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Structural determination of Pb binding sites in *Penicillium chrysogenum* cell walls by EXAFS spectroscopy and solution chemistry

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Fungal cell walls possess strong complexing properties, which make them valuable biosorbents to remove heavy metals from wastewaters. The binding mechanism of Pb to *Penicillium chrysogenum* cell walls was studied by the combination of solution chemistry and EXAFS spectroscopy as a function of the complexation rate, the metal concentration range investigated including two orders of magnitude. The corroborating macroscopic and microscopic results allowed us to identify two different Pb-binding functional groups, carboxyl and phosphoryl groups. The former were the strongest and minor (about 5%) complexing groups, whereas the latter were the weakest and predominant (about 95%) groups.

Keywords: biosorption, cell wall, EXAFS, complexation

1. Introduction

Filamentous fungi can be profitably used in processes for heavy metals removal from wastewater due to their low cost and to the high ion exchange capacity of their cell walls. This property arises from the large density of functional groups present in the cell wall (carboxyl, hydroxyl, amine, phosphoryl, sulfhydryl, ...), creating a negatively charged surface (Krantz-Rulcker *et al.*, 1994). The sorption properties of cell walls have been widely studied by solution chemistry (Volesky, 1990; Wase & Forster, 1997), but the chemical nature of complexing groups is not known. In the present work, the nature of Pb binding sites on the cell walls of the filamentous fungus *Penicillium chrysogenum* was investigated at the macroscopic level by sorption isotherm, and at the molecular level by extended X-ray absorption fine structure (EXAFS) spectroscopy by varying the metal concentration by two orders of magnitude, down to 4.8 10^{-3} mmol Pb/g. The study of such diluted samples, which allowed us to probe the high affinity sites of the cell wall, was feasible owing to the high photon flux delivered by the 3rd generation synchrotron radiation source of the European Synchrotron Radiation Facility ESRF) and to the use of a high counting rate 15-element array germanium detector.

2. Materials and methods

Dead cells of *P. chrysogenum*, who have been previously characterized (Fourest *et al.*, 1994) were supplied by Gist-Brocades (Seclin, France). The sorption isotherm and the preparation of the Pb-cell wall samples were carried out at pH 6 ± 0.1 in 0.1M $NaNO_3$ background electrolyte (Sarret *et al.*, 1998). Pb L_{III}-edge EXAFS experiments were performed on the BM32 CRG/IF beamline at ESRF. Spectra were recorded in fluorescence or transmission mode depending on the metal concentration. Pb Lα fluorescence was recorded using a Canberra 30-element array germanium detector with a total counting rate of 300 000 photons/s. Phase and amplitude functions for Pb-O pair were extracted from αPbO spectrum (Leciejewicz, 1961), and Pb-C and Pb-P phase functions were calculated *ab initio* using FEFF7 code (Rehr *et al.*, 1991).

3. Results

3. 1. Sorption isotherm

Pb sorption isotherm exhibits two distinct plateaus (Fig. 1). The first one at low Pb^{2+} concentration (about 0.02 mmol /l) unequivocally indicates the presence of a strong binding site (Pb1), which is saturated at low metal concentration. The second plateau at about 2 mmol Pb^{2+} /l arises from the presence of a weaker site (Pb2), which is the predominant functional group of the cell wall and is occupied at high metal concentration. This isotherm could be fitted with two Langmuir equations, indicating that Pb did not precipitate as metal hydroxide. The associated sorption constants were $K_{D\text{-}Pb1}$ = 0.003, $C_{(Pb1)tot}$ = 0.01 mM, and $K_{D\text{-}Pb2}$ = 0.35, $C_{(Pb2)tot}$ = 0.20 mM, where C is the total sorption capacity of a site. Thus, the high affinity Pb1 sites represents about 5% of total sites.

