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X-Ray Absorption Spectroscopy of Light Elements* in Biological Systems

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Summary

Soft X-ray absorption spectroscopy of light elements has, until recently, fallen almost exclusively within the purview of materials science. Recent developments in techniques now allow access to this spectral range by biological scientists. This review will discuss some the recent work on X-ray absorption spectroscopy of light elements in biological systems and discuss some of the possibilities for future work.

Introduction

X-ray absorption spectroscopy (XAS) is a well established tool for examining the local atomic environment of heavy atoms, such as transition metal ions. XAS studies of light atoms are much less common, and are fraught with experimental difficulties which are not encountered at higher energies. This review will examine the possibilities for X-ray absorption spectroscopy of light elements in biological systems (we will refer to elements with atomic numbers smaller than 18 (potassium) as "light" elements). This excludes the majority of X-ray absorption studies on metalloenzymes, including the recent studies of first and second transition element L-edges [1-3] which fall in the same X-ray energy range as the spectra from light elements. There are excellent reviews of XAS of heavy elements in biological systems *e.g.* [4].

A typical X-ray absorption spectrum is shown in Fig. 1, illustrating the main features of the spectrum. The spectrum can be divided into two distinct spectral regions; the edge spectrum, and the extended X-ray absorption fine structure (EXAFS) spectrum. The edge spectrum (which is sometimes referred to as a pre-edge spectrum or near-edge structure) is primarily comprised of dipole-allowed transitions to bound states, plus continuum resonances at somewhat higher energies. For the K-edges the bound state resonances are primarily dipole allowed $1s \rightarrow p$ transitions, and the edge spectra are thus highly sensitive to electronic structure [*e.g.* 5]. Unfortunately edge spectra are often difficult to interpret in a quantitative manner. In contrast, EXAFS is relatively simple to interpret, and can give structural information upon the first shell coordination with accurate interatomic distances, together with estimates of the type (atomic number) and number of the surrounding atoms.

Experimental Aspects

For X-ray spectroscopy, the availability of synchrotron radiation provides an ideal source of X-rays. The energies of the absorption edges of some light elements are listed in table

1. Atmospheric attenuation of the incident X-ray beam is a very significant problem. At the sufur K-edge, for example, a pathlength of 1 cm of nitrogen at atmospheric pressure will absorb 25 % of the incident beam, and at the magnesium K-edge 85% of the incident X-rays would be absorbed. Because of these difficulties, experiments are generally conducted in an atmosphere of helium gas or in vacuum at the softer (lower) X-ray energies.

Table 1. X-ray Absorption edge energies of some light elements

Element	K-edge energy (eV)	Biological studies
C	284.2	8
N	409.9	7
O	543.1	–
Na	1070.8	–
Mg	1303.0	*
Al	1559.0	–
Si	1839.0	–
P	2149.0	15
S	2472.0	17,19,20-24
Cl	2833.0	–

* See Fig 2 B.

While conducting experiments in vaccuo is not a problem in many disciplines, biological samples often require an aqueous solution environment, which is incompatible with high vacuum. The use of a helium atmosphere is possible for the Mg K-edge and up, but for the lighter elements (at lower X-ray energies) even helium attenuates the X-ray beam quite significantly. Possible solutions which have been attempted are to use thin windows, windowless frozen solutions at low temperatures or to use dehydrated preparations of the most robust samples. The elements magnesium and aluminium present additional difficulties in that there is no readily available method of obtaining monochromatic X-rays in the appropriate energy range, as most of the commonly available monochromator crystals degrade quickly in the X-ray beam and contain nearby elements (*e.g.* Si in quartz) truncating the available energy range.

Carbon and Nitrogen XAS

The utility of the K-edges of carbon, nitrogen and oxygen is severely hampered by the fact that most biological molecules contain many instances of these elements. In most cases the large number of overlapping spectra would be expected to be impossible to resolve. Despite these difficulties, Mitra-Kirtly *et al.* [6,7] have recently showed the utility of nitrogen K-edge XAS for investigating biological molecules. In particular, these workers demonstrate that the intense low-energy π^* resonance associated with the herterocyclic ring of nucleic acids provides a potential method for separating nucleic acid from protein in X-ray microscopy [7]. More recently Ade *et al.* [8] have observed similar, but rather less pronounced, chemical shifts between protein and nucleic acid at the carbon K-edge. In a particularly elegant study, these workers succesfully used the carbon K-edge chemical shifts to image the DNA in dried chromosomal material from the bean *Vicia faba* at a resolution of 55nm. As pointed out by Mira-Kirtly *et al.* [7] one promising aspect for X-ray microspectroscopy is afforded by the characteristic nitrogen K-edge chemical shifts observed for the different nucleotide bases which might allow sensitivity to nucleic acid composition.

