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Metal loading and enzymatic degradation of fungal cell walls and chitin

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The capacity of chitin (from crab shells) and of fungal cell walls from *Trichoderma harzianum* to accumulate zinc, cadmium and mercury was studied as well as the effects of adsorbed metals on the enzymatic hydrolysis by Novozym 234 of the two substrates. The total adsorbing capacity with respect to these metals was estimated to be at least 10 mmol kg^{-1} chitin (dry weight) and 50 mmol kg^{-1} fungal cell walls (dry weight), respectively, at pH 6.1. Enzymatic digestion of fungal cell walls preloaded with mercury and cadmium was significantly reduced, while zinc did not cause any significant inhibition. The effect of metal complexation by chitin on the enzymatic digestion was not as pronounced as for fungal cell walls. This could reflect the fact that chitin sorbed a lower total amount of metals. The inhibitory effect of metals on the enzymatic hydrolysis was caused by the association of the metals with the two substrates and not by the presence of free metals in solution.

Keywords: degradation, fungi, metals, *Trichoderma harzianum*

Introduction

The distribution and spreading of metals in soils are processes of environmental importance, and the mechanisms are subjects of increasing interest. Microorganisms can directly affect the speciation of metals by controlling their oxidation state, formation of alkylated forms, accumulation, etc., and also indirectly by the production of complexing organics. However, there is inadequate knowledge about the total effects of microorganisms on the distribution of metals in the environment. Soil fungal biomass has recently been shown to have a high ability to accumulate metals, especially at low soil pH (Krantz-Rülcker *et al.* 1993a). Metals associated with fungi are mainly bound to the surface of the organisms (Townsley 1985, Gadd 1989). Tsezos & Volesky (1982) reported that chitin (a β-1,4-linked polymer of *N*-acetylglukosamine) is the active constituent responsible for the biosorptive capacity for metal cations of the mycelial cell wall of a *Rhizopus arrhizus* strain. Chitin together with β-1,3-glucans are the major components of fungal cell walls (Farkás 1985) and it is well documented that chitin possesses high metal sorption capacity (Muzzarelli 1973).

The ability to decompose chitin is widespread in nature. Chitinolytic activity has been detected in both procaryotes as well as in eukaryotes, e.g. fungi and plants (Cabib 1987, Gooday 1988). The large pool of chitin constitutes an important energy, carbon and nitrogen source. Much of the degradation of chitin occurs extracellularly due to the polymer structure of chitin. There are other important functions for chitinases beside decomposition. Intracellular fungal chitinases are important for the apical growth of hyphae, hyphal branching and cell separation, e.g. release of spores (Bartnicka-Garcia 1973, Gooday 1977). Furthermore, the production of chitinases in plants has been suggested to act as a defense against fungal infections (Boller 1985, Mauch *et al.* 1988). Metal complexation with organic compounds has been shown to inhibit their biodegradation (Firestone & Tiedje 1975, Madsen & Alexander 1985, Brynhildsen & Rosswall 1989) and it has been suggested that the recalcitrance of humic compounds to degradation may be due to complexation with metal cations (Juste *et al.* 1975). Aluminum protected a model humic substance from degradation almost completely, while iron protected the organic substance to a lesser degree. Martin *et al.* (1972) showed that complexation of Al^{3+}, Cu^{2+}, Fe^{2+} and Zn^{2+} with polysaccharides greatly reduced the microbial degradation of these organics.

Chitin appears to be rapidly degraded in the oceans, since no major accumulation in sediments is observed (Gooday *et al.* 1991). Since chitin forms strong complexes with metals it has been proposed to be an important agent for transporting metals from surface to deep waters in the

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oceans. The metal complexes would be formed in shallow waters, sink and be degraded in deep waters, and thereby release the associated metals (Yoshinari & Subramanian 1976, Subramanian 1978). This hypothesis was, however, based on the assumption that metal–chitin complexes and free chitin exhibit the same biodegradability, which is not necessary the case.

