V. Extended X-Ray Absorption Fine Structure

Refs:


EXAFS is a relatively new technique that is a hybrid of both absorption and scattering phenomena. The principles of the method were known for a long time but with the relatively dilute (<< 1 mM) solutions of biological molecules that are available the application of the method required the development of very intense light sources in the X-ray region of the spectrum. The light source requires a linear accelerator to generate a stream of high energy electrons that are fed into a storage ring, a circular chamber in which a ring of magnets deflects the electrons so that they change from a linear path to a circular path. Because they are moving at constant velocity in this circular path they are continuously accelerating and consequently are emitting radiation. This radiation is in the range 2.5-25 KeV (1 eV = 23 kcal; wavelength is about 0.1nm); because radiation of less than 2.5 KeV is not yet sufficiently intense these methods are limited to atoms for which Z > 17 (i.e. potassium or higher). With a suitable monochromator (cut from a block of graphite) one can select "light" of the wavelength of interest and hence X-ray absorption spectroscopy can be performed.

The phenomenon is best introduced via Figs. 1 and 2. Fig 1 shows how the transmitted intensity of an X-ray beam is reduced as a function of beam energy (i.e. reciprocal wavelength) in the range 7100-7130 ev when that beam is directed at a sample of an iron compound (KFeF₃). An abrupt increase in absorption occurs when the X-ray energy is sufficient to liberate inner shell (e.g. 1s, 2s) electrons thus converting a bound electron into a photoelectron (called a photoelectron because it is created by the action of a photon on the material). This abrupt increase is called the absorption edge. It occurs when the photon energy (hv) exceeds the ionization potential (E₀) of a bound electron (Fig V-2) as indicated by the (almost) vertical dotted line in Fig. V-2.
For metal 1s electrons this requires a photon from the hard X-ray region of the spectrum; for iron, the energy is about 7100 eV, for copper about 9000 eV. The position of the edge depends upon the oxidation state of the metal ion and moves to higher energies as the oxidation state is increased (the effects are small however and can even vanish in highly covalent compounds).

Below the edge there is background absorption due to the corresponding transition of all of the atoms in the material with a lower atomic number than the one being examined plus transitions involving higher shells (e.g. 2s) of all the atoms present (extreme left of Fig V-3, left) The edge region contains structure (i) at the pre-edge due to bound state transitions (e.g. 1s → 3d, 4s, 4p); (ii) the edge itself due to the 1s → continuum promotion; and (iii) the post-edge arising from interference between the outgoing photoelectron wave and the wave that is backscattered from, primarily, the first shell of atoms around the central absorbing atom (to the right of Fig V-3, right).

Features in the first 50-100 eV past the edge are due mainly to multiple scattering phenomena; this is called the XANES region. Analysis of this region is not well developed. Beyond 50-100 eV the oscillations in the absorption profile are due to single scattering events and comprise the EXAFS region. (Compare the smooth exafs for a monatomic gas, krypton (Fig. V-3 left), with the structured exafs of a metal ion surrounded by ligand atoms, Cu-porphyrin (Fig. V-3 right))

Unlike most spectroscopies in which the useful data is immediately available, in this technique a fair amount of data massaging is first required (Fig V-4).
(a) First one isolates the relevant x-ray absorption ($\mu$) from the background contributions ($\mu_{\text{back}}$) by subtracting the latter from the total absorption ($\mu_{\text{tot}}$) by fitting a smooth curve to the data to the left of the absorption edge and subtracting this smooth curve from all the data. The exafs data (to the right) now looks like a gently decreasing curve that bears small oscillations.

(b) These oscillations are extracted by first fitting a second smooth curve ($\mu_s$, a cubic spline) to the data after the edge and subtracting this smooth curve from the raw intensity data. This quantity, ($\chi$ or $\Delta\mu$), is expressed as ($\mu - \mu_s$)/$\mu_o$, the difference between the raw and smooth curves normalized to the absorption coefficient of the free atom ($\mu_o$).

(c) $\chi$ is plotted versus $k$, the photoelectron (angular) wavenumber$^1$, which has dimensions of Å$^{-1}$. $k$ is proportional to momentum (Fig. V-5, left).

(d) Fourier transforming this data into Å space yields a "spectrum" hopefully containing some large peaks plus smaller peaks usually interpreted as noise (Fig V-5, center). By rejecting data both before and after the region of interest (indicted by arrows in Fig. 5, center) one eliminates unwanted data arising from other shells, from noise and from low frequency drift.

