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Chitin and Chitosan in Fungi

Prof. Dr. Martin G. Peter
University of Potsdam, Institute of Organic Chemistry and Structure Analysis, and Interdisciplinary Research Center for Biopolymers, Karl-Liebknecht-Str. 25, D-14476 Golm, Germany; Tel.: +49-331-977-5401; Fax: +49-331-977-5300;
E-mail: peter@serv.chem.uni-potsdam.de

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AA	amino acid residues
CDA	chitin deacetylase
CHS	chitin synthase gene
CS	chitin synthase
CSN	chitosanase gene
DP	degree of polymerization
ER	endoplasmic reticulum
F_A	mole fraction of *N*-acetylglucosamine residues
FMOC	9-fluorenylmethoxycarbonyl
FT-IR	Fourier transformation infrared spectroscopy
GlcN	2-amino-2-deoxy-D-glucopyranose, β-(1-4)-linked in chitin/chitosan
GlcNAc	2-acetamido-2-deoxy-D-glucopyranose, β-(1-4)-linked in chitin/chitosan
GlcNase	glucosaminidase
HPLC	high-performance liquid chromatography
M	molecular mass (daltons)
MALDI TOF MS	matrix-assisted laser desorption ionization time-of-flight mass spectrometry
M_v	viscosity average molecular mass

M_w	mass average of molecular mass
ORF	open reading frame
PITC	phenylisothiocyanate
UDP-GlcNAc	uridine diphospho-*N*-acetylglucosamine
WGA	wheat germ agglutinin

1 Introduction

The *aminoglucan* chitin (poly-GlcNAc) is widely distributed in nature, whereas the related polysaccharide chitosan (poly-GlcN) occurs in certain fungi only. Fungal chitin shows some special features, in particular with respect to chemical structure and biosynthesis. Thus, it appears appropriate to look at fungal chitin and chitosan in their own rights and to treat them in a separate chapter.

The basic principles of chitin and chitosan are treated elsewhere in this volume, and readers who are not familiar with these aminoglucans should consult also Chapter 15, this volume (Chitin and Chitosan from Animal Sources).

2 Chemical Structure

The chitin of fungi possesses principally the same structure as the chitin occurring in other organisms (see Chapter 15, this volume). However, a major difference results from the fact that fungal chitin is associated with other polysaccharides which do not occur in the exoskeleton of arthropods. Furthermore, the occurrence of chitosan is apparently restricted to fungi.

2.1 Chitin

The molecular mass of chitin in fungi is not known. However, it was estimated that bakers' yeast synthesizes rather uniform chains containing 120–170 GlcNAc monomer units which corresponds to ca. 24,000–34,500 Daltons (for a reference, see Valdivieso et al., 1999). This is roughly one order of magnitude lower than the estimated molecular mass of chitosan isolated from Mucoraceae (see below).

In *Saccharomyces cerevisiae*, terminal reducing ends of chitin chains are attached through β-(1,4)- or β-(1,2)-linkages to the nonreducing end of β-(1,3)-glucan branches which are linked to β-(1,6)-glucan. Attachment of chitin to glucan is catalyzed by chitin synthase 3 (Hartland et al., 1994). A mannoprotein is attached to β-(1,6)-glucan through a glycosylphosphatidylinositol anchor containing five α-linked mannosyl residues (Kollar et al., 1995, 1997). A mutant of *S. cerevisiae* with a reduced β-(1,3)-glucan content shows increased cross-linking of mannoproteins to chitin trough β-(1,6)-glucan (Kapteyn et al., 1997).