The objectives of the present study were 2-fold. The first was to determine the accumulation of zinc, cadmium and mercury by fungal cell walls and pure chitin. The other objective was to study the effects of metal (zinc, cadmium and mercury) complexation on the enzymatic degradation of the cell walls and chitin. The cell walls were prepared from a fungas, *Trichoderma harzianum*, previously shown to accumulate significant amounts of metals (Krantz-Rülcker *et al*. 1993b). The effects of metal complexation on enzymatic degradation of fungal cell walls and chitin were studied using a lytic enzyme mixture (Novozym 234) produced by a *Trichoderma* sp. This enzyme mixture is mainly composed of chitinases, glucanases and proteases (Hamlyn *et al*. 1981), and is widely used to degrade fungal cell walls for fungal protoplast production (Collings *et al*. 1988).

Materials and methods

Organism and growth

Trichoderma harzianum was obtained from the Swedish University of Agricultural Sciences, Department of Microbiology, Uppsala, Sweden (J 65). The organism was maintained on 4% malt extract agar (MEA) slants. After 10–14 days incubation at 20 °C, the culture was stored at 4 °C. This procedure was repeated at 3 month intervals.

Conidia for the inoculation of liquid media were obtained by growing the fungus on 4% MEA for 14 days at 20 °C. The conidia were harvested by covering the agar plate with sterile Milli-Q water with Tween 80 (detergent) added and dislodging the spores with a glass rod. The conidial suspension was filtered through sterile glass wool to remove hyphae and other debris. The filtrate was centrifuged (15000 × *g*; 15 min) and washed three times in sterile 0.1 M KCl. The last pellet was suspended in a freeze medium [in $g l^{-1}$; KH_2PO_4, 0.18; K_2HPO_4, 0.82; Na-citrate, 0.59; $MgSO_4 \cdot 7H_2O$, 0.25; glycerol (87%), 172 $ml l^{-1}$] and frozen in portions of 1 ml with 4×10^6 spores ml^{-1} at −70 °C. These suspensions were used as standardized inocula throughout the experiments. The mycelium for cell wall preparations was grown in a glucose mineral salt medium (GMS; Townsley 1985) with the following composition (in $g l^{-1}$): $C_3H_5(OH)_2PO_4Mg \cdot 2H_2O$, 0.5; $(NH_4)_2SO_4$, 2.0; KCl, 0.5; $CaCl_2 \cdot 6H_2O$, 0.25; 2(*N*-morpholino)-ethane sulphonic acid (MES buffer), 9.76; glucose, 20.0. Trace metals were supplemented by adding 0.1 $ml l^{-1}$ of a solution containing (in $g l^{-1}$): $FeSO_4 \cdot 7H_2O$, 5; $ZnSO_4 \cdot 7H_2O$, 1.75; $MnSO_4 \cdot H_2O$, 0.076; $CuSO_4 \cdot 5H_2O$, 0.1. The pH was adjusted to 5.5 with KOH or HCl. The spore suspension was washed once with GMS medium and inoculated in 100 ml GMS medium, giving 1×10^6 spores ml^{-1} culture medium. The inoculated medium was incubated on a rotary shaker (120 r.p.m.) for 72 h at 20 °C. The mycelium was harvested by centrifugation (15000 × *g*; 10 min) at late logarithmic phase/onset of stationary phase as determined from an earlier established growth curve.

Cell wall preparation

After incubation, the myecelium was thoroughly washed with sterile Milli-Q water and homogenized with an Ultra Turax homogenizer for 5 min at the highest speed. The mycelium suspension was centrifuged at 15000 × *g* for 10 min. The pellet was resuspended in sterile Milli-Q water and sonicated (Bandelin Sonorex RK 510H, 500/1000 W) for 15–30 min and washed repeatedly until no residual glucose or protein could be detected in the supernatant. The cell wall preparation was freeze dried and stored at 4 °C. The lyophilized powder was washed twice prior to experiments with the solution used in the metal loading studies.