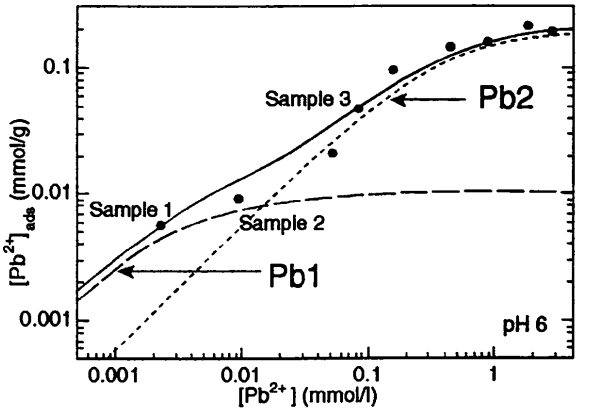

Figure 1
Adsorption isotherm of Pb on *Penicillium chrysogenum* cell walls. $[Pb^{2+}]$ is the concentration in solution at equilibrium.

3. 2. EXAFS results

EXAFS spectra for Pb complexed by *P. chrysogenum* cell walls are presented in Fig. 2a. A loss of amplitude is observed with increasing Pb concentration from sample 1 to sample 2, suggesting an increased structural disorder. The phase of EXAFS spectra also varies with Pb concentration as noted on the first and third oscillations, suggesting a modification of the type of Pb binding groups at increasing Pb concentration. Pb-EXAFS spectra have a low amplitude due to the particular coordination

chemistry of lead, which is characterized by a large and sometimes anharmonic distribution of interatomic distances (Manceau *et al.*, 1996). This spread in distances is likely enhanced in polyfunctional system like the present one. For this reason, EXAFS distances were determined only for the first shell.

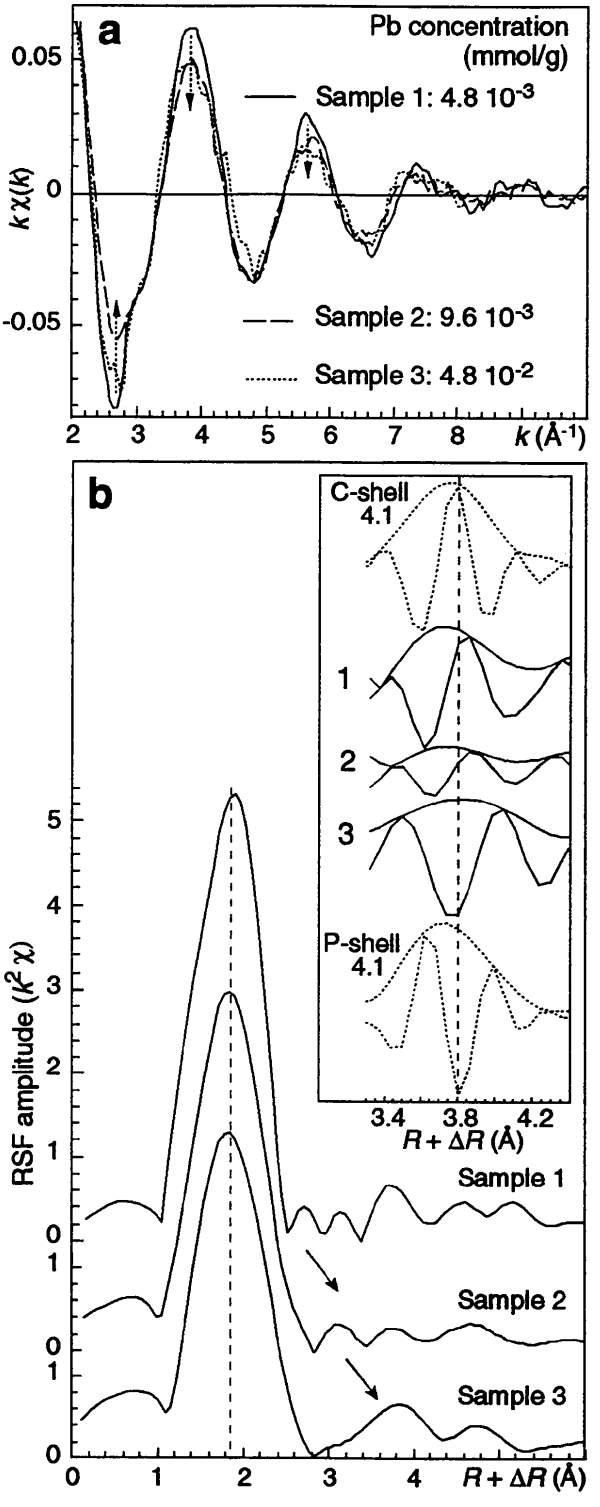

Figure 2
Pb L_3-edge EXAFS spectra (a) and RSFs (b, uncorrected for phase shift ΔR) for *P. chrysogenum* cell walls calculated in the [3.4-10.0 $Å^{-1}$] k range. Inset: Modulus and imaginary parts of Fourier transforms.

