Bioinorganic Chemistry Course

National Taiwan University

Spring 1999

Instructor: Sunney I. Chan

Hours: Class will meet at 1 – 3 p.m. on the following Fridays:

Class dates: February 26
March 5, 12, 19, and 26
April 2, 16, 23, 30
May 14, 21, 28
June 4

Grades: Grades will be determined by a ten-page term paper.

Course content

February 26	Introduction. Important metals in biochemistry. Important issues in bio-inorganic chemistry. X-ray spectroscopy.
March 5	EXAFS. Copper proteins. Electronic spectroscopy of copper centers.
March 12	EPR spectroscopy.
March 19	Binuclear and trinuclear copper proteins. Hemes and heme-proteins: myoglobin; hemoglobin; cytochrome c.
March 26	Electronic, EPR, and Raman spectrum of heme proteins. Cytochrome c peroxidase; horseradish peroxidase; and cytochrome P450
April 2	Non-heme iron proteins: hemerythrin; ribonucleotide reductase; purple acid phosphatases; and soluble methane monooxygenase.
April 16	Cytochrome c oxidase: structural aspects. ENDOR spectroscopy.
April 23	Cytochrome c oxidase: dioxygen chemistry; electron transfer kinetics; proton pumping. Bacterial terminal oxidases.
April 30	Iron sulfur clusters. Mossbauer spectroscopy.
May 14	Copper methane monooxygenase. Magnetic susceptibility.
May 21	Nickel/iron hydrogenases. Photosynthesis. Photooxidation of water. Manganese clusters.
May 28	Nitrogen fixation. Structure of nitrogenase.
June 4	Long-range electron transfer in chemistry and biology.

BIOINORGANIC CHEMISTRY

Bio-inorganic chemists, who

investigate the role of inorganic elements in nutrition and health, and the promotion of diseased states;

study the transport, storage, scavenging and homeostases of metal ions in organelles and cells;

study the interaction of inorganic complexes with biomolecules, including proteins, nucleic acids, carbohydrates, and lipids, etc.;

study the chiral recognition of inorganic complexes with DNA and RNA's, and the chemistry that might ensue;

study the role of metals in promoting the transport of organic molecules, e..g., valinomycin, siderophores, across cell membranes; or in the activation or suppression of genes when they become bound to transcription factors (promoters or suppressors);

the role of metal ions as messengers and signalling agents;

the structural role of certain metal ions in proteins;

the catalytic role of metal ions in enzymes;

inorganic complexes as structural and/or functional biomimetics for the metal sites in metal-containing enzymes;

develop physical and chemical methods to study the above problems.

Lecture 1

INTRODUCTION

Bio-inorganic chemistry vs Inorganic biochemistry

Bioinorganic chemistry: Inorganic chemistry of metal-containing biological systems

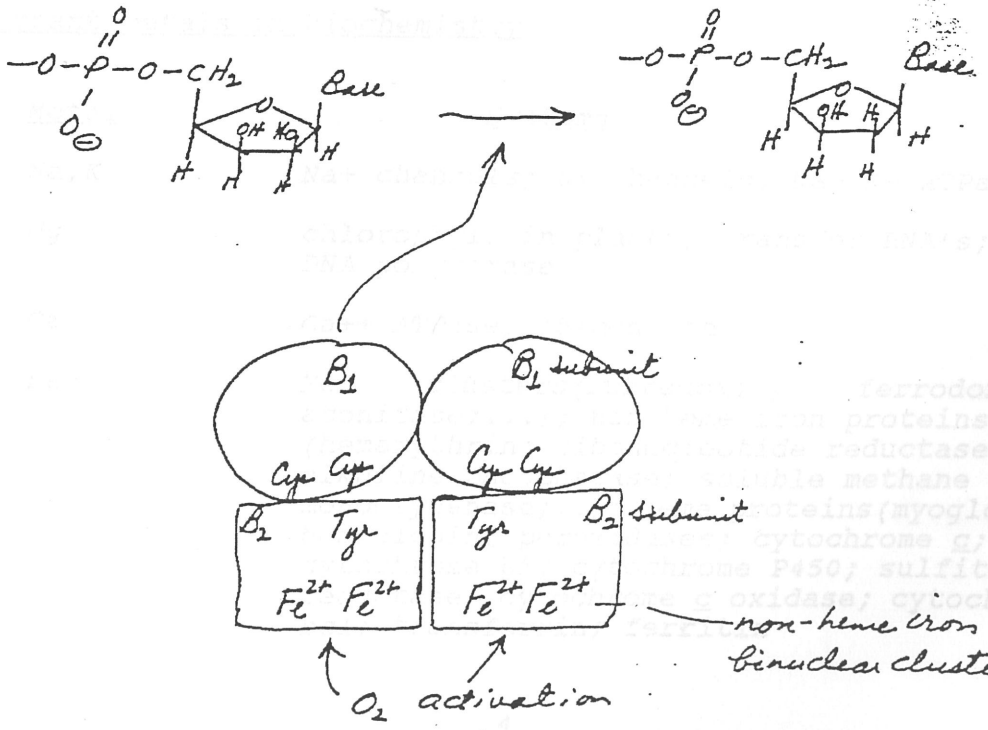
Proteins: metalloproteins; metalloenzymes; metal-containing cofactors
DNA/RNA: interactions of metal ions and inorganic complexes with RNA and DNA, including recognition and cleavage of specific sequences by such complexes
Carbohydrates: binding of metal ions to sugars, carbohydrates, glycolipids and glycoproteins