Chitin preparation

Colloidal chitin was prepared from practical grade crab shell chitin (Sigma C-3387) according to Rodriguez-Kabana *et al*. (1983).

Adsorption

Adsorption isotherms were determined for zinc, cadmium and mercury at 10^{-8} to 10^{-4} M to determine the maximum metal loading capacity of the fungal cell walls and chitin. A radiotracer batch distribution technique was applied using ^{65}Zn, ^{109}Cd and ^{203}Hg as tracers (from Amersham, UK, all as chlorides) and non-radioactive carriers of the same elements to achieve the total concentrations desired. The metal binding studies were made in 50 ml polyallomer centrifuge tubes containing a total sample volume of 20 ml. All equipment was cleaned by soaking in an acidic wash solution (3 M HNO_3; 0.1 M HCl) overnight and then rinsed eight times in Milli-Q water. Solutions of chitin and powder of fungal cell walls were added to the electrolyte solution (0.1 M KCl) to give a final amount of solid phase in each sample of 2 $g l^{-1}$ cell walls and 1.5 $g l^{-1}$ chitin, respectively. An acidic metal stock solution was added to give a final well-defined total metal concentration in the range 10^{-4} to 10^{-8} M and pH was adjusted with 0.1 M KOH to 6.1. The volume was adjusted to 20 ml by adding 0.1 M KCl. Phase separations were made by centrifugation after a contact time of 19 h (on rotary shaker of 120 r.p.m. and 20 °C). Aliquots (1 ml) of the supernatants were sampled and analyzed for their metal content by using a well-type scintillation counter (Compu Gamma; LKB Products, Bromma, Sweden). Also, the pellets (cell walls or chitin) were collected and their radioactivity measured. Metal adsorption on the vessel walls was estimated from the total mass balance.

Desorption

The desorption of metals, after changing the liquid phase, was measured in order to determine the metal concentration in solution and on the substrate during the enzymatic assay. The kinetics of desorption from metal-loaded fungal cell walls and chitin were studied in 50 mM MES buffer. This buffer was chosen since it does not form strong complexes with the present metals (Townsley 1985). Sorption of zinc, cadmium and mercury was first determined at a metal concentration of 10^{-4} M, as described for the isotherm studies. The solid phase was 40 mg l^{-1} (0.20% w/w) cell walls and 30 mg l^{-1} (0.15%, w/w) chitin, respectively. Samples of 1 ml from the batch distribution vessels were collected after 24 h and analyzed for their metal contents. The solid phase was separated from the liquid by centrifugation (15000 × *g*; 15 min), and the pellets were resuspended in 20 ml of 50 mM MES buffer (pH 6.1). The desorption of the metals was then followed for 6 h by measuring the amount of metals in solution, as described for the isotherm studies.

Metal loading

Fungal cell walls and chitin were loaded with zinc, cadmium and mercury in separate batches for subsequent studies of the effects of bound metals on the enzymatic degradation. The cell walls or chitin was suspended in sterile 0.1 M KCl (adjusted with 0.1 M KOH to give a final pH of 6.1 after addition of metal solution), yielding a 0.15% chitin solution and 0.2% fungal cell wall solution, respectively. Portions of 2 ml of these solutions were added to 10 ml polypropylene test tubes. Metal solutions of zinc, cadmium and mercury were added to give a total concentration in solution of 1×10^{-4} M. The test tubes were centrifuged at 15000 × *g* for 15 min after a contact time of 19 h on a rotary shaker (120 r.p.m.). The pellets were suspended in 2 ml of 50 mM MES buffer (pH 6.1).

