus*

Tissue homogenization and assay conditions were as described by Gooday & de Rousset-Hall (1975). The designation of each fraction was based on its appearance by light microscopy and electron microscopy. Samples from the four preparations were assayed immediately; the pellets were resuspended in resuspending medium and stored frozen overnight, and the supernatant was dialysed overnight against resuspending medium; all four samples were re-centrifuged and re-assayed as before.

Designation of fraction	Centrifugation [integrated field-time (g-min) at r_{av} 6 cm]	Chitin synthase activity (% of total)		Sp. activity of re-centrifuged prepn [nmol min ⁻¹ (mg protein) ⁻¹]
		Original preparation	After re-centri- fugation	
Wall	2×10^4	27.6	32.3	2.3
Mitochondrial	1×10^5	6.7	1.3	0.2
Microsomal	6.3×10^6	61.0	56.2	7.7
Supernatant	$> 6.3 \times 10^6$	4.7	10.2	0.1
Total activity recovered (pmol min ⁻¹ stipe ⁻¹)	—	3372	2750	—

Table 3. *Solubilization of chitin synthase activity from the wall fraction of homogenized stipe of Coprinus cinereus*

Conditions were as described in Table 2. The wall fraction was resuspended in resuspending medium with or without 10% (w/v) digitonin, re-centrifuged, and the pellet and supernatant were re-assayed. The pellet of washed wall material was then re-washed as before.

	Enzyme activity (nmol min ⁻¹ fraction ⁻¹)					
	Washed with medium + digitonin			Washed with medium (control)		
	Pellet	Super- natant	Total recovered	Pellet	Super- natant	Total recovered
Original pellet	168	—	168	191	—	191
After one wash	129	77	206	179	3	182
After two washes	51	41	169	158	5	166

Chitin synthase can thus be pictured as an integral protein spanning the cell membrane as a multimolecular aggregate. Digitonin solubilizes this aggregate with retention of activity. Treatment with a high concentration of salt causes a dissociation of the aggregate to give the basic enzyme unit with a molecular weight of about 150000. By analogy with other allosteric systems, this may consist of a number of sub-units, which may or may not be identical, but which together can bind four molecules of UDP-*N*-acetylglucosamine.

The chitin formed by the enzyme *in vivo* is in the form of crystalline assemblies of glycan chains in microfibrils, and so the postulated aggregate of enzyme molecules may be arranged in such a way as to give rise directly to the ordered array of a microfibril. The substrate UDP-*N*-acetylglucosamine will be supplied from the cytosol, and the resultant chitin will be formed directly into the cell wall. The sugar units are probably added to the non-reducing end of the glycan chain. To give the (1→4)- β configuration, each successive sugar molecule must be upside down compared with the previous one, so perhaps each chitin synthase unit has two catalytic sites arranged to add two sugar molecules coordinately in the one chain. However, there is an extra aspect to be considered in the relationship between synthesis and final structure, between polymerization and crystallization. As the chitin in the

fungal wall is apparently α -chitin, adjacent chains run in opposite directions; this must result from some ordered mechanism, perhaps a folding of the chains on themselves as they are synthesized.

Chitinase and polyoxin

The two major antimicrobial agents described by Fleming, lysozyme in 1922 and penicillin in 1929, both act on the same component of the bacterial wall, the peptidoglycan, by disrupting its structure and its synthesis respectively (Fig. 3 *a*). Chitin, as a vital and characteristic component of the fungal wall, is clearly closely analogous to the bacterial peptidoglycan. What are the antifungal agents analogous to lysozyme and penicillin?

Chitinase is the enzyme that hydrolyses the (1 $\rightarrow$ 4)- β glycosidic bond in chitin. It is found in all chitin-containing organisms, where it must have a morphogenetic role, and it can be induced in the range of micro-organisms that can use chitin as a source of nutrients. Incubation of fungi with purified chitinase does lead to some dissolution of walls but, as with the action of lysozyme on bacteria, different fungal species and different kinds of fungal cells show a wide range of susceptibility. In some cases there is sufficient lysis of the wall to lead to loss of hyphal structure, for example with *C. cinereus* (Moore, 1975), but usually there is little visible effect, presumably as the accompanying wall components protect the chitin or have enough strength in themselves to stabilize the wall. Just as lysozyme is used against bacteria, there have been suggestions of using chitinase therapeutically as an anti-fungal agent. Most lysozymes themselves have a low activity on chitin (Jollès *et al.*, 1974), and may in this way act as endogenously against fungi as Fleming showed they can against bacteria. Kamaya (1970) has shown that egg white lysozyme strongly inhibits growth of cultures of *Candida albicans*.