Magnesium and Aluminium XAS

Magnesium is thought to be an essential element for all organisms. In biological systems magnesium is always present as the Mg^{2+} cation, and is known to play very important roles in diverse biological processes. It is important in cellular metabolism, especially in ligating the phosphate groups of adenosine triphosphate (ATP), as the so-called MgATP complex. In most cases ATP is biologically active only as the magnesium complex (or sometimes *in vitro* as a complex with an alternative divalent metal ion such as Mn^{2+}). Magnesium also has roles in biomineralization [9] and (at least) occasionally as its ATP complex [10]. A good example of the essentiality of Mg^{2+} for ATP utilizing systems is provided by the kinases, these are a very widespread group of enzymes which transfer phosphate from ATP to phosphorylate aliphatic –OH groups. They function in diverse roles, from participation in major metabolism to cascade switches [11] and are inactive in the absence of Mg^{2+}, but the precise role that magnesium plays in catalysis remains uncertain. Magnesium is also well known for its role in photosynthesis; the magnesium porpyrin chlorophyll being the primary photoreceptor in photosynthesis of green plants. Despite it's widespread importance, very little is known concerning the coordination chemistry of magnesium in biological systems. This is mostly due to the fact that magne-

sium is a spectroscopically silent element in that, until very recently, there were no there are no useful and easily available spectroscopic probes of the element. The recent availability of YB_{66} X-ray monochromator crystals [12] has now made magnesium XAS a readily available probe of magnesium coordination. Fig. 1 shows the K-edge XAS spectrum of a sample of MgO obtained by using YB_{66} monochromator crystals Fig, 2 A shows the magnesium K-edge spectra of three different octahedral coordinations of the Mg^{2+} cation, clearly illustrating the sensitivity of the edge spectrum to the coordination environment of magnesium. Magnesium is known to have a considerable coordination chemistry [13] and it can be anticipated that the availability of a sensitive probe of coordination will provide much insight into the role of this metal in biolgy.

Unlike magnesium, aluminium has no known biological function. However aluminium is certainly very important in biology. Toxic levels of Al^{3+} can cause dialysis encephalopathy in humans, and the metal has been implicated with other degenerative disorders such as the senile dementia known as Alzheimer's disease (but see [14]). Recent evidence suggests that serum transferrin from people afflicted with Alzheimer's disease may be deficient in it's ability to bind Al^{3+}. Aluminium has also been implicated in renal disease and pancreas acinar pathology. Additionally, aluminium toxicity is strongly implicated in the toxic effects produced by acid rain [15]. Despite it's biological importance very few studies of aluminium chemistry in biological systems have been published. The primary reason for this is that aluminium, like magnesium, can be regarded as a "spectroscopically silent" element in that there are few convenient spectroscopic probes. ^{27}Al nuclear magnetic resonance (NMR) spectroscopy can be used as a powerful probe of the coordination environment of the metal. X-ray absorption spectroscopy should provide a powerful tool that is complimentary to ^{27}Al NMR in providing structural information on aluminium in biological systems.

Phosphorus and Sulphur XAS

Phosphorus is very widespread and important in biology. ^{31}P nuclear magnetic resonance spectroscopy provides a highly sensitive probe of the chemical nature of phosphorus, and it might be argued that phosphorus XAS provides no advantages. Because of this, and because of experimental difficulties in obtaining monochromatic light at the phosphorus K-edge, there are very few reports of phosphorus K-edge XAS of biological systems. Phosphorus K-edge XAS of a range of different compounds, have been investigated using a Ge(111) monochromator by George *et al* [16]. Subtle shifts in the position of the major peak of the

phosphate edge spectrum were observed between ATP, ADP, AMP and cyclic AMP and these may be of use in investigating the biochemistry of these compounds.