Inhibition of enzymatic activity

The effects of metals in solution on the activity of Novozym 234 (Novo Nordisk, Denmark) with metals present was assayed by measuring the release of *p*-nitrophenol from *p*-nitrophenylchitobiose, *p*Np-$(GlcNAc)_2$ (Roberts & Selitrennikoff 1988). The *p*-nitrophenylchitobiose (Sigma, St Louis, MO) was dissolved in 50 mM MES buffer (pH 6.1). An aliquot from a metal stock solution was added to 100 µl pNp-$(GlcNAc)_2$ to obtain a final concentrations of 10^{-4} and 10^{-5} M, respectively of each metal. Novozym 234 was then added (1 µl, 10 mg ml^{-1}) and incubated at 37 °C. The reaction was terminated by adding 5 µl of 1 M NaOH and the absorbance was measured at 410 nm.

The effect of metals on the hydrolysis of fungal cell walls and chitin by Novozym 234 was measured as the release of reducing sugars. The reaction mixture, containing 2 ml of 0.15% chitin (metal loaded) or 0.2% fungal cell walls (metal loaded) suspended in 50 mM MES buffer (pH 6.1) and with 20 µl enzyme solution (10 mg ml^{-1} Novozym 234) was incubated for 1–3 h at 37 °C under continuous shaking. After the incubation period the samples were centrifuged (15000 × *g* for 15 min) and the amount of reducing sugars released into the supernatant was determined (Dygert *et al.* 1965) using *N*-acetylglucosamine as standard. The enzymatic releases of reducing sugar from cell walls and chitin without metals were run as standards. Also cell walls, chitin and enzyme solution dispersed in buffer were examined for the presence of reducing compounds as controls. Enzymatic activity was defined as µmoles of reducing sugar released per hour.

Calculations

The background of reducing compounds originating from fungal cell walls and the enzyme mixture was measured in each experiment. The median values of these measurements were subtracted from the amount of reducing sugar due to enzymatic hydrolysis of the substrates. A corresponding subtraction was made for the chitin experiments. The background medians were based on six to 19 observations and ranged from 8 to 19 µg l^{-1}.

A statistical analysis was made on the enzymatic assay data. A two-factor general analysis of variance (ANOVA) was made on the data for fungal cell walls, as the enzymatic activity varied somewhat between the three experiments. Simple contrast between experiments with no metal and metals was calculated and joint univariate 95% confidence limits were established. For chitin a general one-factor ANOVA was calculated, since this study was limited to one experiment. The test was made on ranks of the data to avoid assumptions of normality and equal variance. The ANOVA analysis was made with the SPSS® for Windows™ program (SPSS Inc.) using the Advanced Statistics module (Norušis 1992).

A calculation was made for all possible pairwise relative differences between no metal and metals within each experiment. For each metal the median of these relative differences was taken as typical enzymatic inhibition. A confidence interval was established based on all relative differences. This confidence limit underestimates the true limit somewhat.

Results

The sorption of zinc, cadmium and mercury by fungal cell walls as well as chitin could be described by linear isotherms at metal concentrations below 10^{-4}–10^{-5} M except for the chitin/mercury system (Figure 1a and b). The total complexing capacities for these metals were estimated to be at least 50 mmol kg^{-1} fungal cell walls (dry weight) and 10 mmol kg^{-1} chitin (dry weight), respectively. No pronounced differences in the affinity order at a metal concentration of 10^{-4} M could be distinguished for the two substrates (Figure 1a and b). However, at lower metal concentrations (10^{-7} M) the affinity for mercury was higher than for the other two metals.