Likewise, the cell wall of *Aspergillus fumigatus* is highly complex, containing in the alkali-insoluble fraction a linear β-(1,3/1,4)-glucan, galactomannan, chitin, and β-(1,3)-glucan, whereas β-(1,6)-glucan is absent. The β-(1,3)-glucan shows 4% of β-(1,6)-branching. As in *S. cerevisiae*, chitin is β-(1,4)-linked to β-(1,3)-glucan (Fontaine et al., 2000). The content of GlcNAc in cell walls of

A. fumigatus in only 50% of that in *Aspergillus nidulans* (Guest and Momany, 2000).

Staining with fluorescent lectins reveals distinct distributions of mannoproteins, glucans and chitin in the cell wall of *Candida albicans* (Ruiz-Herrera et al., 1994). The cell wall of *Fusarium oxysporum* is composed of an outer layer of glycoproteins covering an inner layer of chitin and glucan (Schoffelmeer et al., 1999). Cell walls of the geophilic Dermatophytes *Microsporum fulvum* and *Epidermophyton stockdaleae* contain 4.0–6.5% of glucomannan and 44.2–71.0% of a glucan–chitin complex (Guarro et al., 1993).

2.2 Chitosan

Chitosans isolated from Mucorales typically show M_v in the range 4×10^5 to 1.2×10^6 Daltons and F_A values between 0.2 and 0.09. Amino acid analysis of chitosan prepared from *Aspergillus niger* reveals covalently bound arginine, serine, and proline (Lestan et al., 1993). The chitosan–glucan complexes from mycelia of *A. niger*, *Humicola lutea*, and *Fusarium moniliforme* contain 0.05–0.06% of amino acids, mostly as lysine and histidine (Velichkov and Sotirov, 1990).

Bacillus pumilus chitosanase is more effective than *Streptomyces griseus* chitinase in digestion of the cell wall of *Fusarium oxysporum*. Besides GlcN-GlcNAc as the main product, maltose is also observed as a degradation product, which indicates the presence of α-(1,4)-linked glucan (Fukamizo et al., 1996).

2.3 Polyphenolic Pigments

Cell walls or sporophore capsules of fungi contain often polyphenolic pigments which presumably function to enforce the polysaccharide–protein network by oxidative cross-linking or impregnation with a hydrophobic polymer. According to chemical logic, the polyphenols should be covalently bound to other biopolymers, though this has not been proven unequivocally. Precursors of fungal pigments are L-tyrosine and its hydroxylation product, 3,4-dihydroxy-phenylalanine (DOPA). Black and brown pigments, which are sometimes incorrectly named melanins, are often also derived from 1,8-dihydroxy-naphthalene or from simple catechols (Prota, 1992). Several fungi use γ-glutaminyl-4-hydroxybenzene as a precursor (Pierce and Rast, 1995). Wood- inhabiting fungi, e.g., *Inonotus hispidus*, produce a phenolic biopolymer that is derived from the stryrylpyrone hispidin (for a general review of fungal pigments, see Gill and Steglich, 1987).

3 Occurrence

Chitin is widely distributed in fungi, occurring in Basidiomycetes, Ascomycetes, and Phycomycetes, where it is a component of the cell walls and structural membranes of mycelia, stalks, and spores. The amounts vary between traces and up to 45% of the organic fraction, the rest being mostly proteins, glucans and mannans (Roberts, 1992). However, not all fungi contain chitin, and the polymer may be absent in one species that is closely related to another. Variations in the amounts of chitin may depend on physiological parameters in natural environments as well as on the fermentation conditions in biotechnological processing or in cultures of fungi.

Chitin is the major component in primary septa between mother and daughter cells of *S. cerevisiae*, and also one of the main components of the hyaline outer wall of spores of four arbuscular mycorrhizal *Glo-*

mus species (Sbrana et al., 1995). Hyphal walls of the Oomycete *Pythium ultimum* contain cellulose and chitin, whereas only cellulose is present in another Oomycete, *Phythophthora parasitica*. Both polysaccharides are present in cell walls of the Ascomycetes *Ophiostoma ulmi* and *Colletotrichum lindemuthianum*, whereas the Ascomycete *Fusarium oxysporum* and the Basidiomycete *Rhizoctonia solani* contain only chitin (Cherif et al., 1993). The zoopathogenic fungi *Cryptococcus neoformans*, *Pityrosporum canis* and *Rhizopus oryzae* contain chitin, but not β-(1,3)-glucan (Nicholas et al., 1994).

