



Università degli Studi di Siena

EPR di Metalli di Transizione

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Caratterizzazione di specie paramagnetiche Transition metal ions

- Electronic configuration for transition – metal ions important in biological ESR

Mo⁵⁺

4d¹

↑				
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S = ½

Fe³⁺, Mn²⁺

3d⁵

↑	↑	↑	↑	↑
↑↓	↑↓	↑		

S = 5/2 (high spin)

S = ½ (low spin)

Fe²⁺

3d⁶

↑↓	↑	↑	↑	↑
↑↓	↑↓	↑↓		

S = 2 (high spin)

S = 0 (low spin)

Co²⁺

3d⁷

↑↓	↑↓	↑	↑	↑
↑↓	↑↓	↑↓	↑	

S = 3/2 (high spin)

S = 1/2 (low spin)

Cu²⁺

3d⁹

↑↓	↑↓	↑↓	↑↓	↑
↑↓	↑↓	↑↓	↑↓	↑↓

S = ½

Cu⁺

3d¹⁰

↑↓	↑↓	↑↓	↑↓	↑↓
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S = 0



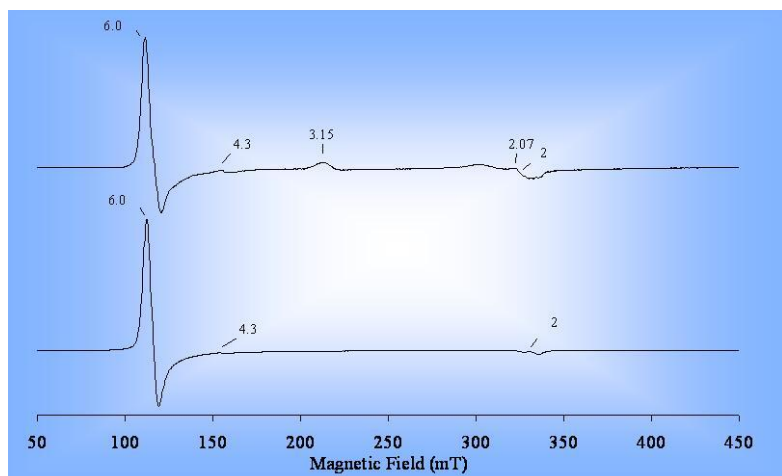
Caratterizzazione di specie paramagnetiche

Transition metal ions

- EPR spectra are more complex than those of free radicals and spin labels.
- The unpaired electrons are primarily localized in the **d** orbitals of the transition metal ion
 - Strong contribution to the electron magnetic moment from the orbital angular momentum of the d-electron.
- Anisotropic spectra with g values very different from the free electron g_e
- The electrostatic and covalent bonding interactions of the ligands with the metal ion produce energy splittings between the different metal-ion orbitals, which affects the distribution of electrons between these configurations and hence the EPR properties of the ion.
 - Small splitting → high spin complexes
 - Large splitting → low spin complexes
- The EPR spectrum contains a considerable amount of information about the liganding and spin state of the transition-metal ions.
- Fast relaxation times (sometimes the spectra can only be observed at low temperature, liquid N or liquid He)



Caratterizzazione di specie paramagnetiche Perossidasi - EPR spectra of native enzyme



The EPR spectra of the resting state of *Bjerkandera* and *Pleurotus* VPs at 20 K confirm the FeIII spin states revealed by electronic absorption spectra.

