Notes

A XANES and EXAFS Investigation of the Speciation of Selenite following Bacterial Metabolization

B. B. Buchanan,[1a] J. J. Bucher,[1b] D. E. Carlson,[1a] N. M. Edelstein,[1b] E. A. Hudson,[1c] N. Kaltsoyannis,[1b] T. Leighton,[1d] W. Lukens,[1b] D. K. Shuh,[1b,*] H. Nitsche,[1e] T. Reich,[1e] K. Roberts,[1f] P. Torretto,[1f] J. Woicik,[1g] W.-S. Yang,[1h] A. Yee,[1f] and B. C. Yee[1a]

Department of Plant Biology, University of California, Berkeley, California 94720, Chemical Sciences Division, Lawrence Berkeley Laboratory, Berkeley, California 94720, G. T. Seaborg Institute for Transactinium Science, Lawrence Livermore National Laboratory, Livermore, California 94551, Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, Forschungszentrum Rossendorf e. V., Institut für Radiochemie, Postfach 51 01 19, D-01314 Dresden, Germany, Earth Sciences Division, Lawrence Berkeley Laboratory, Berkeley, California 94720, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, and Department of Biology, Jackson State University, Jackson, Mississippi 39217

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Introduction

X-ray absorption spectroscopy (XAS) techniques are useful, nondestructive probes of both oxidation state and local chemical environment.[2] In the hard X-ray regime they are well-suited to the study of radioactive and hazardous materials, as the samples may be safely contained for *in situ* measurements at storage ring facilities. The sensitivity of XAS methods permits investigation of dilute samples, both solutions and amorphous materials, thereby providing an ideal means for detailed electronic and structural studies of environmentally relevant systems.

The concentration and immobilization of hazardous materials by microbial agents offer great potential for use in environmental remediation technologies.[3,4] In the case of selenium, possible future applications of bioremediation technologies range from oil refinery waste streams containing selenite to agricultural drainage which has contaminated sites such as the Kesterson Reservoir in California. The aerobic soil bacterium *Bacillus subtilis* has been found to detoxify selenite although the metabolic mechanism is not fully understood.[5] As in other cases,[6,7] selenium oxyanions appear to be reduced to a red elemental form, although there is no direct *in situ* spectroscopic evidence to support this contention.

This paper reports the X-ray absorption near edge structure (XANES) spectroscopic characterization of the oxidation state of selenium following microbial metabolism of selenite from aqueous solution.[8] Extended X-ray absorption fine structure (EXAFS) measurements of the amorphous red and gray allotropes of elemental selenium are also discussed, with the aim of contributing to the substantial but incomplete body of evidence concerning their structure.

Experimental Section

The XANES measurements were performed at the storage rings of the Stanford Synchrotron Radiation Laboratory (SSRL) on wiggler beamline 4-3 and the National Synchrotron Light Source (NSLS) on bending magnet beamline X23A2. Selenium K edge (~12.7 keV) spectra were recorded with Si(311) monochromator crystals at the NSLS, while at the SSRL Si(220) crystals (detuned 50% to reduce higher order harmonic content) were employed. The convoluted experimental resolution is estimated at ~1 eV. Ionization chamber detectors were used for transmission experiments at both facilities. All spectra were collected simultaneously with gray selenium as a reference in order to establish the respective chemical shifts and to ensure photon energy calibrations. The inflection point of the main absorption edge (as determined by the first-derivative method) was taken to be the edge position in all cases. Data analysis was performed with the EXAFSPAK program suite, written by Graham N. George of the SSRL.

