EXTENDED X-RAY ABSORPTION FINE STRUCTURE — ITS
STRENGTHS AND LIMITATIONS AS A STRUCTURAL TOOL

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1. INTRODUCTION

Extended x-ray absorption fine structure (EXAFS) refers to oscillations of the x-ray absorption coefficient on the high energy side of an absorption edge. Such oscillations can extend up to 1000 eV above the edge and may have a magnitude of 10% or more. As an example, the absorption spectrum near the K edge in crystalline Ge is shown in Fig. 1. The phenomenon of EXAFS has been known for about half a century, and the basic physical explanation has been provided by Kronig as being due to modification of the final state of the photoelectron by the crystal (Kronig 1931) or, in the case of gaseous molecules, by atoms surrounding the excited atom (Kronig 1932). However, when this field was last reviewed in this journal (Azaroff 1963), a great deal of confusion still existed over the question of whether a long-range order theory formulated in terms of Bloch waves (Kronig 1931) or a short-range order theory in terms of scattering by neighboring atoms (Kronig 1932, Hartree et al. 1934, Shiraiwa et al. 1958) is more appropriate. A major source of the confusion was that quantitative comparison between theory and experiment was nonexistent.

Over the past few years a dramatic improvement in our theoretical understanding of EXAFS has occurred. As we shall see it is now established that a single scattering short-range order theory is adequate under most circumstances. The oscillatory part of the absorption coefficient $\Delta \mu$ normalized to the structureless (atomic-like) background $\mu_0$ is given by

$$\chi(k) = \frac{\Delta \mu}{\mu_0} = - \sum_j \frac{N_j}{kd_j^2} |f_j(k, x)| \sin[2kd_j - \psi_j(k)] e^{-2y_j^2 k^2} \right(1.1$$

for excitations of an $s$ state in a system in which the orientation of the sample has been spherically averaged. Equation (1.1) describes the modification of the photoelectron wave function at the origin due to scattering by $N_j$ neighbors located at a radial distance $r_j$ away. This is
A Workshop on PEP as a Synchrotron Radiation Source was held by SSRL on October 20–21, 1987. PEP is a colliding beam storage ring that has already been used for a few weeks for synchrotron radiation research parasitically during high energy physics runs.

Its main features as a synchrotron radiation source are high energy (up to 16 GeV with presently installed RF), long straight sections (6 arc 117 meters long), large circumference (2200 meters) and low bending magnet field (0.32 Tesla at 16 GeV). It has operated briefly in a low emittance mode with an emittance less than 12 nanometer-radial 8 GeV, making it the low emittance synchrotron radiation source.

There were 125 registered participants (77 from govern labs, 23 from universities, 16 foreign institutions and 9
7. APPLICATIONS OF SYNCHROTRON RADIATION

7.1 Introduction

Radiation research is an integral part of synchrotron radiation studies including the imaging, spectroscopy, and storage ring operations. The use of synchrotron radiation at several facilities worldwide is leading to the development of new techniques and applications. This section introduces the various applications of synchrotron radiation and provides examples of recent research findings.

7.2 Detectors

A variety of detectors have been used in synchrotron radiation experiments. These include position-sensitive detectors, time-resolved detectors, and imaging detectors. The choice of detector depends on the specific experimental requirements.

7.3 Applications

Synchrotron radiation has a wide range of applications in various fields such as materials science, biology, and chemistry. The section discusses some of the most significant applications.

7.4 Future Directions

The field of synchrotron radiation is rapidly evolving, with new techniques and applications being developed. The section highlights some of the future directions in this field.
Figure 2: Artistic conception of the first bremsstrahlung implementet in 1974 at the Stanford Synchrotron Radiation Project. Courtesy of W. Zawadowski.
Decomposition into amplitude and phase

Consider an arbitrary modulated sine function

\[ Z(k) = A(k) \sin \theta(k) \]

where \( \theta = 2kr + \phi(k) \)

\[ Z(k) \] can be written as

\[ Z(k) = \frac{1}{2i} A(k) e^{i \theta(k)} - \frac{1}{2i} A(k) e^{-i \theta(k)} \]

For the filtered Fourier transform method (\( Z(k) \) half limited in \( k \)), when \( Z(k) \) is transformed to \( Z(r) \)

\[ e^{i \theta(k)} \] corresponds to positive \( r \) value

\[ e^{-i \theta(k)} \] corresponds to negative \( r \) value.

