Binding of Cd ions to the cell wall of B. Subtilis-- an EXAFS study

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Introduction

Understanding metal complexation in aqueous solutions and biological systems is essential to studying metal contaminant distributions in the environment, as well as for developing remediation techniques. Cell walls have been shown to facilitate the precipitation of metals [1,2,3], but the exact mechanism and binding sites for the metal ions is largely unknown. The ability of the Extended X-ray Absorption Fine Structure (EXAFS) technique to probe the local surroundings of the absorbing atom is being used to study the complexation of Cd on the cell walls of B.Subitilis in a changing pH environment. Sorption studies suggest that Cd binds to different functional groups at different pH regions [1], but microscopic studies have not been done.

Methods and Materials

Sample preparation is described elsewhere [1]. Briefly, it involves culturing the *B. subtilis* cells in an appropriate medium, then pelleting them by centrifugation, after which a series of rinsing procedures are followed to strip the cell walls of any metal cations possibly present in the growth medium. The rinsing includes two times rinsing in distilled deionized water (DDI), soaking for one hour in 0.1 HMO_3, DDI rinse, 0.001 N EDTA soaking overnight and a five times final DDI rinse. The cells remain viable after all of the above procedures are applied. An aqueous solution containing a known concentration of Cd in a 0.10 M $NaNO_3$ is placed in contact with a known weight of bacteria, allowed to equilibrate and stirred for about 30 min. The sample is then filtered through a 0.45μm filter. Any decrease in metal concentration in the solution is assumed to be coming from Cd atoms adsorbing to the cell wall. The filtered mass is then placed in specially designed sample holders for fluorescence EXAFS experiments. In the end, a series of samples made by running the same concentration Cd solution through the same *B. subtilis* cell mass at different pH values were made. EXAFS measurements were done in fluorescence mode in a Stern-Heald type detector, with 100% Kr gas sealed in the ion chamber. 5 to 6 scans from 4 different sample spots were taken and averaged.

For calibration standards used in the XAFS analysis, spectra from various cadmium standard compounds were measured. These include CdO, $Cd(OH)_2$, Cd acetate, Cd phosphate, CdS and $CdSO_4$. EXAFS parameters were extracted from them using their known crystallographic structure, to be used later in the analysis of the unknown samples.

MRCAT beamline is equipped with a double-crystal Si (111) monochromator and undulator which allows XAFS measurements over the energy range from 5 KeV to 30 KeV. A harmonic rejection mirror was used to eliminate the third and higher x-ray harmonics from the monochromator; the contamination of the beam by these higher energies greatly complicates the data interpretation. Fast-scanning mode was used which minimizes the radiation damage to the film.

Results and Discussion

The step height in an absorption edge spectrum in fluorescence mode is proportional to the amount of absorber in the sample, provided that gases and gains of the detectors are kept constant. Given below (fig.1) are the step heights for the various pH samples, with error bars being the standard deviation of all scans for that pH.

The graph clearly shows regions of steeper rises (pH=4 to 5.5, then 7 to 8.5) and plateaus in between. The data point at pH=9.2 shows a drop in adsorption of the metal which could be due to precipitation or to complexation of Cd to hydroxyl groups so that less amount of Cd is available to be adsorbed to the cell. The same trends at similar pH's are seen in the adsorption studies [1] which are interpreted as the "switching-on" and saturation of

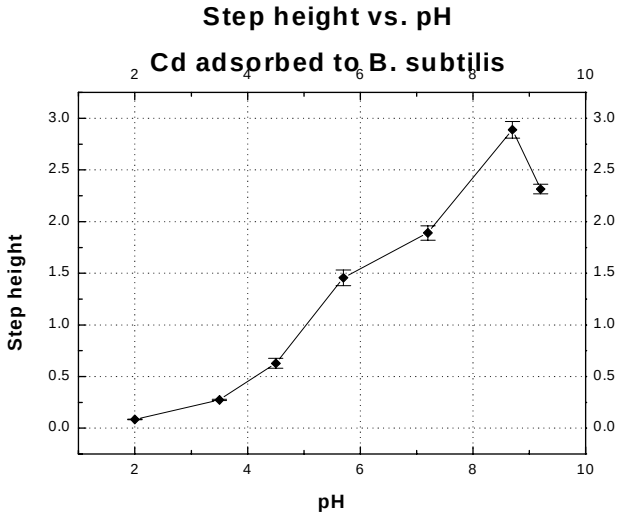

Fig.1 Step height vs. pH for *B. subtilis* samples

different binding sites in the cell walls. In this way we are able directly and independently to confirm that the trends in the pH dependence come from the Cd actually adsorbed to the cell wall.