Note that the focus of bioinorganic chemistry is on the metal coordination chemistry and the chemistry catalyzed by the metal site.

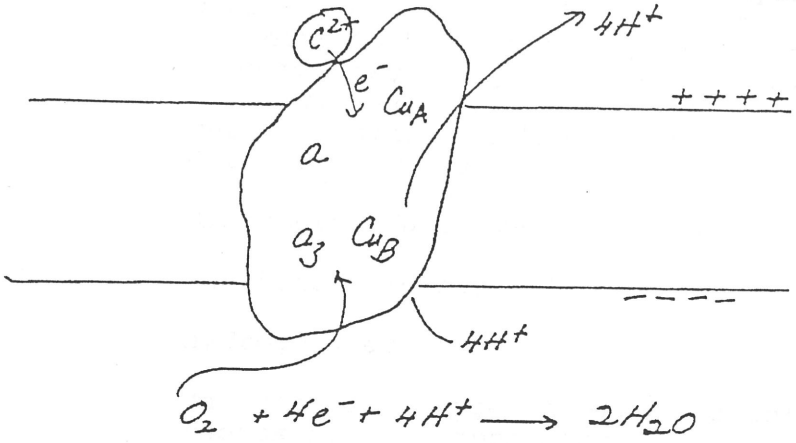
Inorganic biochemistry: overall chemistry associated with metal-containing biological systems, in addition to chemistry at the metal coordination site(s)

Two examples:

(1) ribonucleotide reductase(an enzyme that catalyzes the conversion of ribonucleic acids to deoxyribonucleic acids)



(2) cytochrome c oxidase (an enzyme that acts as a free energy transducer converting redox energy to a transmembrane protonmotive force)



In both of these systems, the bioinorganic chemist will focus his/her attention on the structure of the metal site(s) and the chemistry catalyzed by the metal ion(s); the biochemist will study the structure of the protein as a whole and will determine those structural and dynamical properties of the biological macromolecule that are responsible for the biological activity of the system.

Important metals in biochemistry

Metal	Systems
Na,K	Na+ channels; K+ channels; Na+/K+ ATPase
Mg	chlorophyll in plants; transfer RNA's; DNA polymerase
Ca	Ca++ ATPase; calmodulin
Fe	FeS clusters (rubredoxin; ferrodoxins; aconitase;...); non-heme iron proteins (hemerythrin; ribonucleotide reductase; alkaline phosphatase; soluble methane monooxygenase;..); heme proteins (myoglobin; hemoglobin; peroxidases; cytochrome c; cytochrome b5; cytochrome P450; sulfite reductase; cytochrome c oxidase; cytochrome bc1; transferrin; ferritin

Cu	Normal copper proteins (superoxide dismutase; diamine oxidase; dopamine beta-monooxygenase; galactose oxidase; phenylalanine hydroxylase); blue copper proteins (plastocyanin; azurin; stellacyanin; amicyanin; rusticyanin; umecyanin); binuclear copper proteins (molluscan hemocyanins; arthropodan hemocyanins; tyrosinase); multicopper oxidases (laccase; ascorbate oxidase; ceruloplasmin; nitrous oxide reductase; cytochrome c oxidase)
V	Bromoperoxidase; vanadium nitrogenase
Mo	xanthine oxidase; nitrogenase
Ni	hydrogenases
Mn	Mn catalase; oxygen evolving complex in photosynthetic apparatus
Zn	Superoxide dismutase; DNA polymerase; carboxypeptidase; carbonic anhydrase; zinc fingers

The course could be organized according to the above elements. However, it is more interesting and instructive to organize the subject matter along functional lines.