Replace negative part of \( Z(r) \) by zero and back transform to \( \hat{Z}(k) \).

\[ \hat{Z}(k) = \frac{1}{2i} A(k) e^{i \theta(k)} \]

\[ A(k) = \frac{1}{2} |\hat{Z}(k)| \]

\[ \theta(k) = \text{Arg} \{\hat{Z}(k) + \hat{Z}(k)^*\} \]
Fig. 13
Use of chemical transferability of phase

\[ \Phi^m, \Psi(x) \text{ are known for a selected model compound} \]

Total phase shift for model ice

\[ \Phi^m(x) = 2k R^m + \Psi(x) \]

and for the system under study

\[ \Phi(x) = 2k R + \Psi(x) \]

The difference in phase is

\[ \Delta \Phi = \Phi - \Phi^m = 2k(\nu - R^m) + (\Psi(x) - \Psi^m(x)) \]

If chemical transferability holds

\[ \Psi(x) - \Psi^m(x) = 0 \]

The a plot of the phase difference vs \( x \)

is a straight line, the slope of which

is \( 2(k \nu - R^m) \) from which \( R^m \) can be obtained.

In practice, \( \nu^0 \) is varied in a linear

least squares fit such that the fit passes through the origin. \( \nu^0 \approx 1 \nu \)
Fig. 16

Phase difference (radians)

(a) \( \Delta E_0 = -5\text{eV} \)

Phase difference (radians)

(b) \( \Delta E_0 = 0\text{eV} \)
Amplitude function -

contains information on N, geometry, kind of atoms, amount of disorder, etc.

Simplest case considered here:
a single shell with N identical atoms

\[ A(k) = \frac{N}{R^2} f(k,R) e^{-2k^2 \beta^2} e^{-2k^R(k)} \]

Use principle of amplitude transferability

log of ratio of amplitudes of known \((m)\) and unknown \((n)\) as

\[ \ln \left( \frac{A_m(k)}{A_n(k)} \right) = \ln \left( \frac{N_m}{N_n} \frac{R_n^2}{R_m^2} \right) + 2k^2 [6\alpha^2 - 6\beta^2] + 2 \left[ \frac{R_n}{f_n(k)} - \frac{R_m}{f_m(k)} \right] \]

If \( \beta \approx \alpha \) and \( kR_n \gg 1 \), then the third term is negligible. Plot \( \beta \) left hand term vs. \( k^2 \) gives a value of slope of intercept from which \( N_n \) and on can be respectively obtained; \( R_n, \beta, \alpha \) known...
subtelin - small cation-sulfur protein
M.W. = 6000

Fe-S center consists of cation tetrahedrally coordinated to four sulfur atoms.

Diffraction work on crystalline protein yields three Fe-S bond distances with same value; one shorter Fe-S bond.

3. Fe-S distance = 2.30 ± 0.04 Å

1. Fe-S distance = 2.05 ± 0.04 Å

Considerable strain energy was thought to be stored in short bond, this could affect redox potential of protein thence else transport properties.

Analysis of subtelin by EXAFS yielded bond length difference of 0.00 ± 0.01 Å.

The equivalent Fe-S distances have since been confirmed in a redetermination of X-ray diffraction data.
Hemoglobin

For high and low affinity forms, a

difference in binding energy for O2 of

c. 3 kcal/mole split.

Two questions:

1) Is structural difference localized

in immediate vicinity of active

site of the two high affinity forms?

2) Pathway or mechanism for conversion

between high and low affinity forms

of this protein.

For (1) Fe-O distance was measured

in low affinity hemoglobin and

in hemoglobin kenza (a high affinity

mutant). Distances were found to

be identical to 1.90 ± 0.1. No

significant difference at active site

of these two forms.
(2) **Paul's hypothesis:**

Fe out of pyrophoric plane in
 Dentif. flour

Fe

Fe fired with plane on O2

Dentif. flour

Motion of Fe on O2 bonding as much as 0.7 Å.

Postulate that this motion would cause
change in Fe - N (plane) distance when O2
bonds. Propagation of the strain energy
resulting from the Fe motion in particular
acting as a "plunge."† was proposed as
a model for corrosion or O2 bonding.