The chitin of the cell wall of the white-rot fungus *Rigidoporus lignosus* is degraded by enzymes excreted as a defense response by the host cell, and therefore is not detectable during the process of infection (Nicole and Benhamou, 1991). The fungal sheaths of another white-rot fungus, *Phellinus noxius*, do not contain chitin (Nicole et al., 1995).

The mycelia, and the caps and stalks of fruiting bodies of four edible mushrooms, *Lentinus edodes*, *Lycophyllum shimeji*, *Pleurotussajor-caju*, and *Volvariella volvacea* contain chitin as a minor component (Cheung, 1996).

Chitosan occurs naturally in the Mucorales, in particular *Mucor, Absidia*, and *Rhizopus* species. There is apparently only one report on the presence of chitosan in a Basidiomycete, *Lentinus edodes* (Shiitake mushroom) (Crestini and Giovannozzi-Sermanni, 1996; Crestini et al., 1996).

Slime molds (Myxomycetes) and bacteria (Schizomycetes) are devoid of chitin.

4
Physiological Function

Chitin serves as a fibrous strengthening element responsible for cell wall rigidity. However, there are other functions of chitin and chitosan, as revealed by mutants bearing a defect in the complex machinery of chitin biosynthesis, intracellular trafficking of chitin synthases, or deposition of the polysaccharide in cell walls, although the morphology of a mutant may be indistinguishable from that of the wild-type. Thus, chsD disruptants of *Aspergillus nidulans* show excessive swelling and lysis of conidia in hypoosmotic media (Specht et al., 1996) and *S. cerevisiae* chs5Δ null mutants fail to undergo cell fusion in mating (Santos et al., 1997) (for details, see Section 6.1).

5
Chemical Analysis and Detection

Determination of fungal chitin in biological samples is important for estimating fungal biomass, e.g., in infected plant tissues. A comprehensive review of the most common methods was given by Muzzarelli (1997) (see also Chapter 15, this volume). Frequently, GlcN is quantified by colorimetric methods in hydrolyzates of alkali-resistant fractions to determine the amounts of chitin and chitosan (Plassard, 1997). GlcN was also determined in acid hydrolyzates by high-performance liquid chromatography (HPLC) of 9-fluorenylmethoxycarbonyl (FMOC)- or phenylisothiocyanate (PITC)-GlcN (Ekblad and Naesholm, 1996; Osswald et al., 1995), or by gas chromatography-mass spectrometry (GC-MS) techniques (Penman et al., 2000).

Localization of chitin in cell walls or spores of fungi is achieved by using dyes that intercalate with polysaccharides. Calcofluor white shows enhanced fluorescence when binding to β-(1,4)-glucans, such as chitin, chitosan and cellulose, whereas β-(1,3)-glucans are selectively stained with aniline blue (Nicholas et al., 1994). Various wheat germ agglutinin (WGA) labeling techniques in combination with fluorochromes or gold

labeling are also described for the detection of chitin in fungi (Sbrana et al., 1995; Hu and Rijkenberg, 1998; Ekramoddoullah et al., 2000).

Fourier transform (FT) Raman spectroscopy of cell walls of fungi was applied to discriminate between different mixed species in culture media (Edwards et al., 1995).

6 Biosynthesis of Chitin and Chitosan

Chitin is biosynthesized in all chitinous fungi, including the relatively few investigated examples of Mucorales which contain chitosan. Chitin synthases as well as chitin deacetylases are reviewed in this section.

6.1 Chitin Synthases (CS)

In contrast to the situation in arthropods, many details of chitin biosynthesis are known in fungi. Most of the current knowledge is based on studies on baker's yeast, *S. cerevisiae*. The earlier literature was discussed comprehensively by Cabib (1987) and, since then, various aspects of chitin synthesis in fungi have been reviewed (Bulawa, 1993; Martinez and Gozalbo, 1994; Ruiz-Herrera and Xoconostle-Cazares, 1995; Bruyere et al., 1996; Cabib et al., 1996; Gooday, 1996; Merz et al., 1999a; Ruiz-Herrera and Martinez-Espinoza, 1999; Valdivieso et al., 1999; Karnezis et al., 2000).