(e) Finally back-transforming this edited data to $k$-space yields the final "Fourier-filtered" exafs spectrum (Fig V-5, right). The amplitude of these oscillations decay with increasing $k$ and so to facilitate visualization it is standard practice to multiply $\chi$ by $k^3$ so that the oscillations have about the same amplitude for all values of $k$.

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$^1$ The bound electron is promoted by the incident photon which has energy $E$ (i.e. $h\nu$). This energy is larger than the ionization potential, $E_o$, and so the emitted photoelectron has a kinetic energy $= E - E_o$. This is equal to $1/2 \ m_e v^2 = p^2/2m$ ($p =$ momentum). Thus the photoelectron has a wavelength $\lambda = h/p$; $k = 2\pi/\lambda$, so $k = [(8\pi^2 m_e h^2 (E - E_o))/J]/[0.26(E - E_o)]^{1/2}$ if $E$ and $E_o$ are given in electron volts with $k$ being in reciprocal Angstroms.
What do we do with this exafs "spectrum"? It is analyzed by a fitting technique in which chemical intuition is used to systematically vary the identity, number and distances of atoms within 5Å of the scattering ion using a computer to predict the exafs spectrum for this "guessed" structure and varying the structure until the "guessed" spectrum and the observed spectrum are the same, using the smallest number of atoms that can reasonably account for the data.

The fitting equation is V-1 and the terms in the fitting procedure are:

\[ \chi(k) = \frac{1}{k} \sum s N_s \frac{f_s(\pi, k)}{R_{as}^2} \exp(-2\sigma_{as}^2 k^2) \sin[2kR_{as} + \alpha_{as}(k)] \]

Eqn. V-1
(i) An interference term \( \Rightarrow f_s(k)\sin\{2\mathbf{kR} + \alpha(k)\} \). The argument to the sin, \( \{2\mathbf{kR} + \alpha(k)\} \), describes the total change in phase between the outgoing and reflected waves \( \alpha(k) = \delta + \alpha \) (eqn 1), and \( f_s \) is the inherent back-scattering amplitude for a selected atom. If the relative phases between the outgoing and incoming wave was only governed by the distance, \( \mathbf{R} \), traveled by the scattered wave then the oscillatory part of the exafs would depend only on \( \sin(2\mathbf{kR}) \) (Fig V-6).

![Fig V-6](image)

As the energy of the incident photon increases the energy of the emitted photoelectron increases. The wavelength of the photoelectron thus decreases, \( \mathbf{k} \) increases, and the product \( \mathbf{kR} \) will cycle through multiples of \( \pi \). When \( \mathbf{kR} \) is an exact multiple of \( \pi \) the reflected wave, upon arriving back at the absorber will interfere constructively with the emitted wave; otherwise the reflected wave will produce destructive interference at the absorber (Fig V-6, right) to an extent that depends on the difference between \( \mathbf{kR} \) and the multiples of \( \pi \).

This is the term that yields the distance between the two atoms. A complication is that the traveling electron is perturbed by the electrostatic potential of both the central atom and the scattering atom during its journey and this introduces a second phase shift represented by \( \alpha(k) \). This is the term that identifies the scatterer, for the magnitude of \( \alpha(k) \) depends explicitly on the chemical identity of the scatterer-absorber combination and we know the absorber. The total amplitude of the interference \( A(k) \) (= \( N_s f_s \) see item iii below) also depends on the chemical identity of the scattering atom; it increases approximately as \( Z/k^2 \) (\( k > 4^{-1}\text{Å}^{-1} \)) and once the scattering atom has been identified, via \( \alpha \), \( A(k) \) yields the number of scattering atoms of the particular type. So the important correlations are:

Frequency \( \rightarrow \) Distance
\( \alpha \) \( \rightarrow \) Identity
\( A(k) \) \( \rightarrow \) Number and Identity.

(ii) Because the absorber-scatterer distance is not absolutely fixed the amplitude of the exafs oscillations decays as \( \mathbf{k} \) gets larger. This is accommodated by multiplying the sine term by \( \exp(-2\sigma^2k^2) \). This term is called the Debye-Waller term; it defines the rms variation \( (\sigma^2) \) in \( \mathbf{R} \). It has two contributions. The first is simply thermal motion i.e. kinetic disorder, which arises from the vibrations involving the two atoms and can be calculated from the vibrational spectrum. The second contribution allows for static disorder; i.e. not all molecules have identical bond distances because e.g. of packing forces. It also can be calculated from first principles.