Turning to the synthesis of chitin, the polyoxins are a group of very potent antifungal antibiotics that, in activity, have the closest analogy to penicillin. They are nucleoside antibiotics, produced by *Streptomyces cacaoi* var. *asoenis*, and were discovered in Japan, where they are widely used as agricultural fungicides, particularly against the black spot disease of pears, *Alternaria kikuchiana* (Hori *et al.*, 1974*a*).

Polyoxin is active against representatives of all groups of fungi that contain chitin, even the yeast *S. cerevisiae* in which chitin constitutes less than 1 % of the wall (Table 1). Endo, Kakiki & Misato (1970) suggested that polyoxin inhibits chitin synthase and all subsequent experiments bear this out. It acts as a very powerful competitive inhibitor, and Hori, Kakiki & Misato (1974*b*) suggest a mechanism whereby polyoxin replaces the substrate UDP-*N*-acetylglucosamine at the active site of the enzyme. There are clear points of similarity in their structures (Fig. 4).

The morphological effects of polyoxin can be satisfyingly rationalized with its potent inhibition of chitin synthesis. Growing hyphal apices of many fungi swell and burst when treated with 20 μ M-polyoxin and the growing hyphal apex is precisely the site of action of chitin synthase. Its effect on budding yeast cells of *Saccharomyces* is very similar to the effect of penicillin on dividing bacterial rods, as 'butterfly' shapes are formed as the protoplasm balloons out from between the mother and putative daughter cell. The polyoxin is preventing the formation of the chitin ring in the budscar that would otherwise be formed as an essential structure separating the bud from the parent cell (Bowers, Levin & Cabib, 1974).

A wide range of fungi show a very similar response to polyoxin, but the concentration required to kill them varies. Chitin synthases from these fungi also show a similar response, giving apparent K_i values of about 1 μ M polyoxin, which is about 1000-fold less than the apparent K_m values for UDP-*N*-acetylglucosamine (Table 1).

Fig. 3. The disruption of the synthesis and structure of the walls of (a) bacteria and (b) fungi.

Fig. 4. Structures of polyoxin D and UDP-*N*-acetylglucosamine.

In the case of the stipe of *C. cinereus*, 500 nM-polyoxin D will inhibit elongation, which is consistent with the evidence that chitin synthesis is an essential process during this developmental sequence. Polyoxin D also strongly inhibits the chitin synthase preparations (Fig. 1), giving 50 % inhibition at 5 μ M (Gooday, de Rousset-Hall & Hunsley, 1976). The value of K_i obtained from a Dixon plot is about 3 μ M, and this concentration will completely inhibit development of the tissue. Again, this gives confidence that the properties of the extracted enzyme can be compared with those of the enzyme in the growing cell.

The final result of polyoxin treatment of the *Coprinus* stipe is almost total autolysis of the tissue. Once one essential activity in the cell is interrupted, control of all other activities must be lost progressively; in this case, when the lysis:synthesis interrelationship is disrupted, the lysis can carry on unchecked, to initiate the destruction of the cell.

Not all chitin-containing fungi are susceptible to the polyoxins when tested *in vivo*. Unfortunately they have little or no reported activity against human pathogens. In Japan, resistant strains of *A. kikuchiana* have been isolated from orchards after several years of spraying polyoxin. However, the chitin synthase of these strains is still susceptible, and the mechanism of resistance is not clear (Hori *et al.*, 1974*a*). The action of polyoxin *in vivo* is also antagonized by the presence of simple peptides, which has explained its reported lack of activity in some cases (Bowers *et al.*, 1974), and its lack of promise as a therapeutic antifungal agent.

Conclusion

Nevertheless, the very existence of polyoxin does hold out hope that we can use chitin synthesis as a target for a rational therapy for fungal diseases (Fig. 3*b*). We have seen that chitin is specific to fungi; it is an essential part of the structure of the fungal cell; it is produced precisely at the site of invasion of tissue, at the hyphal apex; and its synthesis is accomplished by one enzyme that can be specifically inhibited.

