Photoabsorption Study of *Bacillus megaterium*, DNA, and Related Biological Materials in the Phosphorus K-edge Region

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Introduction

The *Bacillus megaterium* spore has served as a model system for the investigating ionizing radiation damage and its effects on cell survival [1, 2]. Cell survival is greatly affected by the destruction of bonds within the DNA backbone, including those involving phosphorus [3]. Preliminary work has been done on the phosphorus K-edge region with *Bacillus subtilus* spores [4], but only for three discreet photon energies. Before a detailed study of the role of selective phosphorus excitation in overall *B. megaterium* cell survival can be performed, the continuous spore absorption coefficient spectrum must be known.

We have measured the absorption coefficient of the *B. megaterium* spore along with several biological compounds, including DNA, ATP, DOPC, Na_3PO_4, and elemental phosphorus. The absorption spectra provide insight into phosphorus bonding and electronic excitations involving phosphorus atoms.

The results from this work will be useful in understanding not only radiation damage studies performed with photons at the phosphorus K edge but also macromolecular crystallography studies that employ anomalous dispersion in this photon energy range [5].

Methods and Materials

The measurements were performed at the APS beamline 2-ID-B end station. Radiation produced by the 5.5-cm period undulator was passed through a spherical grating monochromator employing a 600-line/mm grating and exited into air through a Si_3N_4 window.

The samples were mounted either on top of a polycarbonate film (*B. megaterium* and DNA) or within a polyurethane polymer film (ATP, DOCP, Na_3PO_4, and elemental P). Transmitted photons were detected by using a calibrated absolute photodiode (IRD AXUV-100Ti_2) with and without the sample in the beam.

Absorption A was calculated via $A = -ln(I/I_0)$. For the spore and DNA sample, the absorption coefficient μ was calculated by using sample thickness z according to $\mu = A/z$.

Results

The measured absorption spectra are shown in Fig. 1. They are plotted by using a relative absorption scale, where the pre-edge absorption at 2140 eV is used as a reference. Only relative absorption is reported because five of the seven samples were diluted in a polymer film; thus, the thickness of all substances was not measurable.

Discussion

All spectra except elemental phosphorus display two features: a well-defined peak at lower energy and a wider, less intense peak at higher energy. The first peak is due to transistions of P 1*s* electrons to antibonding orbitals associated with various types of phosphorus-oxygen bonding. The second peak is due to resonant scattering of ionized P 1*s* electrons. In a free molecule, this type of transition is termed a shape resonance. We see a shift in the threshold of the first peak as phosphorus atom oxidation increases. In partucular, the inflection point of the phosphate threshold lies 4.2 eV above that of elemental phosphorus.

The spore spectrum is shifted 0.9 eV higher in energy from the DNA spectrum. However, when the spore spectrum is shifted in energy and rescaled vertically so as to be overlayed onto the DNA spectrum, the shapes are nearly identical. Therefore, it is supposed that most of the phosphorus in the *B. megaterium* spore is bonded in a manner similar to that of the phosphorus in DNA, and much less of the phosphorus in the spore is bonded in inorganic phosphates.

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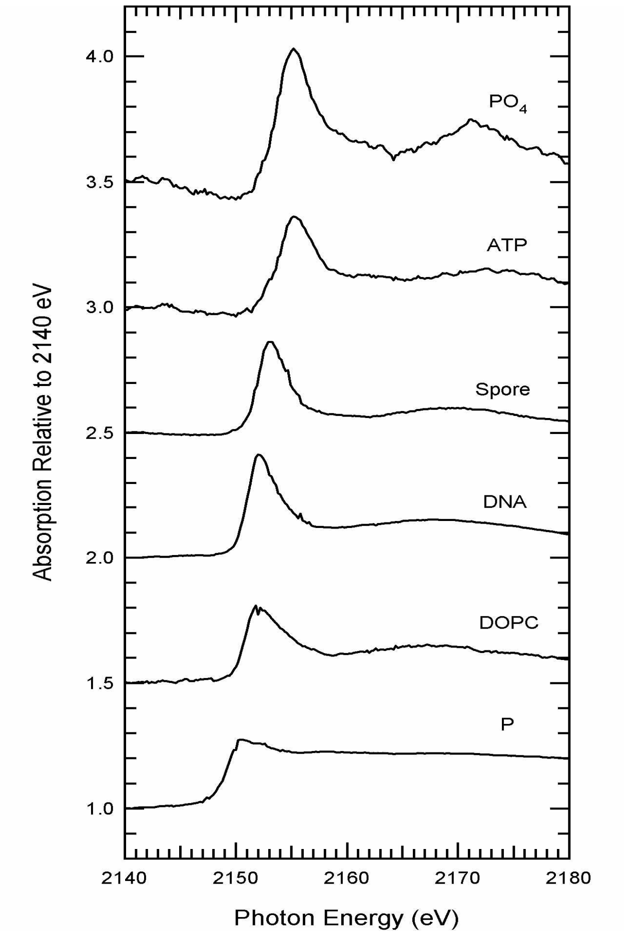

FIG. 1. Measured absorption spectra. The absorption relative to 2140 eV as the phosphorus K edge is crossed is shown for B. megaterium, DNA, ATP, DOPC, phosphate, and elemental phosphorus. The normalized elemental phosphorus and phosphate absorption values were divided by 4 and shifted vertically by 0.75 and 3.25, respectively. All other absorption values were shifted by a corresponding multiple of 0.5.

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