The desorption of zinc, cadmium and mercury as a function of time is depicted in Figure 2 (a and b). The

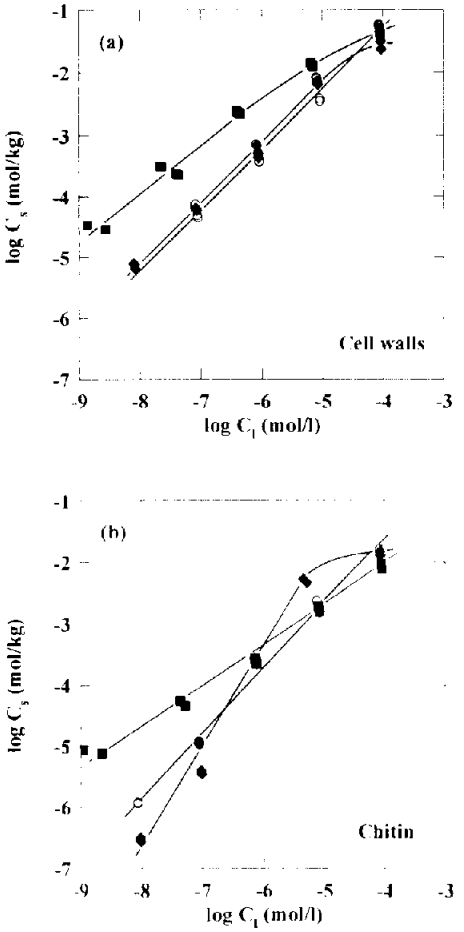

Figure 1. Adsorption isotherms (C_S, concentration of metals on the solid phase; C_l, concentration of metals in the aqueous phase) for the accumulation of zinc, cadmium and mercury by fungal cell walls (a) and chitin (b) (5 mg solid phase in 20 ml 0.1 M KCl; 19 h at room temperature and pH 6.1). Symbols: ◆, zinc; ○, cadmium; ■, mercury.

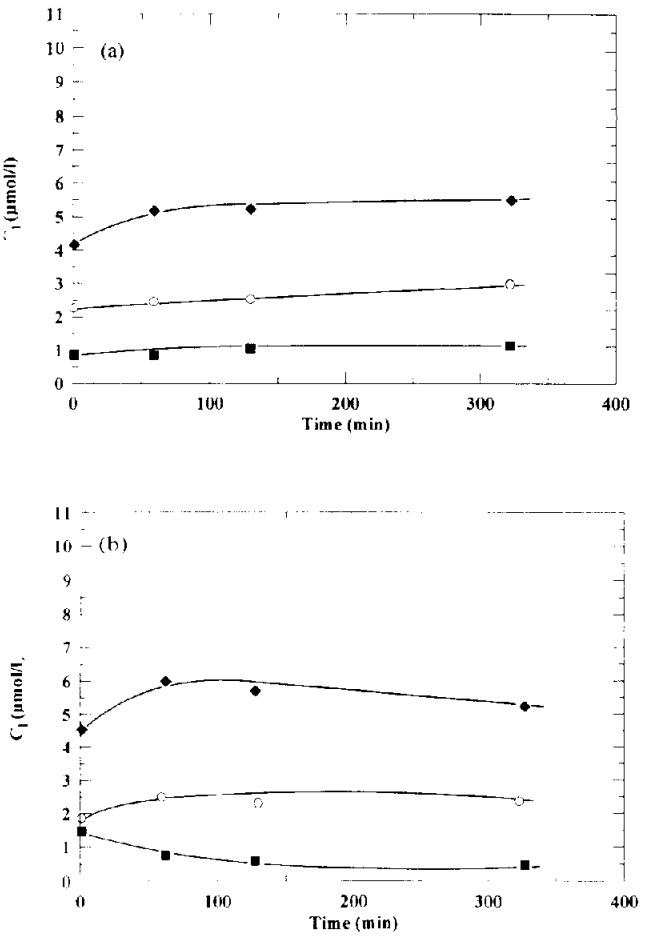

Figure 2. Kinetics of the desorption of zinc, cadmium and mercury from cell walls (a) and chitin (b) in 50 mM MES buffer at pH 6.1 (the symbols represent the mean values of three parallels). Symbols: ◆, zinc; ○, cadmium; ■, mercury.