Significant issues in bioinorganic chemistry

(1) Structure and mechanism of carriers

a. Electron carriers
blue copper proteins; iron sulfur clusters; cytochrome c; cytochrome b's

b. Dioxygen carriers
Myoglobin; hemoglobin; hemocyanins; hemerythrins

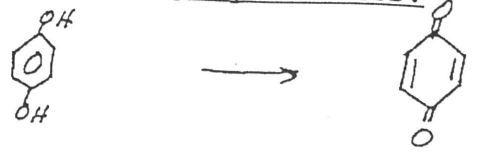
(2) Activation of dioxygen

a. Oxidases I
Laccase; cytochrome c oxidase; ascorbate oxidase; cytochrome bo

$$RX: \quad 4\ e^- + 4\ H^+ + O_2 \longrightarrow 2\ H_2O$$

Source of reducing equivalents:

Laccase:



Cytochrome c oxidase: Ferrocytochrome c ⟶ Ferricytochrome c

Ascorbate oxidase: Ascorbate ⟶ Dehydroascorbate

Cytochrome bo:

b. Oxidases II

Galactose oxidase; monoamine oxygenase

RX: $2\ e^- + 2\ H^+ + O_2 \longrightarrow H_2O_2$

Source of reducing equivalents:

Galactose oxidase: 6-CH_2OH of galactose ⟶ 6-$C(=O)-H$

Monoamine oxygenase: $RCH_2NH_2 \longrightarrow RC(=O)-H + NH_3$

c. Monoxygenases

Cytochrome P450; soluble methane monooxygenase; particulate monooxygenase

RX: $RH + O_2 + 2\ e^- + 2\ H^+ \longrightarrow ROH + H_2O$

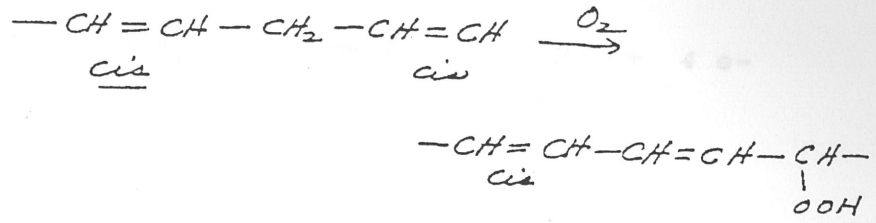
Methane monooxygenase: $CH_4 \longrightarrow CH_3OH$

$CH_3-CH=CH_2 \longrightarrow CH_3CH(O)CH_2$

d. Dioxygenases

Lipoxygenase; benzene dioxygenase

Lipoxygenase:



Benzene dioxygenase:

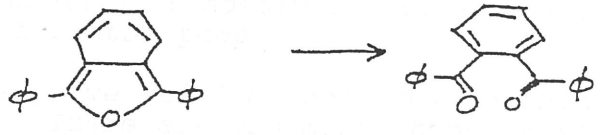
3. Activation of H_2O_2

a. Peroxidases

Cytochrome c peroxidase; lactose peroxidase; chloroperoxidase; horseradish peroxidase

RX: $2\ e^- + 2\ H^+ + H_2O_2 \longrightarrow 2\ H_2O$

Horseradish peroxidase:



diphenylisobenzofuran

b. Catalases

Fe heme catalase; Mn catalase

RX: $H_2O_2 \longrightarrow O_2 + 2\ e^- + 2\ H^+$

$$H_2O_2 + 2\ e^- + 2\ H^+ \longrightarrow 2\ H_2O$$

$$2\ H_2O_2 \longrightarrow O_2 + 2\ H_2O$$

4. Superoxide dismutation

Superoxide dismutase

RX: $2\ O_2^- + 2\ H^+ \longrightarrow H_2O_2 + O_2$

5. Nitrogen fixation

Nitrogenase

RX: $H_2 + 8\ H^+ + 8\ e^- + 16\ MgATP$

$\longrightarrow 2\ NH_3 + H_2 + 16\ MgADP + 16\ Pi$

6. Photooxidation of water

Mn cluster in oxygen evolving complex of photosynthetic apparatus

RX: $2\ H_2O \xrightarrow{4h\nu} O_2 + 4\ H^+ + 4\ e^-$

Why study these systems?

The goal is to uncover the systematics of the catalytic chemistry mediated by these enzymes and to attempt to develop biomimetics of these systems(artificial catalysts).

Chemists are at a disadvantage here. Biological macromolecules have many more degrees of freedom and can therefore tune reaction specificities and reaction rates. The branching of kinetic pathways is more likely with a macromolecule. This kind of kinetic control is absolutely essential for a molecular machine such as a proton pump.