Sulphur has a particularly rich X-ray absorption edge spectrum, with chemical shifts spanning more than 14 eV [17,18]. We note in passing that, in addition to applications to biology, sulphur XAS has been used extensively in the investigation of chemical types of non-volatile sulphur in coals and other heavy hydrocarbons [17,19]. In combination with measurements at the copper K-edge, George *et al* [20] have used sulphur K-edge EXAFS to examine the sulphur environment of copper metallothionein. Quantitative analysis of the EXAFS gave a sulphur-copper distance of 2.23Å, and a sulphur-carbon distance of about 1.90Å, which agreed well with the Cu-S distance determined from analysis of the copper K-edge EXAFS [20]. Hedman *et al.* [21,22] have used sulphur K-edge XAS to probe the chemical environment of the sulphur in the iron-molybdenum cofactor of the nitrogen fixing enzyme nitrogenase from *Azotobacter vinelandii*. These workers used *in-situ* electrochemical control to examine the XAS of both the oxidized and the reduced cofactor [22]. In a particularly elegant study which combined measurements at the copper K-edge, sulphur K-edge and self-consistent-field Xα scattered-wave calculations, Shadle *et al.* [23] have used the sulphur K-edge XAS spectrum of poplar plastocyanin to probe the electronic environment of the copper-thiolate site in this protein. These workers found that the sulphur K-edge spectrum possessed an intense pre-edge resonance, which they assigned as a sulphur $1s \rightarrow$Cu $3d$ transition, where the Cu 3d orbital is actually a molecular orbital with Cu $3d_{x^2-y^2}$ and sulphur $3p$ orbital character. The intensity of this transition provides a direct probe of the covalency of the Cu-S bond and clearly shows that the copper-thiolate coordination in plastocyanin is very highly covalent. As pointed out by Shadle *et al* [23] these observations provide an explanation of the unusually low copper EPR hyperfine coupling which is highly characteristic of type I copper proteins.

Frank *et al.* [18,24] have used sulphur K-edge XAS to probe the chemical forms of sulphur in plasma cells of the tunicate *Ascidia ceratodes* and found a large reservoir of sulfate and aliphatic sulfonic acid. Sulphur K-edge XAS can also be used to probe the forms of sulphur in living bacteria [25]. Fig. 3 shows the sulphur K-edge XAS spectrum of suspensions of whole cells from three different organisms. Using curve-fitting methodologies developed for analysis of sulphur XAS of coals and related materials [19], the differences in the spectra can be quantitatively interpreted [25] in terms of constituent components, which can be interpreted to

reflect the differing modes of metabolism of the three organisms. Analysis of sulphur K-edge XAS provides a direct, non-invasive, probe which can be exploited to determine the metabolic fates of sulphur in whole cells.

Conclusion

In summary, while it is true that X-ray absorption spectroscopy of light elements in biological systems has, to date, been very little used, we can anticipate that recent technological developments will facilitate studies not only at the sulphur K-edge, but also at the magnesium and aluminium K-edges. The chemical sensitivity of XAS, possibly combined with applications in microscopy, is expected to prove an important tool not only in the investigation of purified proteins, but also in intact specimens.

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Figure Captions

Fig. 1. Typical X-ray absorption spectrum showing the pre-edge edge and EXAFS regions.

Fig. 2. **A** Magnesium K-edge spectra of compounds with $Mg(O)_6$ $Mg(OH)_2O_4$ and $Mg(OH)_4O_2$ coordination. Illustrating the sensitivity of the spectrum to minor changes in coordination environment. **B** Magnesium K-edge spectra of chlorophyll. The peak near 1307 eV broad

Fig. 3. Sulphur K-edge X-ray absorption spectra of whole cells of *Rhodospirillum rubrum*, *Rhodobacter capsulatus* and the marine archaebacterium *Beggiatoa*.