REFERENCES

- BARTNICKI-GARCIA, S. (1970). Cell wall composition and other biochemical markers in fungal phylogeny. In *Phytochemical Phylogeny*, pp. 81–103. Edited by J. B. Harborne. London: Academic Press.
- BARTNICKI-GARCIA, S. (1973). Fungal cell wall composition. In *Handbook of Microbiology*, vol. II, pp. 201–214. Edited by A. I. Laskin and H. A. Lechavalier. Cleveland: CRC Press.
- BARTNICKI-GARCIA, S. & LIPPMAN, E. (1972). Inhibition of *Mucor rouxii* by polyoxin D: effects on chitin synthetase and morphological development. *Journal of General Microbiology* **71**, 301–309.
- BHATTACHARYA, A., PURI, M. & DATTA, A. (1974). Induction of N-acetylglucosamine kinase in yeast. *Biochemical Journal* **141**, 593–595.
- BOWERS, B., LEVIN, G. & CABIB, E. (1974). Effect of polyoxin D on chitin synthesis and septum formation in *Saccharomyces cerevisiae*. *Journal of Bacteriology* **119**, 564–575.
- BULL, A. T. & TRINCI, A. P. J. (1977). The physiology and metabolic control of fungal growth. *Advances in Microbial Physiology* **15**, (in the Press).
- CAMARGO, E. P., DIETRICH, C. P., SONNEBORN, D. & STROMINGER, J. L. (1967). Biosynthesis of chitin in spores and growing cells of *Blastocladiella emersonii*. *Journal of Biological Chemistry* **242**, 3121–3128.
- CARLILE, M. J. (1977). Bacterial, fungal and slime mould colonies. In *Biology and Systematics of Colonial Organisms*. Edited by G. Larwood and B. R. Rosen. London: Academic Press.
- CHATTAWAY, F. W., HOLMES, M. R. & BARLOW, J. E. (1968). Cell wall composition of the mycelial and blastospore forms of *Candida albicans*. *Journal of General Microbiology* **51**, 367–376.
- DURÁN, A., BOWERS, B. & CABIB, E. (1975). Chitin synthetase zymogen is attached to the yeast plasma membrane. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 3952–3955.
- ENDO, A., KAKIKI, K. & MISATO, T. (1970). Mechanism of action of the antifungal agent polyoxin D. *Journal of Bacteriology* **104**, 189–196.
- FLEMING, A. (1922). On a remarkable bacteriolytic element found in tissues and secretions. *Proceedings of the Royal Society B* **93**, 306–317.
- FLEMING, A. (1929). On the antibacterial action of cultures of a *Penicillium* with special reference to their use for the isolation of *B. influenzae*. *British Journal of Experimental Pathology* **10**, 226–236.
- FLEMING, A. (1945). Penicillin. In *Nobel Lectures: Physiology or Medicine 1942–1962*. Amsterdam: Elsevier.
- FLEMING, A. & SMITH, G. (1944). Some methods for the study of moulds. *Transactions of the British Mycological Society* **27**, 13–19.
- GALE, E. F. (1974). The release of potassium ions from *Candida albicans* in the presence of polymer antibiotics. *Journal of General Microbiology* **87**, 20–36.
- GLASER, L. & BROWN, D. H. (1957). The synthesis of chitin in cell-free extracts of *Neurospora crassa*. *Journal of Biological Chemistry* **228**, 729–742.
- GOODAY, G. W. (1971). An autoradiographic study of hyphal growth of some fungi. *Journal of General Microbiology* **67**, 125–133.
- GOODAY, G. W. (1975). The control of differentiation in fruit bodies of *Coprinus cinereus*. *Reports of Tottori Mycological Institute (Japan)* **12**, 151–160.
- GOODAY, G. W. (1977). The enzymology of hyphal growth. In *The Filamentous Fungi*, vol. III. Edited by J. E. Smith and D. R. Berry. London: Arnold.
- GOODAY, G. W. & DE ROUSSET-HALL, A. (1975). Properties of chitin synthetase from *Coprinus cinereus*. *Journal of General Microbiology* **89**, 137–145.
- GOODAY, G. W., DE ROUSSET-HALL, A. & HUNSLEY, D. (1976). The effect of polyoxin D on chitin synthesis in *Coprinus cinereus*. *Transactions of British Mycological Society* **67**, 77–83.
- GULL, K. & TRINCI, A. P. J. (1973). Griseofulvin inhibits fungal mitosis. *Nature, London* **244**, 292–294.
- HARE, R. (1970). *The Birth of Penicillin and the Disarming of Microbes*. London: Allen and Unwin.
- HORI, M., EGUCHI, J., KAKIKI, K. & MISATO, T. (1974a). Studies on the mode of action of polyoxins. VI. Effect of polyoxin B on chitin synthesis in polyoxin-sensitive and resistant strains of *Alternaria kikuchiana*. *Journal of Antibiotics* **27**, 260–266.
- HORI, M., KAKIKI, K. & MISATO, T. (1974b). Interaction between polyoxin and active center of chitin synthetase. *Agricultural and Biological Chemistry, Japan* **38**, 699–705.
- HUNSLEY, D. & BURNETT, J. H. (1968). Dimensions of microfibrillar elements in fungal walls. *Nature, London* **218**, 462–463.
- HUNSLEY, D. & BURNETT, J. H. (1970). The ultrastructural architecture of the walls of some hyphal fungi. *Journal of General Microbiology* **62**, 203–218.
- HUNSLEY, D. & KAY, D. (1976). Wall structure of the *Neurospora* hyphal apex: immunofluorescent localization of wall surface antigens. *Journal of General Microbiology* **95**, 233–248.
- JOLLÈS, P., BERNIER, I., BERTHOUD, J., CHARLEMAGNE, D., FAURE, A., HERMANN, J., JOLLÈS, J., PÉRIN, J. P. & SAINT-BLANCARD, J. (1974). From lysozymes to chitinases: structural, kinetic, and crystallographic studies. In *Lysozyme*, pp. 31–54. Edited by E. F. Osserman, R. E. Carfield and S. Begchoke. New York and London: Academic Press.