6.1.1 Enzymology and Subcellular Localization of CS

The biosynthesis of chitin takes place vectorially in a membrane-bound protein complex. Chain elongation occurs by sequential transfer of GlcNAc from UDP-GlcNAc to the nonreducing end of the growing polymer. CS (chitin-(UDP-GlcNAc)-transferase, EC 2.4.1.16) belongs to family 2 glycosyltransferases which catalyze glycosyltransfer with inversion of the anomeric configuration (Coutinho and Henrissat, 1999). Further classification is based on sequence similarities or identities, and to date five CS classes have been assigned (cf. Table 1).

In general, β-glycosyltransferases, including cellulose and chitin synthases, have a highly conserved common motif 'D, D, D35Q(R,Q)XRW'. The second residue (R or Q) in the Q(R,Q)XRW sequence is probably involved in determining the degree of polymerization (DP) of the glucan chain (Saxena and Brown, 1997). Alignment with the deduced protein sequences of most known chitin synthase genes (CHS) reveals five to seven conserved domains (Xoconostle-Cazares et al., 1996).

CS is detected in membrane fractions and in chitosomes, the latter constituting small secretory vesicles (ca. 100S) which function as conveyors of CS to the cell surface (for a review, see Ruiz-Herrera and Martinez-Espinoza, 1999). Chitosome-membrane trafficking of CS was also observed in *Neurospora crassa* (Leal-Morales et al., 1994b). Solubilization of CS is achieved with detergents, such as digitonin, yielding a catalytically active 16S protein complex of molecular mass ca. 500 kDa. CS isolated from a microsomal fraction of *Absidia glauca* is a 30-kDa zymogenic polypeptide (Machida and Saito, 1993). A catalytically active CS was isolated as a 60-kDa polypeptide from 100S chitosomes of *Mucor rouxii* (Merz et al., 1999b).

In *S. cerevisiae*, CSI is more abundant than CSII, and both are localized in low- density chitosomes ($d = 1.15$ g mL^{-1}) as well as in high-density membrane fractions ($d = 1.21$ g mL^{-1}) (Leal-Morales et al., 1994a). Likewise, the transcripts from the *Ustilago maydis* gene, UmCHS1, appear to be present at a higher level than those from UmCHS2, and both transcripts appear to be more

Tab. 1 Chitin synthase genes from fungi (alphabetical listing by name of organism)