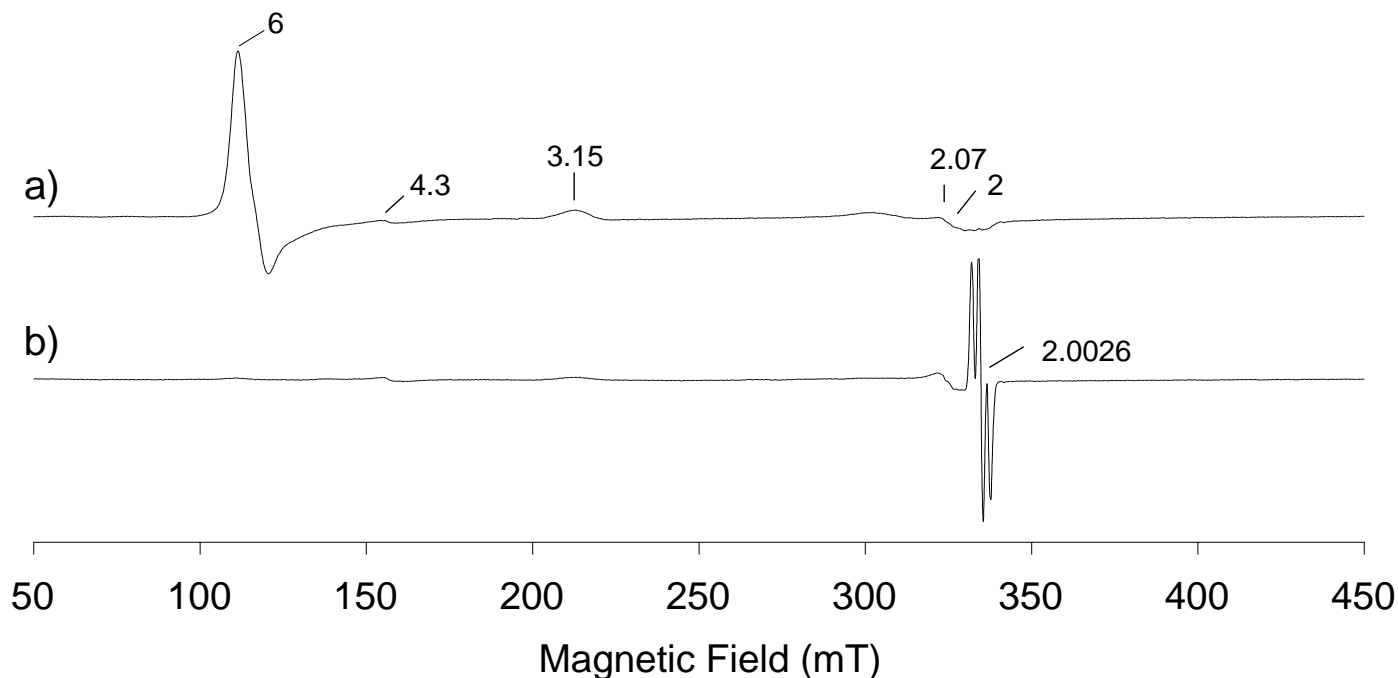
The spectra show the presence of a **dominant high spin FeIII species ($g_{\perp} = 6.00$ and $g_{\parallel} = 2.00$)**. The *Bjerkandera* spectrum is characterized by a **low spin FeIII species ($g = 3.15$ and 2.07)**. The third value of this latter species is too weak to be observed. The feature at **$g = 4.3$ corresponds to a non-heme iron impurity** often seen in protein samples.

The presence of LS species only in *B. adusta* VP could be due to the different enzyme origin: VP from *B. adusta* was extracted by fungal culture, while VP from *P. eryngii* was obtained using *E. coli* expression.

R. POGNI, M. C. BARATTO, S. GIANSANTI, C. TEUTLOFF, J. VERDIN, B. VALDERRAMA, F. LENDZIAN, W. LUBITZ, R. VAZQUEZ-DUHALT, R. BASOSI, "A Tryptophan Based Radical in the Catalytic Mechanism of Versatile Peroxidase from *Bjerkandera adusta*", *Biochemistry*, 44, 4267-4274, 2005.



Caratterizzazione di specie paramagnetiche Perossidasi – Formazione del Compound I

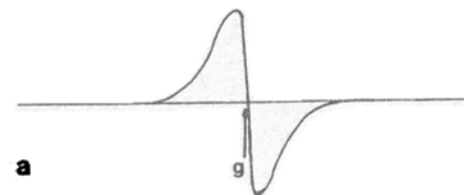


After the addition of an excess of H_2O_2 (1:8 enzyme/ H_2O_2 molar ratio) and rapid cooling the ferric high- (and low-) species disappeared almost completely and were replaced by an intense radical signal centered at a g value of 2.0027(1), corresponding to a protein radical species

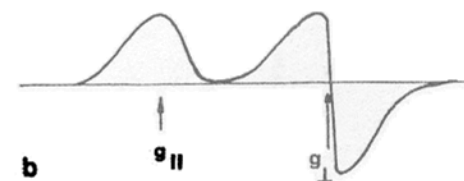


- Symmetry around the metal center gives the anisotropy degree

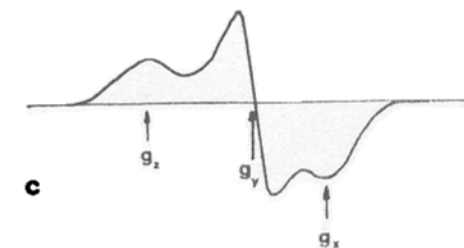
spheric symmetry



axial symmetry



rhombic symmetry



- **METALLO PROTEINS**

- They are involved in many biological processes:
 - O₂ carrier functions (hemoglobin, myoglobin)
 - Metal transfer (transferrin, ferritin)
 - Redox reactions and O₂ metabolism (cytochrome c, superoxide dismutase, oxygenase)
- In order to increase sensibility EPR spectra of metallo proteins are performed at low temperature.
- g value differs from 2.0023 as orbitalic contribution is present
- The intensity of the signal is proportional to the electron concentration