- KAMAYA, T. (1970). Lytic action of lysozyme on *Candida albicans*. *Mycopathologia et mycologia applicata* **42**, 197–207.
- KELLER, F. A. & CABIB, E. (1971). Chitin and yeast budding: properties of chitin synthetase from *Saccharomyces carlsbergensis*. *Journal of Biological Chemistry* **246**, 160–166.
- KERRIDGE, D., KOH, T. Y. & JOHNSON, A. M. (1976). The interaction of amphotericin B methyl ester with protoplasts of *Candida albicans*. *Journal of General Microbiology* **96**, 117–123.
- KRAVCHENKO, N. A. (1967). Lysozyme as a transferase. *Proceedings of the Royal Society B* **167**, 429–430.
- LEHLE, L. & TANNER, W. (1975). Formation of lipid-bound oligosaccharides in yeast. *Biochimica et biophysica acta* **399**, 364–374.
- LEHMANN, P. F., WHITE, L. O. & RIDE, J. P. (1974). Aspects of systemic *Aspergillus fumigatus* infection in untreated and immunosuppressed mice: use of a chitin assay to measure fungal growth. *Proceedings of the Society for General Microbiology* **2**, 30.
- MARSHALL-WARD, H. (1888). A lily-disease. *Annals of Botany* **2**, 319–382.
- MATSUMAE, A. & CANTINO, E. C. (1971). Sensitivity of the spores of *Blastocladiella emersonii* and related fungi to antibiotics and some other drugs. *Journal of Antibiotics* **24**, 77–84.
- McMURROUGH, I. & BARTNICKI-GARCIA, S. (1971). Properties of a particulate chitin synthetase from *Mucor rouxii*. *Journal of Biological Chemistry* **246**, 4008–4016.
- MOORE, D. (1975). Production of *Coprinus* protoplasts by use of chitinase or helicase. *Transactions of the British Mycological Society* **65**, 134–136.
- DE ROUSSET-HALL, A. & GOODAY, G. W. (1975). A kinetic study of a solubilized chitin synthetase preparation from *Coprinus cinereus*. *Journal of General Microbiology* **89**, 146–154.
- RUDALL, K. M. (1969). Chitin and its association with other molecules. *Journal of Polymer Science, C* **28**, 83–102.
- RUDALL, K. M. & KENCHINGTON, W. (1973). The chitin system. *Biological Reviews* **48**, 597–636.
- RUIZ-HERRERA, J., SING, V. O., VAN DER WOUDE, W. J. & BARTNICKI-GARCIA, S. (1975). Microfibril assembly by granules of chitin synthetase. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 2706–2710.
- RUPLEY, J. A. & GATES, V. (1967). Studies on the enzymic activity of lysozyme. II. The hydrolysis and transfer reactions of *N*-acetylglucosamine oligosaccharides. *Proceedings of the National Academy of Sciences of the United States of America* **57**, 496–510.
- SPELLER, D. C. E. & MACIVER, A. G. (1971). Endocarditis caused by a *Coprinus* species: a fungus of the toadstool group. *Journal of Medical Microbiology* **4**, 370–374.
- WINTERBURN, P. J. (1974). Polysaccharide structure and function. In *Companion to Biochemistry*, pp. 307–342. Edited by A. T. Bull, J. R. Lagnado, J. O. Thomas and K. F. Tipton. London: Longman.