Organism	Gene	Comments	Reference
Agaricus bisporus	CHS1	CS class III; 2727 bp (ORF); 909 AA;	Sreenivasaprasad et al. (2000)
Ampelomyces quisqualis	AqCHSA	CS class I; 2786 bp; 910 AA	Weiss et al. (1996)
Aspergillus fumigatus	CHSD	CS-like; low but significant similarity to other CS	Mellado et al. (1996)
Aspergillus nidulans	CHSA CHSB	1013 AA 916 AA	Yanai et al. (1994)
A. nidulans	CHSD CHSE	CS class V and CS class IV; high sequence identity to ScCHS3 and CaCHS3	Specht et al. (1996)
Beauveria brongniartii	BbCHS1	Fragment; CS class II; 95.8% similarity with CHS2 of *Metarhizium anisopliae*	Nam et al. (1997)
Candida albicans	CaCHS1A	775 AA	Sudoh et al. (1995)
Fonsecaea pedrosoi	FpCHS1 FpCHS2 FpCHS3	600 bp and 366 bp; CS class I and II; homology to *S. cerevisiae* CS	Karuppayil et al. (1996)
Metarhizium anisopliae	MaCHS1 MaCHS2 MaCHS3	CS class I CS class I CS class III	Nam et al. (1998)
Mucor circinelloides	McCHS1	CS class TI; expressed during exponentially growing hyphal stage	Lopez-Matas et al. (2000)
Neurospora crassa	CHS2	Similar to CHS from other fungi	Din and Yarden (1994)
Paracoccidioides brasiliensis	CHS2	CS class II; 1043 AA	Nino-Vega et al. (1998)
Penicillium chrysogenum	PcCHS1 PcCHS2 PcCHS3 PcCHS4	CS class I CS class II CS class II CS class III	Namgung et al. (1996)
P. chrysogenum	CHS4	CS class III; 915 AA (ORF); close relationship between *P. chrysogenum* and *Aspergillus* CHS	Park et al. (2000)
Phialophora verrucosa	PvCHS1 PvCHS2 PvCHS3	CS class I and II; 614 bp CS class III; 366 bp; 88.2% similarity and 78.4% identity; with the *S. cerevisiae* enzyme	Peng et al. (1995)
Pyricularia oryzae	Fragment	340 bp; 86% homologous to *A. fumigatus* CHSE	Hwang et al. (1997)
Rhizopus oligosporus	CHS3	CS class IV; sequence similarity to CHS3 of *S. cerevisiae*; 46.7% identity with class IV CS of *N. crassa*	Motoyama et al. (1998)
Saccharomyces cerevisiae	CHS4	696 AA	Trilla et al. (1997)
S. cerevisiae	CHS5	671 AA	Santos et al. (1997)
S. cerevisiae	CHS6	See text	Ziman et al. (1998)
S. cerevisiae	CHS7	See text	Trilla et al. (1999)
Saprolegnia monoica	CHS2	Oomycetes and chitinous fungi have conserved CS	Mort-Bontemps et al. (1997)
Tuber borchii	–	CS class II; ca. 600 bp	Lanfranco et al. (1995)

Tab. 1 (cont.)

Organism	*Gene*	*Comments*	*Reference*
T. magnatum	TmCHS4	1230 AA; 62% homology to class IV CHS of *N. crassa*	Garnero et al. (2000)
Ustilago maydis	UmCHS1 UmCHS2	See text	Xoconostle-Cazares et al. (1996)
U. maydis	UmCHS5	Predicted CHS class IV; high similarity CHS3 from *S. cerevisiae* and *C. albicans*, CHS4 from *N. crassa*, CHSE from *A. nidulans*	Xoconostle-Cazares et al. (1997)
Wangiella dermatitidis	WdCHS4	High homology with CS class IV (Chs3p) of *S. cerevisiae*	Wang et al. (1999)

abundant in the mycelial form (Xoconostle-Cazares et al., 1996).

In *N. crassa*, CSII is compartmentalized in chitosomes which are abundant in the vicinity of the hyphal tip. Immunological studies have revealed that several peptides of microsomal membrane fractions react with a polyclonal antibody to CSII (Sietsma et al., 1996).

Though not proven conclusively *in vivo*, it appears that CSI and CSII are activated by proteolysis, occurring in a zymogenic form which contains a cytosolic amino-terminal region. The CS from *Saprolegnia monoica* is stimulated by digitonin and remains zymogenic after dissociation (Leal-Morales et al., 1997). Proteolytic activation of CSIII is observed in the presence of substrate (for discussion and references, see Merz et al., 1999a; Valdivieso et al., 1999).

As a rule, CSI and CSII are activated by divalent metal ions as co-factors, most commonly Mg^{2+} or Mn^{2+}. However, CSII of *S. cerevisiae* requires Co^{2+} rather than Mg^{2+} (Leal-Morales et al., 1994a).

In the cellulosic, nonfibrillar α-chitin-producing Oomycete *Saprolegnia monoica*, CS is found in high-density membrane components, but not in chitosomes (Leal-Morales et al., 1997).