desorption, after the change of the liquid phase from potassium chloride to MES buffer, reached a constant level within 1 h and no further change was observed after 6 h. The desorption of mercury was low for cell walls (5%) as well as for chitin (10%) (Figure 2a and b), indicating that mercury was the metal most strongly bound by both fungal cell walls and by chitin. A negative slope of the curve showing the desorption of mercury from chitin was obtained. However, since the desorption of mercury in general was very low, the values obtained are close to the detection limit and thus uncertain. The release of adsorbed cadmium from the cell walls and chitin was 10–15%, suggesting a similar degree of cadmium binding between the two components. The highest release, 60%, was detected for zinc from chitin, while the desorption of zinc from fungal cell walls was 20%.

The amounts of metals adsorbed by the cell walls after changing the liquid phase were similar for the three metals (Figure 3). The total amount was lower for chitin compared with cell walls, except for cadmium. However, the cadmium data are based on one single experiment. The

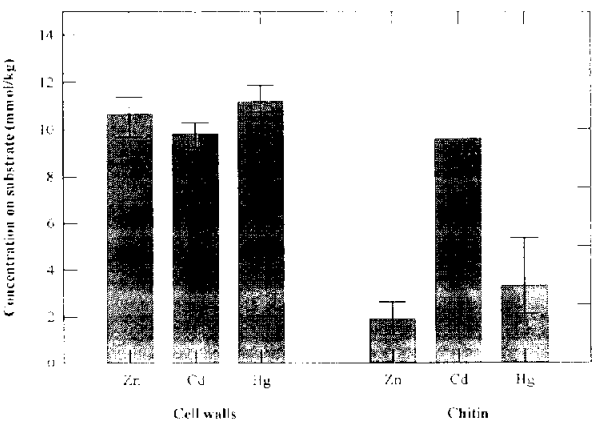

Figure 3. The mean concentration of zinc, cadmium and mercury adsorbed by fungal cell walls and chitin after desorption (6 h) expressed as mmol kg^{-1} (solid phase ratio: 0.20%, cell walls and 0.15%, chitin in 0.1 M KCl; initial metal concentration of 1×10^{-4} M; 19 h at room temperature and pH 6.1). The error bars represent minimum and maximum values.

relationship between metal concentrations remaining on the two substrates agrees with the adsorption capacities determined in the isotherm studies (Figure 1a and b).

Measurements of the chitinase activity of Novozym 234 with metals in solution showed that the release of *p*-nitrophenol by Novozym 234 was inhibited only in the presence of mercury at 10^{-4} M (data not shown). No reduction in enzymatic activity could be observed in the presence of zinc and cadmium even at the highest studied metal concentration (10^{-4} M; data not shown).

The release of reducing sugar from both fungal cell walls and pure chitin was partially inhibited by the presence of bound metals (Figure 4). The degree of inhibition for cell walls loaded with zinc, cadmium and mercury was 2, 26 and 48%, respectively, while the same measurements for chitin revealed an inhibition of 17, 22 and 22%, respectively.

Statistical analysis confirms the difference in release of reducing sugar from fungal cell walls between experiments with and without metals ($P < 0.001$). A significantly lower amount of reducing sugar is found with mercury is present, compared both with the case without metal and the two other metals ($P < 0.05$). The presence of cadmium on cell walls might also have an inhibitory effect ($P < 0.10$), while no significant difference was found between experiments with zinc and experiments without metals. It is harder to draw any definite conclusion about chitin since there were no differences at the 5% significance level ($P = 0.09$).