Vegetative cells of *B. subtilis* and of an unidentified bacillus isolated from the Kesterson Reservoir were exposed to aqueous growth media containing selenium of different oxidation states, either selenite (Se(IV)) or selenate (Se(VI)). The bacteria metabolized selenium from the selenite growth solution, but there was no metabolism of selenate. The bacteria were isolated and separated from the growth medium by repeated steps of centrifuging and washing in Tris-HCl buffer, pH 7.4. The centrifugal pellets were prepared for XANES by lyophilization and subsequent double encapsulation by 0.0025 in. thick Mylar tape within 0.002 in. thick polyethylene bags. On a dry weight basis, the pellets containing the *B. subtilis* and Kesterson bacillus cells contained 2.6% and 4.2% selenium, respectively, based on total selenium determined by ICP-AES. Amorphous red selenium was prepared by the reaction of sodium selenite with ascorbic acid and then extensively washed with distilled water and lyophilized. Gray selenium was obtained from Allied Chemical.

Results and Discussion

Selenium K edge XANES spectra were obtained for elemental selenium (red and gray allotropes) and Na_2SeO_3 and Na_2SeO_4 powders, all of which contain selenium in well-defined oxidation states. The edge positions in selenite and selenate are shifted to higher energy by 4.2 and 9.0 eV, respectively, relative to elemental selenium (at 12 658 eV) (data not shown). Figure 1 presents the XANES spectra of red and gray selenium, together with those of the bacteria, and clearly demonstrates that the selenium is present in elemental form in both *B. subtilis* and

(1) (a) Department of Plant Biology, University of California. (b) Chemical Sciences Division, Lawrence Berkeley Laboratory. (c) G. T. Seaborg Institute for Transactinium Science, Lawrence Livermore National Laboratory. (d) Department of Molecular and Cell Biology, University of California. (e) Forschungszentrum Rossendorf e. V., Institut für Radiochemie. (f) Earth Sciences Division, Lawrence Berkeley Laboratory. (g) National Institute of Standards and Technology. (h) Department of Biology, Jackson State University.

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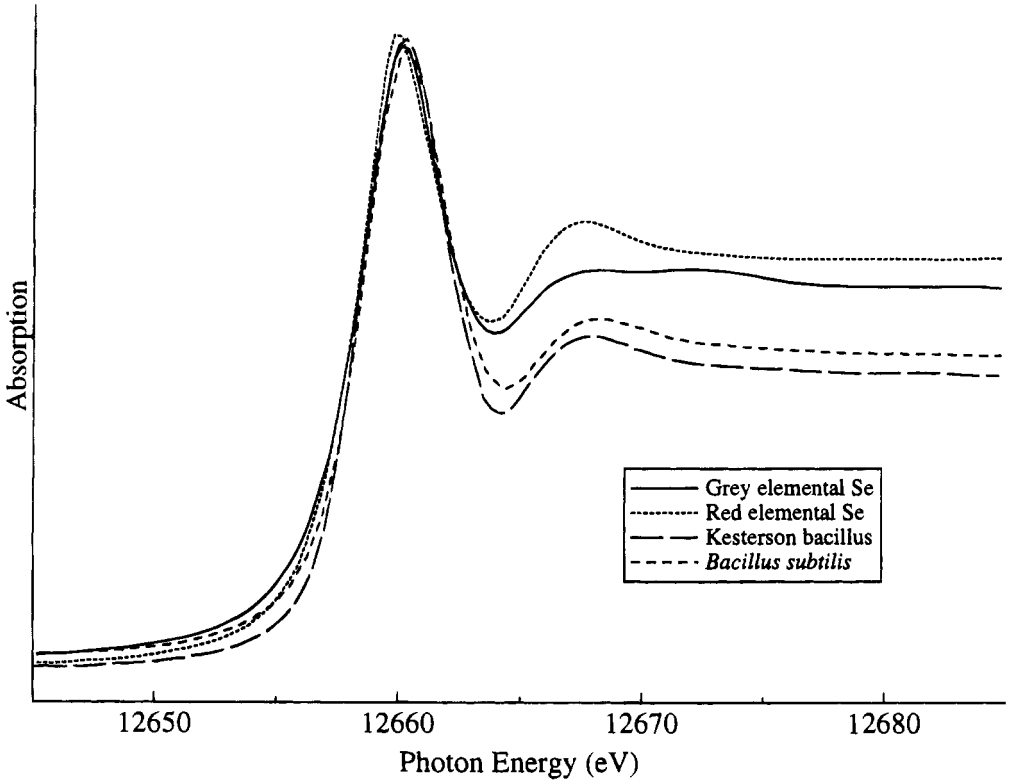

