 ROBL-CRG	Experiment title: Interaction between microorganisms and metals using bacteria	Experiment number: 20-01-625
	Beamline: BM 20	Date of experiment: 04.03.-05.03.05; 03.12.-06.12.05; 24.07.-26.07.06
Shifts: 18	Local contact(s): Dr. Andreas Scheinost	<i>Received at ROBL:</i>
Names and affiliations of applicants (* indicates experimentalists): M. Merroun*, K. Pollmann, J. Raff, A. Scheinost*, A. Rossberg*, C. Hennig*, H. Funke*, S. Selenska-Pobell		

Report: The S-layer of *Bacillus sphaericus* strain JG-A12, isolated from a uranium-mining site, exhibits a high metal-binding capacity, indicating that it may provide a protective function by preventing the cellular uptake of heavy metals and radionuclides. This property has allowed the use of this and other S-layers as self-assembling organic templates for the synthesis of nano-sized heavy metal cluster arrays /1, 2/. However, little is known about the molecular basis of the metal protein interactions and their impact on secondary structure. In this work, X-ray absorption spectroscopy was used to determine the structural parameters of the Pd complexes formed by this S-layer protein.

Experimental: For sorption of Pd(II), the protein was dialysed against H₂O and 10 mg of it were incubated in 100 ml of a solution of 2 mM Na₂PdCl₄ (pH = 3.1), which was prepared 24 h before the use and kept in dark. After 3 hours of incubation at room temperature under shaking in the darkness the sample was centrifuged and the pellet was resuspended in H₂O. Residual salts were removed by dialysis of the metallised proteins against H₂O. For XAS analysis, the protein samples were dried in a vacuum oven (48 h, 80°C) and pulverised.

Results: Figure 1 shows the XANES regions of the XAS spectrum obtained with the Pd(II)-bound S-layer from strain JG-A12 and for reference compounds containing two oxidation states of palladium: Pd(II) (PdO, [Pd(NH₃)₄]Cl₂, PdCl₂, Na₂PdCl₄) and metallic Pd (0.025 mm thick palladium foil). Comparison of the experimental spectrum to the reference spectra clearly shows that Pd is present as Pd^(II) in the Pd-loaded S-layer protein sample because the two absorption maxima (ca. 24360 and ca. 24380 eV) characteristics of metallic Pd (feature a, Fig. 1) are absent. The fine structure of XANES of the Pd-loaded S-layer resembles that of [Pd(NH₃)₄]Cl₂ and PdO, indicating that Pd-O and Pd-N bonds contribute to the metal protein binding.

The Pd K-edge EXAFS spectrum of the palladium species formed on S-layer protein from *B. sphaericus* strain JG-A12 and its corresponding FT are shown in Fig. 2. The data exhibit an excellent signal-to-noise ratio allowing the analysis up to 15 Å⁻¹. Four peaks are found at bond distances of 2.01, 2.49, 3.02 and 3.41 Å corresponding to Pd-O, Pd-O, Pd-Pd and Pd-Pd bonds, respectively. The distances were identified using Pd-O and Pd-Pd backscattering phase and amplitude functions obtained from atomic coordinates of PdO using the FEFF 8 programme. The first shell can be fitted to about 3-4 oxygen atoms at a distance of 2.01 Å. EXAFS analysis

indicated the presence of an additional shell at distance of 2.49 Å which was not found in the PdO (used as model for the EXAFS fit). This shell may be due to a side-lobe peak, originating from a truncation effect due to the limited reciprocal space integrated in the Fourier transform (model a) or may have a structural origin (model b). Therefore, the origin of the peak at 2.49 Å cannot be clarified. However, the data interpretation is not impeded by this ambiguity, because there are also no significant differences between the structural parameters of the S-layer bound Pd complexes determined with either model.

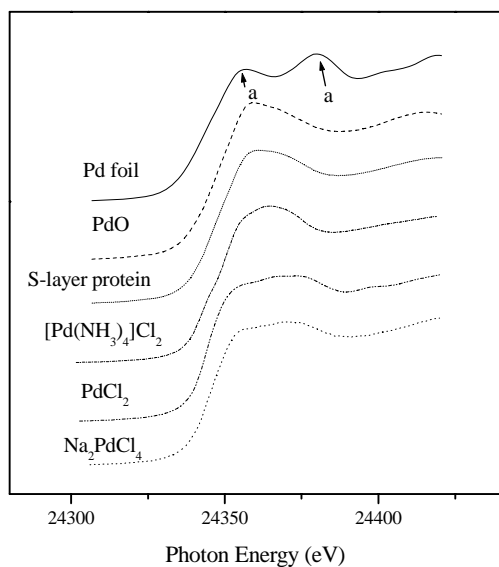


Fig. 1: Pd K-edge XANES region of EXAFS spectra of Pd-loaded S-layer protein of *B. sphaericus* JG-A12 and reference compounds.

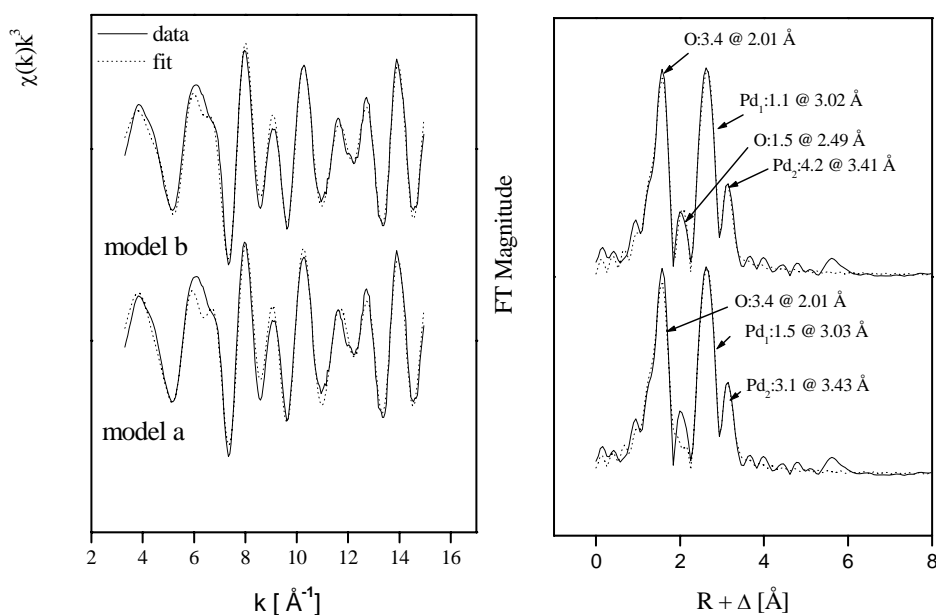


Fig. 2: Pd K-edge k^3 -weighted EXAFS spectra (left) and the corresponding FT (right) of the Pd complexes formed by S-layer of *B. sphaericus* JG-A12.

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