EXAFS measurements on O2 adsorbed
from carrier gas.

Results for Fe - N (plane)
2.055 ± 0.01 Å
1.98 ± 0.01 Å

Large scale motion of Fe appears to be ruled out
hence no "plunge" effect.
Figure 27. Active Site geometry of plastocyanin and azurin: (A) Cu(I) plastocyanin; (B) Cu(II) Plastocyanin; (C) Cu(I) Azurin (reproduced from Sykes, 1990)
Table II is approximately that of \( \Sigma R^2_{\text{non}} \) and represents the best possible fit to the data. The residues of the resulting data are compared to the estimated noise in Fig. 3b. Even though 3.5 S and 0.5 N per Fe, as diagrammed in Fig. 4, is suggested as the best solution by both analysis procedures which involve reasonable estimates and assumptions, the solution of 3.0 S and 1.0 N per Fe cannot be completely excluded.

Recent studies on the amino acid sequence homology of the Rieske iron-sulfur protein from seven different sources, including mitochondria, chloroplasts, and bacteria, have revealed a highly conserved polypeptide segment near the C-terminal. This polypeptide, with amino acid sequences, containing cysteine and histidine residues, has been isolated from yeast Rieske protein by using cysteine and histidine residues in the close vicinity. A sequence homology of typical [2Fe-2S] and [4Fe-4S] iron sulfur proteins indicates that the minimum number of amino acid residues between directly liganding residues is two. Since cysteine and histidine are reasonable residues for S and N ligands, the C-terminal side sequence may provide C12N and C13N. On the other hand, the N-terminal side could provide either C14S and C15S or H15S and C14S. Recently, Gatti et al. [44] also suggested that the Rieske [2Fe-2S] cluster in the yeast mitochondria is liganded by three cysteine residues (C12N, C13S, and C14S) and one histidine (H11S). This suggestion was based on the analysis of 11 respiratory deficient mutants, each of which were identified to have a single amino acid replacement in the highly conserved C-terminal region. They obtained evidence indicating that C14S is one of the ligands to the cluster. These data also suggested the involvement of H11S in the binding of ubiquinone.

It should be pointed out that the amino acid sequence of the Rieske-type protein is very similar to the Rieske protein in mitochondria, chloroplasts, and others [21, 37-43], only this thermophilic protein contains two identical clusters per molecule, while all other proteins contain a single cluster per molecule. Recently, Rieken and Sone [45] reported the isolation of the cytochrome b, complex from another thermophilic, bacterioluminescent PPS, which consists of four poly-peptides with apparent molecular weights of 29 (cytochrome CO2), 23 (Rieske protein), 21 (cytochrome b), and 14 kDa. This complex is similar to all other Rieske-type proteins having only one [2Fe-2S] cluster per protein molecule, but dissimilar to that isolated from T. thermo-

![Fig. 3. Comparison of the residual solutions of Table I and Table II (---)]. Fig. 3b shows the results of the first and higher shell data as one contribution and subtraction of a higher shell contribution similar to phthalate oxidae, other iron-sulfur proteins, and models.

![Fig. 5. Schematic diagram of the FeN and FeS solution for the reduced Rieske protein. A bar over the Fe-S distance indicates that it is the average of the seven contributions. Errors are given in the text.](image)

<table>
<thead>
<tr>
<th>Fe-N</th>
<th>Fe-S</th>
</tr>
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<tr>
<td>( I_{\text{Fe}} )</td>
<td>( I_{\text{Fe}} )</td>
</tr>
<tr>
<td>209</td>
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</tr>
<tr>
<td>211</td>
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<tr>
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**Table II**

Results of two atom-type fitting procedure to the first coordination shell data of reduced Rieske protein with constrained number of ligand values (higher shell assumed to be Fe-Fe contributions).

\( r \) in \( \text{FeS} \): estimated error, \( \pm 0.02 \) A; \( N_r \text{Fe-S} \) per iron atom values held constant during minimization, \( \Delta E_r \text{Fe-S} \) relative to \( \text{Fe}, \text{FeS} \) in eV, estimated error, \( \pm 0.22 \) eV; \( \Delta E_{\text{Fe-N}} \): 3 eV (see text). * denotes single atom-type fit with \( N \) variable.