(iii) The product of (i) and (ii) is (a) divided by \( \mathbf{R}^2 \) because the waves are spherical and the atom is located at an arc of the circumference which decreases with a \( 1/R \) dependence; this becomes
\[1/R^2\] on the round trip, and (b) multiplied by \(N_s\) the number of atoms of a particular type at this distance. The product \(N_s f_s\) is the total back-scattering from this "shell" of atoms = \(A(k)\).

(iv) This procedure is repeated for all types of atoms under consideration and the whole thing is summed. (By type we mean all atoms of the same chemical identity at the same distance).

To be specific: we might do an analysis of a copper compound which, in principle, might have 4O, 3O1N, 2O2N, 1O3N or 4N as the pattern of nearest neighbors. For each possibility all of the quantities described above are known except the distance \(R\) of each atom from the copper. So the computer systematically varies the four \(R\)'s over the range of acceptable values and reports which set of atoms at which distances best reproduces the experimental data.\(^2\)

Examples of the use of EXAFS:

1) Rubredoxin. A small protein from Clostridium pasteurianum that contains 1 Fe attached to 4 cysteine residues. The X-ray structure had shown that the cysteine residues are in a tetrahedral arrangement with three cysteine residues at 2.3 Å but the fourth cysteine was located at 1.95Å, an unusually short distance for sulfur bonded to iron; this led to great speculation over the biological significance of the unique distance. However exafs measurements on frozen solutions could be fit with four equivalent sulfurs of distance 2.256 ± 0.016Å for the oxidized protein and 2.32±0.02Å for the reduced protein. These distances are clearly well outside the prediction of the X-ray analysis and forced the crystallographers to return to the analysis of their data. They subsequently found that the short bond was in error with all bond-lengths falling within 0.1Å of one another. No explanation was ever given for the source of the original error.

2) Ferredoxin I from Azotobacter vinelandii contains 1 classical 4Fe center and 1 3Fe center and was the first protein for which a 3Fe cluster had been determined structurally. It appeared that this cluster was "cyclohexane-like" with 3 alternating Fe and S atoms in a six membered ring. An exafs analysis of a second protein with a 3Fe cluster believed to be similar showed that all iron-sulfur distances were in the range 2.25Å while all iron-iron distances were close to 2.7Å. Fe-Fe distances close to 4Å could not be detected; these long distances were required by the "cyclohexane" structure.

The original structure had a number of questionable features. First the cluster was improbable on chemical grounds as biochemical evidence had suggested that it was formed by the removal of an iron from the traditional 4Fe4S "cube". Second the polypeptide chain folding and cluster connections were quite different to those found in homologous proteins for which structures were available. Third, the crystallographic goodness of fit parameter (R) was disturbingly high and fourth a large number of water molecule had been introduced into the structure to return to the an analysis of their data. They subsequently found that using the initial choice of space group (P43212) a stretch of left handed helix was created. This immediately suggested that the correct space group is the mirror image, P41212. With this new choice of space group a perfectly reasonable structure was obtained with the 3Fe group having the anticipated "cubane like" structure, right handed helices being generated and an improved value for R (when compared under the same conditions).

\(^2\) A complication is that \(E_o\) is not known precisely, being deduced from the position of the initial edge, which is spread over 10 eV. Thus the computer program also varies the value of \(E_o\) about its selected position as part of the fitting algorithm.
3) Peroxidase and catalases are heme-containing enzymes that degrade hydrogen peroxide. The first step in the catalytic sequence is a 2 electron oxidation of the enzyme by the hydrogen peroxide. One electron is provided by the protein or the porphyrin (depending upon the specific enzyme. The second electron is provided by the heme iron.

\[
\text{[Enz-Fe}^{\text{III}}\text{]}^{+1} + \text{H}_2\text{O}_2 \iff \text{[Enz-Fe}^{\text{IV}=\text{O}}\text{]}^{+3} + \text{H}_2\text{O}
\]

The existence of the oxy-ferryl bond (Fe(IV)=O) in HRP compounds I and II was established using exafs to document the presence of a short (1.64Å) iron-oxygen bond.