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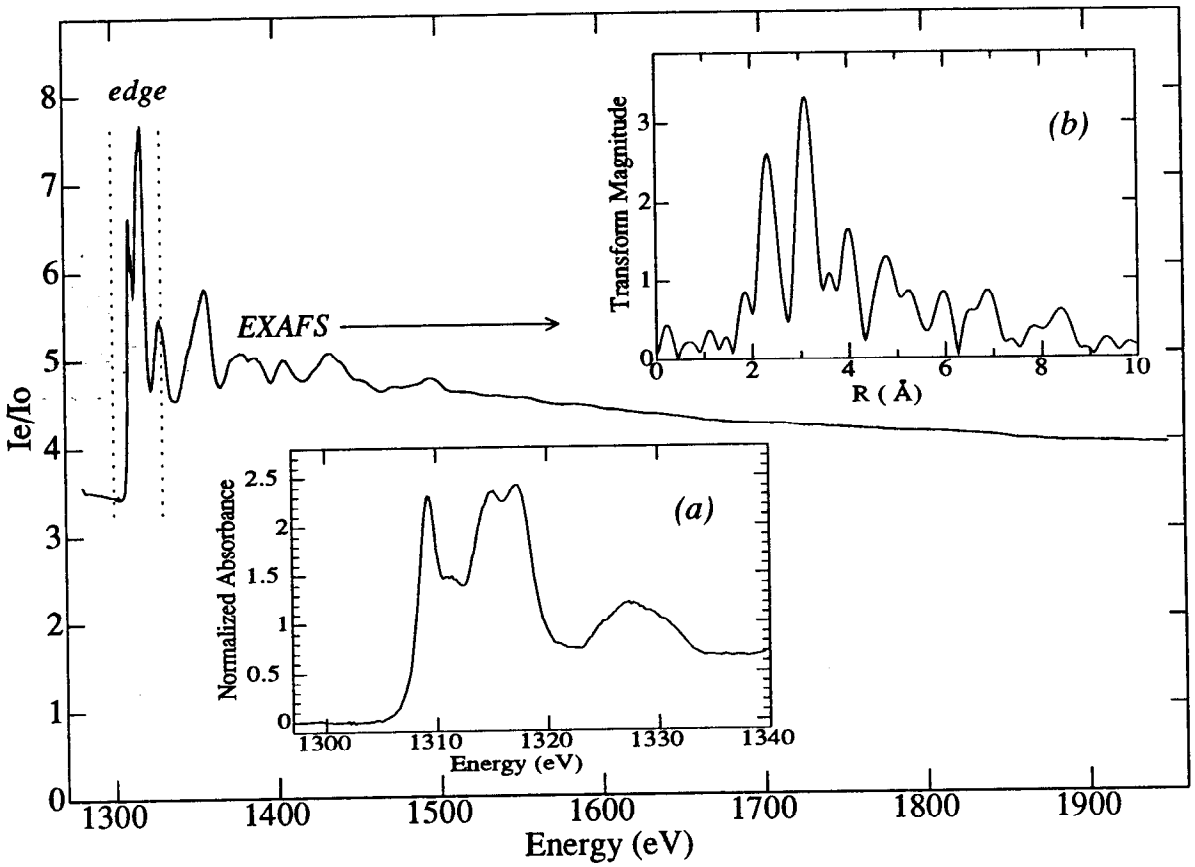
edge
EXAFS
Ie/Io
Energy (eV)
(a)
Normalized Absorbance
Energy (eV)
(b)
Transform Magnitude
R (Å)

Figure 1

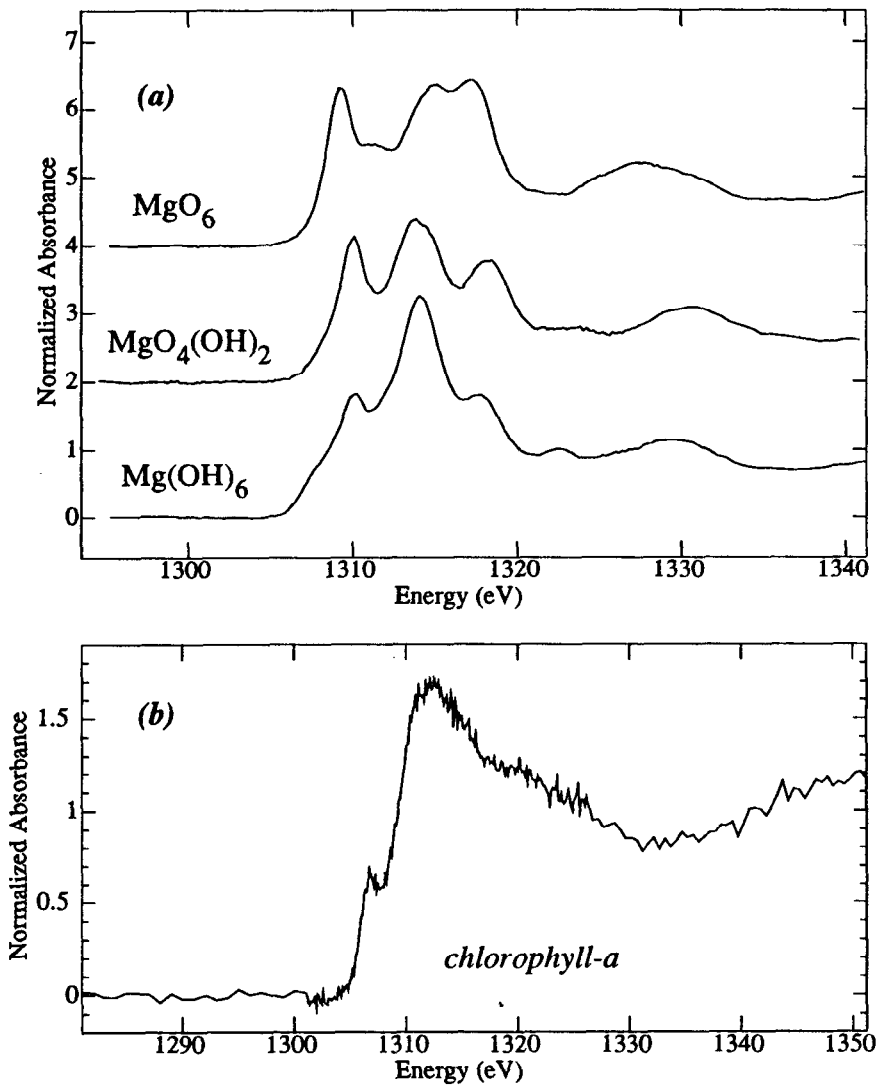
(a)
MgO6
MgO4(OH)2
Mg(OH)6
Normalized Absorbance
Energy (eV)
(b)
chlorophyll-a
Normalized Absorbance
Energy (eV)