6.1.2
Genetics of CS

The genetics of fungal CS are investigated very intensively, often using CHS from *S. cerevisiae* as hybridization probes, yielding useful information for taxonomy and phylogenetic relations, as well as the basis for the understanding of CS functions and their regulation (for reviews, see Bulawa, 1993; Ruiz-Herrera and Xoconostle-Cazares, 1995; Valdivieso et al., 1999). The variety of CHS is illustrated with a few recent examples in Table 1. A comprehensive listing of sequences is available, e.g., in the CAZy database (Coutinho and Henrissat, 1999; see also Campbell et al., 1997).

Much insight into the functions of CHS and their transcripts has been obtained from studies on the effects of gene deletion or disruption. Eight CHS have been described to date in *S. cerevisiae*. ScCHS1 and ScCHS2 are the structural genes for CSI and CSII, respectively, whereas the remaining genes are components of the CSIII complex (Valdivieso et al., 1999). Disruption of ScCHS2 or simultaneous disruption of ScCHS2 and ScCHS3, but not of ScCHS1 or ScCHS3, is lethal. However, a gene suppressing the lethality of disruption of ScCHS2 occurs in a *S. cerevisiae* strain which does not require ScCHS2 for viability. A mutant containing

the suppressor and lacking ScCHS1 and ScCHS2 has normal amounts of chitin in its cell wall. Apparently, the suppressor gene encodes or controls the expression of CSIII (Baymiller and McCullough, 1993). Chs6p is required for anterograde transport of Chs3p from the chitosome to the plasma membrane (Ziman et al., 1998). The CHS7 gene is specifically involved in Chs3p export from the endoplasmic reticulum (ER) (Trilla et al., 1999).

S. cerevisiae CHS show significant homology to insect CHS and to bacterial and vertebrate hyaluronan synthase HAS genes (DeAngelis et al., 1994; Ibrahim et al., 2000; see also Chapter 15 in Volume 5 and Chapter 15 in this volume).

The CS of *C. albicans*, called CaChs1p, CaChs2p, and CaChs3p, are structurally and functionally analogous to the *S. cerevisiae* CS. CaChs1p is involved in septum formation and is required for the viability of *C. albicans*. Inhibition of CaChsp1 with RO-09-3143 (see below) causes cell death in the cachs2Δ null, but not in cachs3Δ null mutants (Sudoh et al., 2000).

In *U. maydis*, six CHS or fragments are presently known which could operate to compensate an eventual loss of one activity by the remaining enzymes, thus maintaining fungal viability. Umchs5 null mutants display significant reduction in growth rate, chitin content, and chitin synthase activity, especially in the mycelial form, and reduced virulence to corn plants (Xoconostle-Cazares et al., 1997).

Inactivation of the *N. crassa* CHS2 gene produces progeny which is indistinguishable from those of the wild-type, though a significant reduction in CS activity and increased sensitivity to the phosphatidylcholine biosynthesis inhibitor edifenphos were observed (Din and Yarden, 1994).

CHSB, but not CHSA, is essential for hyphal growth in *A. nidulans* (Yanai et al., 1994; Borgia et al., 1996). Chitin synthesized by the CHSD-encoded isoenzyme contributes to the rigidity of the walls of germinating conidia, of the subapical region of hyphae, and of conidiophore vesicles, but is not necessary for normal morphology of these cells. Hyphae from both, chsD and chsE disruptants contain ca. 60–70% of the chitin present in wild-type hyphae. The morphology and development of chsE disruptants are indistinguishable from those of wild-type cells (Specht et al., 1996).

An interesting feature of CHS is the presence of a N-terminal myosin motor-like sequence that has first been observed in the *A. nidulans* csmA gene which contains a large open reading frame (ORF) encoding a polypeptide of 1852 amino acids (Fujiwara et al., 1997; see also Zhang and Gurr, 2000). Apparently, the csmA transcript has important roles in polarized cell wall synthesis and maintenance of cell wall integrity (Horiuchi et al., 1999).

6.1.3
Regulation of CS

On the enzymatic level, CS of *Mucor rouxii* is allosterically activated by GlcNAc which shows cooperative binding (Horsch and Rast, 1993; Merz et al., 1999a).