Figure 1. Selenium K edge XANES spectra of amorphous red and gray selenium and from *B. subtilis* and the Kesterson bacillus after growth in a selenite medium. The spectra have been normalized to equivalent peak heights. Gray selenium was employed as a reference in all cases, and the energy scales have been aligned with respect to its absorption edge at 12 658 eV.

the Kesterson bacillus. Furthermore, there is a discernible difference between the red and gray allotropes of selenium in the near edge region (12 665−12 675 eV). The results suggest that both *B. subtilis* and the Kesterson bacillus reduce selenite to red selenium, a finding consistent with the assertion that red selenium is the biological end product.

The origin of the XANES difference between gray and red selenium is not easy to ascertain. In the near edge region, multiple scattering paths dominate the absorption modulations while single scattering becomes increasingly important as the photoelectron wavelength decreases.[9] Although the theoretical modeling of XANES is becoming increasingly widespread,[9] it is very difficult to achieve without well-established structural parameters. Unfortunately these are not available for selenium. Elemental selenium occurs in a number of structural modifications, not all fully characterized.[10] The gray, "metallic" form is thermodynamically the most stable and consists of unbranched helical chains with Se−Se distances of 2.37 Å. Red selenium has three crystalline modifications, all of which feature Se_8 rings and differ only in their packing.[11] The average ring Se−Se separation is 2.34 Å. The more common, amorphous form of red selenium consists of a mixture of distorted chains and Se_8 rings,[12] although recent Raman spectroscopic studies indicate that the ring concentration is very small.[13]

In view of the observed slight difference in the XANES spectra of gray and amorphous red selenium, EXAFS spectroscopy was employed in an attempt to distinguish the two forms. EXAFS was measured to a photoelectron wavevector, k, of 12.7 $Å^{-1}$. The Fourier transform revealed a single frequency in both cases, which were fitted well using the parameters given in Table 1. Theoretical phase and amplitude functions from the tables of McKale et al.[14] and values calculated using FEFF 5[15,16] gave essentially the same results. Thus the EXAFS data do not distinguish between the gray and red allotropes of elemental selenium.

Table 1. Structural Parameters from EXAFS Curve Fitting of Elemental Selenium

allotrope	Se−Se (Å)	coord no.	Debye−Waller factor ($Å^2$)
red	2.43	2	0.0028
gray	2.44	2	0.0026

The observation of exclusively one frequency in the EXAFS is somewhat surprising, given that the closest distance between the chains in gray selenium is only 3.44 Å.[10] It may be that acquisition of data beyond 12.7 $Å^{-1}$ would reveal further scattering shells, but it must be emphasized that there is no indication of this in the available data. It is likely, however, that differences in the atomic positions beyond the nearest neighbors are responsible for the different XANES of red and gray selenium. Distortions of the helical chains or differences in their packing are potential causes of different multiple scattering pathways.

Conclusions

The reduction of Se(IV) to red elemental selenium by both *B. subtilis* and a bacillus isolated from selenium-contaminated

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soil has been documented by comparison of their selenium K edge XANES spectra with those of known selenium materials. The red and gray allotropes of elemental selenium can be distinguished by their XANES, although EXAFS data fail to differentiate between the two forms. The results support the view that organisms like *B. subtilis* offer a promising means for the removal of selenite (Se(IV)) from contaminated aqueous environments such as oil refinery and agricultural waste streams. Future application of XANES spectroscopy to the monitoring of *in situ* selenium bioremediation activity is clearly indicated.

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