Discussion

Fungal cell walls adsorbed zinc, cadmium and mercury to a total capacity of at least 50 mmol kg^{-1} (at pH 6.1) with no pronounced differences in affinity order at the highest metal concentration studied (Figure 1a). Similar results were previously obtained for intact hyphae of *T. harzianum* (Krantz-Rülcker *et al.* 1993b). Furthermore, the entire isotherms for these two systems (cell walls and intact hyphae) are quite similar. The main accumulation of metals by this fungus thus appears to be due to sorption on the cell wall. The adsorption capacity of chitin was at least 10 mmol kg^{-1} (Figure 1b). This difference in capacity in comparison with the fungal cell walls suggests that additional components besides chitin are involved in the sorption of metals by the fungal cell walls. However, it has been shown that the metal binding capacity of chitin from different sources varies substantially. Manskaya & Drozdova (1968) reported that 20% of added uranium was adsorbed by chitin from crab shells, while chitin of a fungal origin adsorbed 60% of the added uranium. This observation may explain the difference in metal binding between fungal cell walls and chitin in the present study, since the chitin was prepared from crab shells.

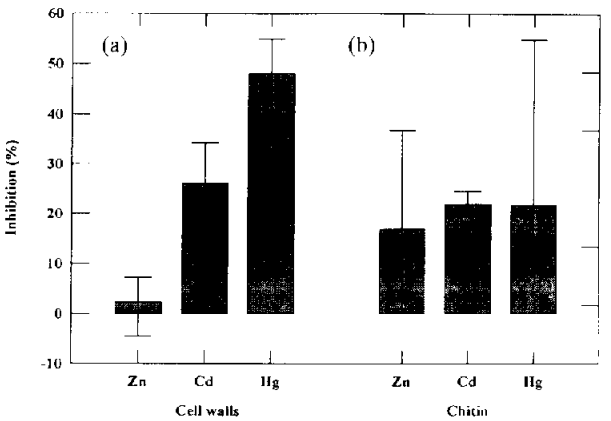

Figure 4. Enzymatic activity of Novozym 234 on cell walls (a) and chitin (b) expressed as the median percentage inhibition of release of reducing sugar by metal complexation (0.20% cell wall, respectively, 0.15% chitin in 50 mM MES buffer, 1–3 h at 37 °C). Error bars represent 95% confidence intervals.

The higher affinity for mercury by both solid phases, at low metal concentrations, indicates the presence of selective adsorption sites. The capacities of these sites must be low since the differences are not distinguishable at higher mercury concentrations.

The enzymatic release of reducing sugars from the two substrates was affected by the accumulated metals but to different extents. Enzymatic digestion of fungal cell walls preloaded with mercury was significantly reduced (by 48%). A lower degree of inhibition was observed for cadmium (26%), while zinc did not cause any significant enzymatic inhibition. The inhibition by mercury of the enzymatic digestion of chitin was not as pronounced as of the fungal cell walls. This can be explained by the fact that chitin adsorbs a lower total amount of mercury compared with the cell walls (Figure 3). In the case of cadmium, the results from the sorption and desorption as well as the effects on the enzymatic digestion were similar to those obtained for cell walls. The data obtained for zinc and chitin indicated a larger decrease in the release of reducing sugar. However, a lower total amount of zinc was adsorbed by chitin than by the cell walls (Figure 3). Possibly, the cell walls contain components other than chitin that could complex zinc. Another enzyme in the enzyme mixture (Novozym 234, e.g. glucanase) may be responsible for the release of reducing sugar from the cell walls. This enzyme may be less sensitive to the complexation of zinc by the cell walls than chitinase, which is the enzyme responsible for the degradation of chitin and *p*-$(GlcNAc)_2$. No far reaching conclusions should be drawn from the differences between the three metals and between the fungal cell wall and the chitin systems since the confidence intervals are large particularly for the chitin experiments (Figure 4). The use of non-parametric confidence intervals makes the observed asymmetric intervals possible. The apparent differences in statistical distribution between the inhibitions of each metal is probably due to the limited number of replicates. A single outlier can have a strong influence on the estimated confidence limit. Still, the intervals give some visual representation of the variation in the data. The negative interval for zinc and cell walls (Figure 4) does of cause only indicate that the inhibition is not different from zero and should not be interpreted as a possible stimulation of the enzyme.