Septum formation in mycelial fungi and yeasts, as well as apical growth of the hyphae of filamentous fungi requires a precisely regulated, complex interplay of CS and chitinases. In *S. cerevisiae*, CSI is involved in repair functions at the end of cytokinesis. CSII deposits a disk of chitin in the mother-bud neck, forming the primary septum at the end of mitosis, and CSIII synthesizes a ring of chitin at the onset of bud emergence. Genomic analysis reveals multigenic control of chitin synthesis (Valdivieso et al., 1999). Post-translational regulation, probably by activation of latent zymogenic forms, appears to be predominant for the three CS of

S. cerevisiae (Choi et al., 1994). Furthermore, Chs2p and Chs3p are spatially and timely regulated, involving also differential trafficking (Chuang and Schekman, 1996). The ScCHS4 gene which encodes a protein with no potential transmembrane domain regulates the catalytic activity of CSIII, as V_{max} is reduced in the enzyme of chs4 null mutants. In addition to the chitin defect, the chs4 mutant shows a severe defect in mating (Trilla et al., 1997). Chitin synthesis in *S. cerevisiae* is suppressed on the transcriptional level by the KNR4 gene (Martin et al., 1999).

Chitin synthesis in *S. cerevisiae* is also under control of the α mating factor (Martin et al., 1999; Santos and Snyder, 1997; for references, see also Valdivieso et al., 1999).

6.1.4 Inhibition of CS

Benzoylphenylureas and tunicamycin are not inhibitors of chitin synthesis in fungi, whereas the nucleotide analogous nikkomycins and polyoxins are highly effective (for reviews, see Cohen, 1993; Munro and Gow, 1995; Palli and Retnakaran, 1999; Zhang and Miller, 1999; Rast et al., 2000). However, Nikkomycin Z is not active against *S. cerevisiae*, as CSIII but not CSII is inhibited (Gaughran et al., 1994).

A potent inhibitor of CSI of *C. albicans* has recently been identified as 8-(6,6-dimethylaminohepta-2,4-diynyl)amino-4*H*-benz[1,4]-oxazin-3-one (RO-09-3143) (K_i for CaChs1p 0.55 nM) which arrests cell growth in wild-type yeast at MIC_{50} 0.27 μM (Sudoh et al., 2000).

A number of natural products isolated from plants or microorganisms show antifungal effects by inhibiting CS or functional components required for CS activity. Further review is beyond the scope of this article, however.

6.2 Glucan Transferase

The formation of branched glucan, glucan–glucan cross-links, and glucan–chitin cross-links in fungal walls involves the action of glucanases, glycosyltransferases, and transglycosylases. Glucosyltransferases from cell walls of *S. cerevisiae* and *C. albicans* were partly characterized. An activated intermediate is formed from a donor β-(1,3)-glucan by cleaving off a disaccharide (Goldman et al., 1995). A chitin–glucan-β-(1,4)-transferase which catalyzes formation of the linkage between the terminal reducing GlcNAc residue of chitin and the nonreducing Glc residue of β-(1,3)-glucan, as well as a potential use of the enzyme for assaying antifungal agents, are described in a patent (Kollar et al., 1996).

6.3 Chitin Deacetylase (CDA)

Enzymatic deacetylation of chitin by CDA [EC 3.5.1.41] is apparently restricted to fungi and bacteria (for reviews, see Kolodziejska et al., 1995; Tsigos et al., 2000). A few earlier reports on the occurrence of CDA in arthropods (Aruchami et al., 1986) have so far been neither confirmed nor disproved. A review of CDA is given in the following section. The selective deacetylation at the nonreducing end of $(GlcNAc)_n$ for Nod-factor biosynthesis as well as applications of deacetylases for the synthesis of partially acetylated chitooligosaccharides, and the deacetylation of small substrates for the terminal metabolism of chitin are excluded, as these topics are discussed in Chapter 15, this volume.

6.3.1 Enzymology of CDA

CDA deacetylates preferentially amorphous chitin of medium F_A (for details, see Chapter