Looking at the Fourrier Transform (FTR) of the EXAFS itself, we see trends in the first peak amplitude (fig.2):

As pH is increased, the first peak amplitude is decreased, except for the pH=9.2 sample (which is the sample with the drop in the amount of adsorbed Cd). This trend is independent of the step height trend and is due to one of the following reasons:

- reduction of the number of the same functional group attached to Cd
- change of the ratio of different groups attached to Cd--this would introduce different signals in the EXAFS which interfere with each other and reduce the peak amplitude.

- simulations with FEFF6 [4] show that small changes in the distance of the O and C atoms in a carboxyl environment (which is the expected environment) could lead to constructive or destructive interference between the similar signals coming from O and C near-neighbor backscatterers.

Detailed fitting using FEFF6 [4] and FEFFIT [5] with calibration parameters determined from the measured standards should be

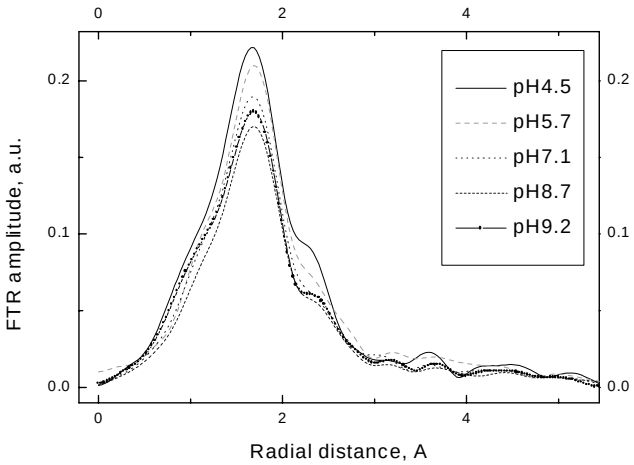

Fig.2 Fourrier transforms of the EXAFS spectra of the *B. subtilis* samples

able to resolve which of the above reasons is responsible for the decrease in amplitude. Some qualitative conclusions can be made by comparing the sample FTRs to the FTRs of the powder standards Cd acetate and Cd phosphate (fig.3):

The two standards (symbols) have the same first peak position, but have slightly different structure at 2.1A and above 3.0A. The structure above 3.0A is due to longer range order present in the

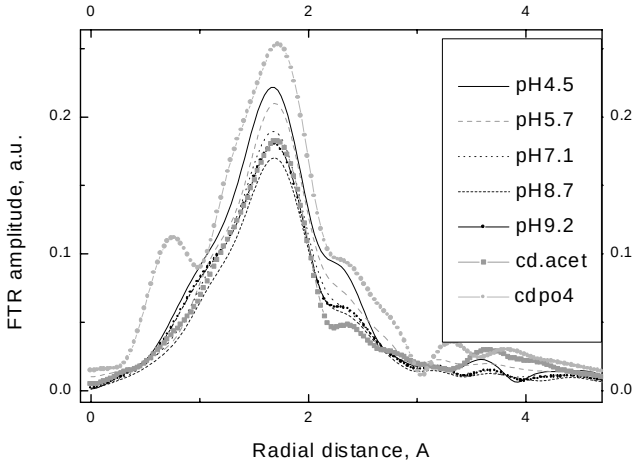

Fig.3. Comparison of the sample FTRs with the FTRs of standard compunds

powder standard but not present in the (liquid) samples. Therefore, the differences in the samples are being sought in the region up to about 2.2A. Sorption studies suggest that as pH increases the binding sites shift gradually from carboxyl to phosphoryl [1]. We see an indication of this in the region about 2.2A, where the acetate has a bigger dip than the phosphate. That trend is seen in the samples as well, where the slope of the curve at 2.2A is almost zero for the pH=4.5 sample and decreases, up to the pH=9.2 sample. Detailed fitting underway will be able to resolve how the environment changes, as mentioned before.

Conclusions

Cd K-edge EXAFS measurements have been done to determine the mechanism of Cd adsorption to the cell walls of *B.subtilis* on the atomic level. A direct and independent confirmation of the results for the amount of Cd adsorbed to the cell wall as a function of pH in [1] has been obtained. Clear trends are seen in the first peak FTR amplitude and shape as pH changes, indicating change in the average local environment of the Cd atoms. Qualitative results are consistent with a gradual change from carboxyl at low pH to phosphoryl environment at higher pH. Detailed fitting using EXAFS parameters determined from measured calibration standards is underway and will ultimately show the mechanism of adsorption.

Acknowledgments

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