Unfortunately, we still do not know to think about such complex systems. There are too many atoms, states, etc. We do not know how to keep track of the coordinates of every atom and the energies of all the states on paper. In principle, a supercomputer can help. First, we need to know the structure of the molecule to some resolution. Note that the equilibrium structure is not necessarily the relevant one here. The equilibrium structure is often the dead end state. Accordingly, we also need to know the family of structures that interconvert among themselves during the catalytic cycle and the rates of interconversion among them.

BENCH-MARK CHEMICAL REACTIONS

1. Dioxygen Reduction (oxidases)

$$O_2 + 4\,e^- + 4\,H^+ \rightarrow 2\,H_2O$$

2. Nitrogen Fixation (nitrogenases)

$$N_2 + 6\,e^- + 6\,H^+ \rightarrow 2\,NH_3$$

3. CO_2 Fixation (methanogens)

$$CO_2 + 8\,e^- + 8\,H^+ \rightarrow CH_4 + 2\,H_2O$$

4. Alkane Hydroxylation (methane monooxygenases)

$$CH_4 + O_2 + 2e^- + 2\,H^+ \rightarrow CH_3OH + H_2O$$

5. Photooxidation of Water (Photosystem II)

$$2\,H_2O + h\upsilon \rightarrow 4\,H^+ + O_2 + 4\,e^-$$

X-Ray Spectroscopy

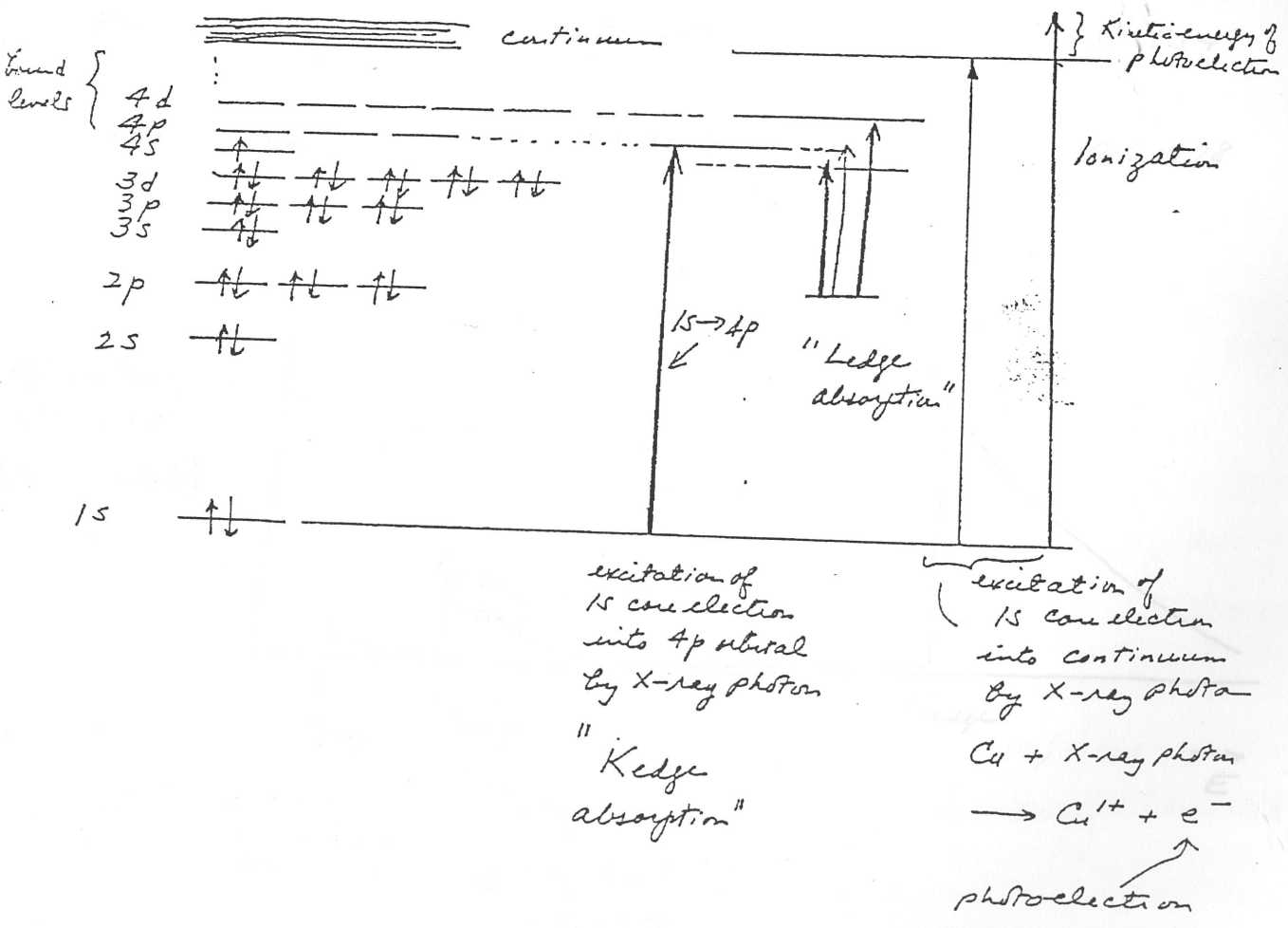
Electronic spectroscopy of core electrons in atoms