The observed decreased enzymatic activity was clearly

not caused by metals in solution. Measured metal concentrations in solution after desorption from the cell walls were 5.5×10^{-6}, 3.0×10^{-6} and 1.1×10^{-6} M for zinc, cadmium and mercury, respectively, and 5.2×10^{-6}, 2.3×10^{-6} and 0.47×10^{-6} M, respectively, for chitin. Inhibition of the enzymatic activity, due to metals in solution, was only observed in the mercury system at a concentration of 1×10^{-4} M, as indicated from the *p*-nitrophenol release experiments. This is about 100 times higher than the measured metal concentration in solution during the enzymatic assay. These results clearly indicate that the observed decrease in degradation of metal loaded cell walls is due to the complexation of the metals by the substrate and not to the metals in solution.

The fact that the metals that were most readily desorbed (Figure 2) caused the lowest degree of enzymatic inhibition (Figure 4) supports the hypothesis that it is the complexation of metal ions by the substrate that is reponsible for the observed reduction in enzymatic activity. It is also obvious that the three metals studied have different effects on the enzymatic hydrolysis of the two substrates. These differences in inhibition can be related to the different chemical properties of the metals. This conclusion is based on the fact that the final amounts of the metals adsorbed by the cell walls were similar despite differences in the amounts desorbed.

The inhibition of the enzymatic digestion of fungal cell walls by adsorbed metals was increasing in the order Zn < Cd < Hg, which follows the size of the metal ions (0.75, 0.95 and 1.02 Å for zinc, cadmium and mercury, respectively; Jesson & Muetterties 1969). This possibly reflects a larger sterical hindrance to the enzyme by a larger metal ion. The metals also have different ligand preferences, which means that the three metals may form complexes with different ligand atoms present on the cell surfaces of the mycelia (e.g. sulfur for mercury, oxygen for zinc).

Knowledge of the inhibitory effects of metal ions on lytic enzymes is poor. However, Balsasubramanian & Manocha (1992) have studied the effects of mercury, copper, manganese and zinc on membrane-bound and cytosolic chitinases from two mucoraceous fungi, *Coanephora cucurbitarum* and *Phascolomyces articulosus*. Their results showed that the cytosolic chitinase was totally inhibited by all metals. The membrane-bound chitinase was totally inhibited by mercury and manganese, while copper and zinc had an inhibitory effect of 98 and 23% (*C. cucurbitarum*), respectively, and of 53 and 66% (*Ph. articulosus*) on the chitinase activity. Whether this inhibitory effect was due to binding of the metals to the chitinases, to the substrate or to both is not possible to distinguish since the substrate and enzyme were added to a solution with a rather high metal concentration (50 mM) without the removal of non-adsorbed metal ions before the enzymatic assay.

The inhibition of enzyme activity by metal binding to the enzyme is well known and has been proposed to be one of the mechanisms of metal toxicity to organisms (Tyler 1981). The effects of the binding of metals to the substrate on the activity of corresponding enzyme are scarcely reported. However, Brynhildsen (1992) reported an almost complete inhibition of the microbial degradation of zinc–, copper– and cadmium–citrate. The experiments were designed to yield 95% of the citrate in the metal-complexed form. The metal complexes were taken up by the cells, but the citrate carbon was not assimilated or incorporated into macromolecules. Disturbed interactions between the enzyme aconitase and the substrate in the conversion of citrate to isocitrate due to the complexed metal ions by the citrate could be a possible explanation. However, this hypothesis could not be confirmed due to methodological problems when working with the aconitase assay and metals.

The consequences of an inhibition of lytic enzymes by metal complexation of the substrates suggested by the present work could be important. The immobilization of metals bound by fungal cell walls and other chitinous materials could be significant, if the complexation of metals results in a reduction of the degradation of the materials. This possible reduction could also cause a limited recycling of an important source of nutrients in nature. Those arguments stress the need of further research of the effects of metals bound to organic substrates on lytic enzymes, such as chitinases.

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