Figure 2

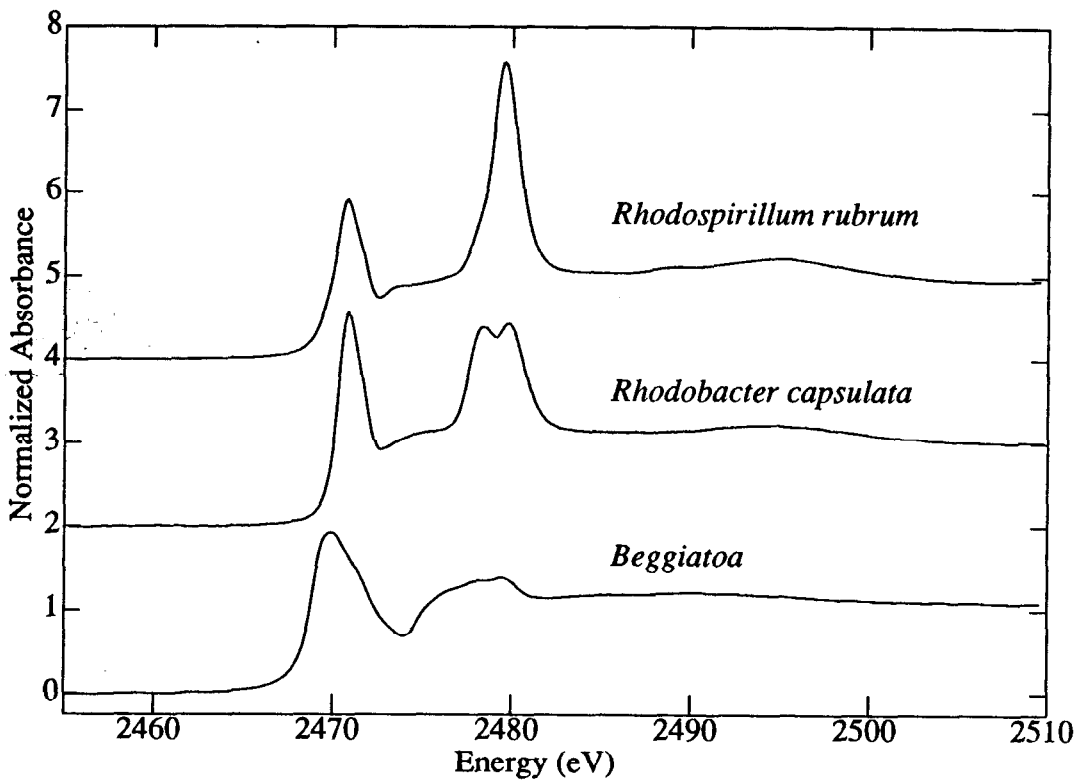
Normalized Absorbance
Energy (eV)
Rhodospirillum rubrum
Rhodobacter capsulata
Beggiatoa
0
1
2
3
4
5
6
7
8
2460
2470
2480
2490
2500
2510

Figure 3