Take a transition metal element, e.g. Cu

Electronic configuration : $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4s^1$

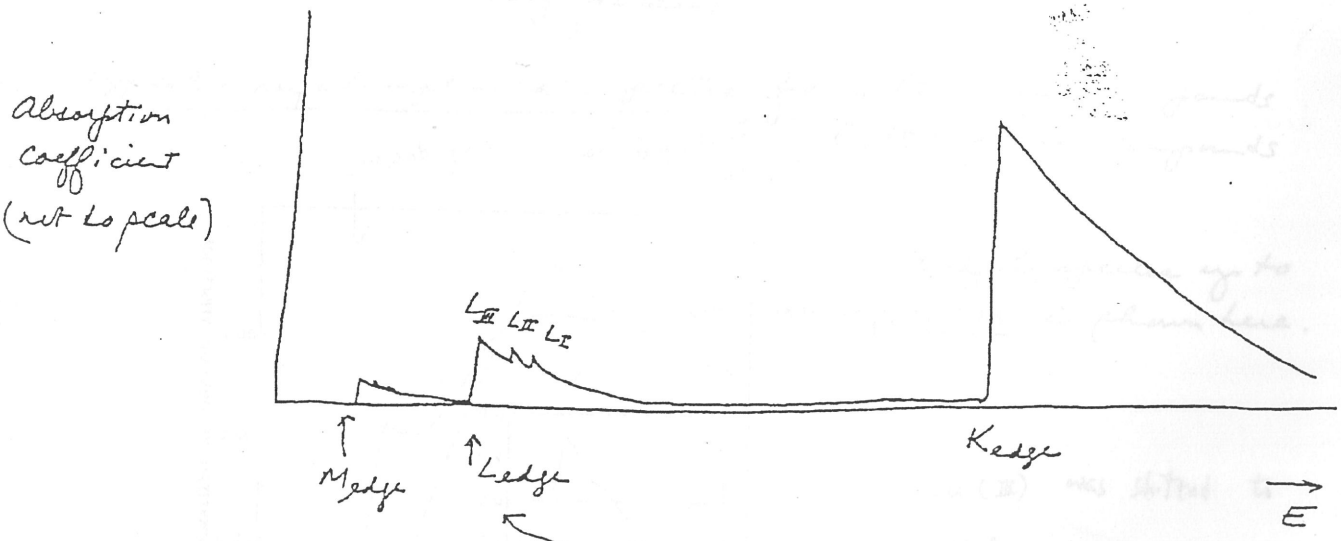
According to Aufbau principle.

Atomic levels are given by



Absorption edges

		Absorption edge (keV)	λ of X-rays (Å)
S	Z = 16	2.470	5.0185
Mn	25	6.5376	1.8964
Fe	26	7.1112	1.7435
Co	27	7.708	
Ni	28	8.332	
Cu	29	8.9803	1.3806
Zn	30	9.6607	1.2834
Mo	42	20.0039	0.61978