The second shell was interpreted semi-quantitatively by examining the modulus and phase of the electronic waves. The position of the first peak is constant in the three samples, and corresponds to an oxygen shell at about 2.39 Å (Figure 2b, Table 1). There was no indication of Pb coordination by sulfur, for which an interatomic distance higher than 2.7 Å would be expected (Sarret *et al.*, 1998), showing that sulfhydryl groups do not participate to Pb binding. As expected, coordination numbers (*CN*) are particularly low and decrease with increasing Pb concentration (2.4 to 1.7, Table 1). Because of the high structural disorder of the Pb coordination shells, these values do not reflect the real coordination numbers of Pb atoms. A second RSF peak is detected for samples 1 and 3 at $R + \Delta R = 3.7$ Å, which may be attributed to either C or P next-nearest neighbors. These two atoms can be conclusively distinguished by analyzing the phase of the Pb-P and Pb-C electronic waves. As illustrated in the inset of Fig. 2b, the imaginary parts of the Fourier transforms for these two pairs are precisely out of phase. Thus, EXAFS spectroscopy allows a clear distinction to be made between phosphoryl and carboxyl groups, even at the Pb L_{III} edge. The inset in Fig. 2b shows that the imaginary part of sample 1 is superimposed on the Pb-C simulation, whereas sample 3 resembles the Pb-P simulation. This analysis unambiguously allows us to conclude that Pb is predominantly bonded to carboxyls at very low concentration, and to phosphoryls at high concentration. The imaginary part of sample 2 is intermediate to the two extremes, which indicates that this sample contains both Pb-COO and Pb-PO_4 complexes. Since Pb-C and Pb-P waves are out of phase, the mixing of the two complexes lowers the resulting wave amplitude. This effect accounts for the very low amplitude of the second RSF peak of sample 2, which is nearly extinguished. This wave phase analysis provides direct spectroscopic insight on the nature of Pb1 and Pb2 sites observed on adsorption isotherms (Fig. 1). Sample 1 is located in the lower region of the isotherm, which allows us to conclude that high affinity Pb1 sites are carboxyls. Inversely, sample 3 is located in the upper part, and in this region Pb is predominantly bound to phosphoryl groups. Sample 2, located on the first plateau of the isotherm, is intermediate: Pb is bound to both COO and PO_4 groups.

Table 1
Structural parameters for the 1st oxygen shell of Pb complexed to *Penicillium chrysogenum* cell walls

Compound	R (Å)	*CN*	$\Delta\sigma$ (Å)	Q
αPbO	2.31	4.0	-	-
Sample 1, 4.8 10^{-3} mmol/g	2.39	2.4	0.02	0.008
Sample 2, 9.6 10^{-3} mmol/g	2.39	1.9	0.02	0.010
Sample 3, 4.8 10^{-2} mmol/g	2.39	1.7	0.02	0.010

R: interatomic distances, *CN*: number of atomic neighbors, $\Delta\sigma$: differential Debye-Waller factor expressed as the difference of σ between the αPbO reference and the sample, Q: figure of merit for the fit : $Q = \sum(k^2\chi_{exp} - k^2\chi_{th})^2 / \sum(k^2\chi_{exp})^2$.

4. Conclusion

Minor ≡COO (~5%) surface ligands of the *P. chrysogenum* cell walls have a high affinity for Pb compared to the predominant ≡PO_4 ligands (~95%). For this reason, the preferential formation of ≡$(COO)_n$-Pb complexes at low Pb concentration, followed by the formation of overwhelming ≡$(PO_4)_n$-Pb complexes at higher concentration, is recognized on the adsorption isotherm by the presence of two distinct plateaus. Pb1 site identified on sorption isotherms corresponds to the minor ≡COO ligands, whereas Pb2 site corresponds to the dominant ≡PO_4 ligands. This application of EXAFS spectroscopy to the study of the complexation

mechanism of metals to fungi cell walls can be extended to many other natural organic systems like soil organic matter, bacteria, higher plants, and opens large perspectives in environmental biogeochemistry

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