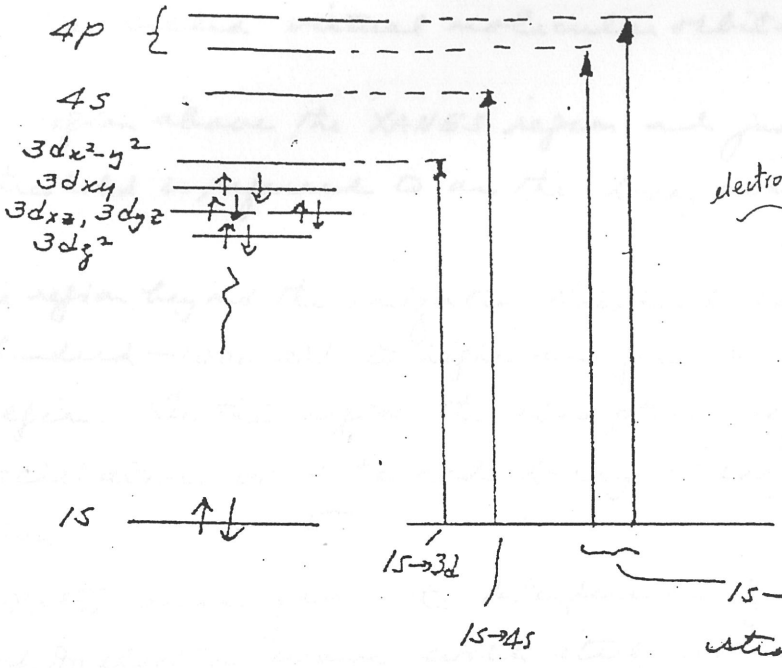
Cu 29

L_I 1.096 keV

L_{II} 0.951 keV

L_{III} 0.933 keV

If Cu is d^9, i.e., Cu(II), and ion is part of a tetragonal complex, then the edge is broadened, and often times exhibits fine structure



chemical bonding

electron repulsion

cause Cu(I) and Cu(II) are different

by selection rule

1s→4p strong

1s→4s weak

1s→3d weak (strictly forbidden)

Typical X-ray absorption edge spectra for Cu(I) ——— compounds
Cu(II) ---- compounds

Coulomb part is more important. 0.02 eV

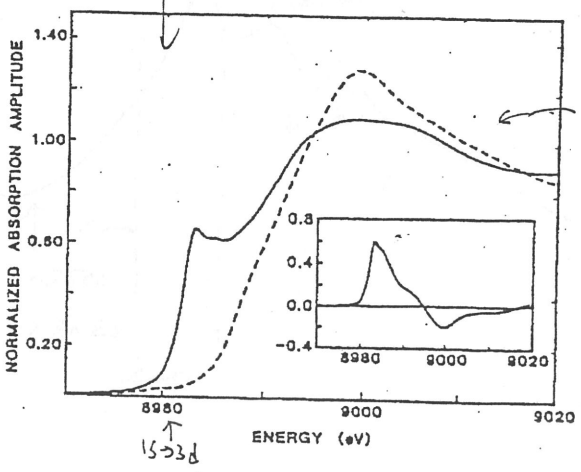


photo-ionization 40 eV

Only the spectra up to 40 eV is shown here.

Cu(II) was shifted to higher energy

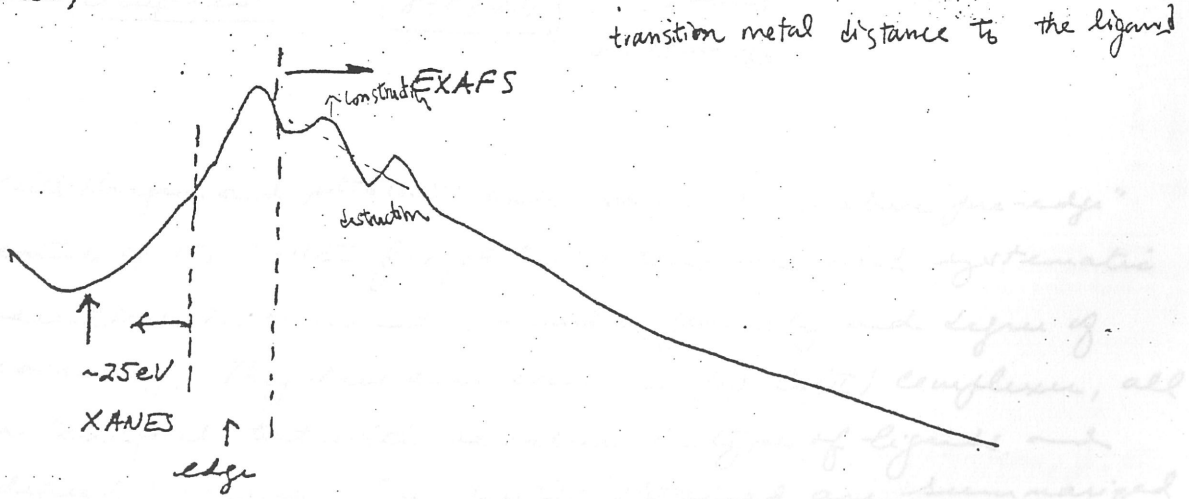
↑
K edge threshold

The first ~25 eV above the edge threshold is the near edge region, often called X-ray absorption near edge structure (XANES). XANES corresponds to the excitation of a (core electron) into a previously unoccupied state (such as a molecular orbital) or a resonance state (often called virtual molecular orbital). XANES

The region above the XANES region and just beyond the ionization threshold is referred to as the X-ray absorption edge.

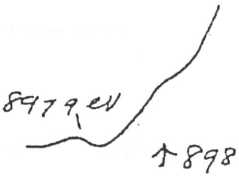
The region beyond the ionization threshold extending over several hundred–1000 eV to higher energies is called the EXAFS region. In this region, the absorption coefficient often exhibits oscillations or extended X-ray absorption fine structures, thus E X A F S.

EXAFS arise from the interference of the outgoing photoelectron wave with itself when it is back-scattered by neighboring atoms in a molecule or in condensed matter (∴ no EXAFS when there is no neighboring atoms)



Will focus on XANES first. EXAFS will be discussed next lecture.

XANES — an example

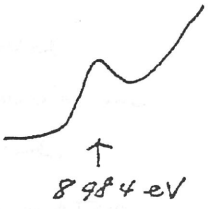


Cu(II)

first intense transition occurs at 8986 eV
a weak feature is observed at 8979 eV

Assignments : a) **8979 eV** $1s \rightarrow 3d$

b) **8986 eV** (i) $1s \rightarrow 4s$
or (ii) $1s \rightarrow 4p$
or (iii) $1s \rightarrow 4p$ simultaneous with a ligand to metal shake down transition



Cu(I)

pronounced feature at 8984 eV

Assignment **8984 eV** $1s \rightarrow 4s$
or $1s \rightarrow 4p$.

Keith Hodgson and students have examined the above "pre-edge" features of the XANES for 19 Cu(I) complexes with systematic variations in coordination number, geometry and degree of covalency. They have also examined 40 Cu(II) complexes, all ~ tetragonal, but with variations in type of ligands and degree of covalency. The results obtained are summarized below.

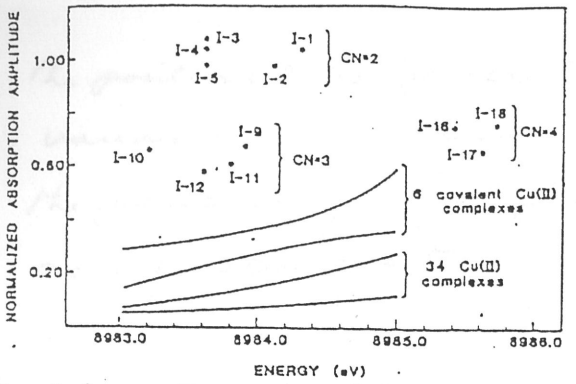


Figure 2. Summary of representative amplitudes of normalized edge spectra for Cu(I) and Cu(II) complexes between 8983.0 and 8986.0 eV. Amplitudes of pre-edge features for Cu(I) complexes are indicated by points. Two amplitude–energy ranges covered by the low energy absorption tails of Cu(II) complexes are indicated: one for 34 normal Cu(II) complexes, the second for 6 covalent Cu(II) complexes (see Results, section A2).

Table II. Compounds Used in K Edge Studies

	compounds[a]	ligation	ref[b]
I-1	$[Cu(xypz)_2](BF_4)_2$	N_2	a
I-2	$[Cu_2(EDTB)](ClO_4)_2$	N_2	b
I-3	$Cu(TMP)_2BF_4$	N_2	c
I-4	$Cu(2\text{-}MIm)_2BF_4$	N_2[c]	c
I-5	$Cu(3,5\text{-}DMP)_2BF_4$	N_2[c]	c
I-6	$_1Cu(BBDHp)](BF_4)_{0.34}(PF_6)_{0.66}$	N_2 (+S_2 at 2.37 Å)	d
I-7	Cu_2O	O_2	e
I-8	$Cu(pze)BF_4$	N_3O	f
I-9	$[Cu(L_1\text{-}pr)](BF_4)$	N_2S	g
I-10	$Cu_2(SOIM(t\text{-}Bu)_2(pz)$	N_2O[c]	h
I-11	$[Cu_2(mxyN_6)](BF_4)_2$	N_3	i
I-12	$Cu(pza)BF_4$	N_3[c]	f
I-13	$[Cu(etu)_3]_2SO_4$	S_3	j
I-14	$[(C_6H_5)_4P]_2[Cu(SC_6H_5)_3]$	S_3	k
I-15	$[(C_6H_5)_4P]_2[Cu_4(SC_6H_5)_6]$	S_3	l
I-16	$[Cu(py)_4]ClO_4$	N_4	m
I-17	$Cu(tepa)BPh_4$	N_4	n
I-18	$[Cu_2(XYL\text{–}O\text{–})]PF_6$	N_3O	o
I-19	$Cu(2,5\text{-}DTH)_2ClO_4$	S_4	p
II-1	$Cu(ImH)_4(ClO_4)_2$	N_4O_2	q
II-2	$Cu(ImH)_4SO_4$	N_4O_2	r
II-3	$Cu(ImH)_2(NO_3)_2$	N_4O_2	s
II-4	$Cu(NH_3)_4SO_4{\cdot}H_2O$	N_4O_2	t
II-5	$Cu_2(fsa)en{\cdot}CH_3OH$	$N_2O_2 + O_4$	u
II-6	$CuFe(fsaen)Cl{\cdot}2.5H_2O$	N_2O_2	v
II-7	$Cu[(prp)_2en]Co(hfa)_2$	N_2O_2	w
II-8	$Cu[(prp)_2en]Cu(hfa)_2$	$N_2O_2 + O_6$	w
II-9	$Cu[(acp)_2en]CoCl_2$	N_2O_2	x
II-10	$Cu[(acp)_2en]CuCl_2$	$N_2O_2 + Cl_2O_2$	x
II-11	Cu citrate dihydrate	O_4O	y
II-12	Cu carnosine	N_2O_2N	z
II-13	$CuSO_4{\cdot}5H_2O$	O_4O_2	aa
II-14	$Cu(acac)_2$	O_4	bb
II-15	anhydrous Cu(formate)$_2$	O_4O	cc
II-16	$Cu(OAc)_2{\cdot}H_2O$	O_5Cu	dd
II-17	anhydrous Cu(propionate)$_2$	O_5Cu	ee
II-18	Cu succinate dihydrate	O_5Cu	ff
II-19	CuO	O_4O_2	gg
II-20	$[Cu_2(L_4\text{-}Et)(N_3)](BF_4)_2$	N_4O	hh
II-21	$[Cu_2(L_4\text{-}Et)(OAc)](ClO_4)_2$	N_3O_2	hh
II-22	$[Cu(L_3)(H_2O)(OClO_3)](ClO_4)$	N_2O_2S	ii
II-23	$[Cu_2(L_4\text{-}Et)(HCOO)](ClO_4)_2$	N_3O_2[c]	hh
II-24	$[Cu_2(L_4\text{-}Et)(NCS)]{\cdot}(ClO_4)_2{\cdot}H_2O$	$N_4O + N_3OS$[c]	hh
II-25	$[Cu_2(L_4\text{-}Et)(NO_2)](ClO_4)_2$	N_3O_2	jj
II-26	$[Cu_2(L_4\text{-}Et)(\text{pyrazolate})](ClO_4)_2$	N_4O[c]	hh
II-27	$[Cu_2(OH)(ClO_4)\supset A](ClO_4)_2{\cdot}CHCl_3$	N_3O_2	kk
II-28	$[Cu(pip)]_2(im)(NO_3)_3{\cdot}2.5H_2O$	N_4O_2	ll
II-29	$[Cu(pmdt)]_3(2\text{-}Meim)(ClO_4)_3$	N_4	ll
II-30	$[Cu(pmdt)]_3(Bzim)(ClO_4)_3{\cdot}H_2O$	$N_4 + N_4O$	ll
II-31	$[Creatinium]_2[CuCl_4]$	Cl_4	mm
II-32	$Cs_2[CuCl_4]$	Cl_4	nn
II-33	$Cu(phen)_2Cl_2{\cdot}3H_2O$	N_4[d]	oo
II-34	$Cu(cyclam)(SC_6F_5)_2$	N_4S_2	pp
II-35	$Cu(14\text{-}ane\text{-}S_4)(ClO_4)_2$	S_4O_2	qq
II-36	$Cu[(C_2H_5)_2NCS_2]_2$	S_4SH	rr
II-37	$Cu(2,5\text{-}DTH)_2(ClO_4)_2$	S_4	p
II-38	Cu[butyraldehyde-thiosemicarbazone]$_2$ green form	S_2N_2[c]	ss
II-39	Cu[butyraldehyde-thiosemicarbazone]$_2$ orange form	S_2N_2[c]	ss
II-40	Cu[2,3-butanedione-bis(thiosemicarbanonato)]	S_2N_2[c]	tt

[a] See the cited references for the abbreviation of each compound. [b] Letters correspond to ref 31 in references and footnotes. [c] Structure not reported; however, the crystal structure of a copper complex with a similar ligand set has been solved. [d] Structure is not determined.

Most important conclusion

We emerge from these experiments is that for all 40 Cu(II) compounds studied, no Cu(II) model has a "pre-edge" maximum below 8985.0 eV, and the intensity of the absorption tail over this spectral region is always significantly lower than the intensity of the peak of any of the Cu(I) complexes.

Therefore, the appearance of a "pre-edge" peak below 8985 eV in the Cu absorption edge spectrum indicates the presence of Cu(I) in the spectrum.

Ref L.-S. Kau, D.J. Spira-Solomon, J.E. Penner-Hahn, K.O. Hodgson & E.I. Solomon, JACS (1987) 109, 6433