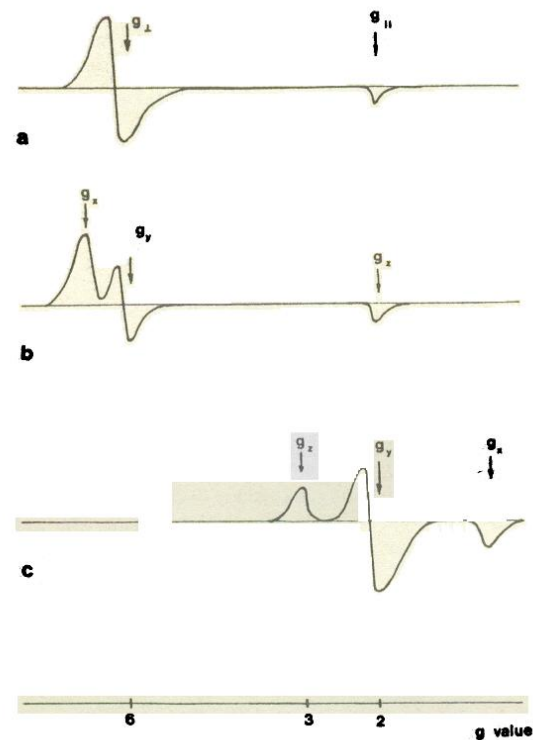
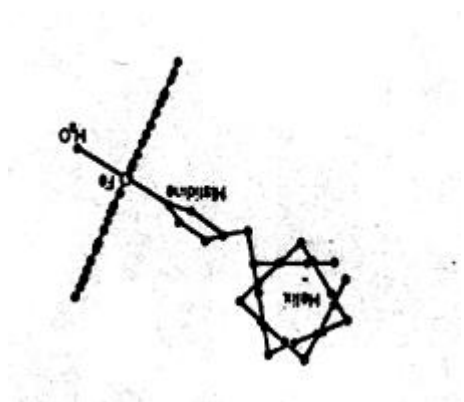
■ **HEME-PROTEINS: MYOGLOBIN**

- They are involved in electron transfer and oxygen carrier functions
- Five of the coordination positions to the iron are provided by nitrogens (from the porphyrin ring and a polypeptide chain histidine). The sixth coordination position in myoglobin can be occupied by water, oxygen or other small molecules.
- EPR studies provide information on coordination to the sixth position of the heme and on the orientation of the heme plane with respect to the rest of the protein.
- The symmetry around the metal center depends strongly on the properties of the ligand on the z axis.



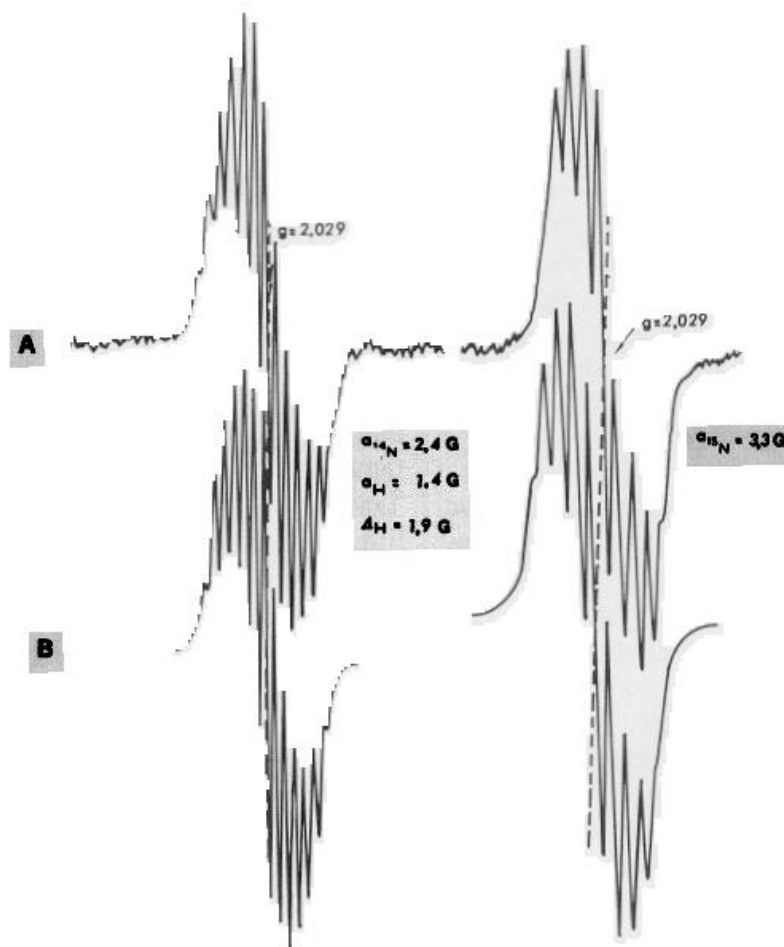
Caratterizzazione di specie paramagnetiche Transition metal ions

- a) If z ligand produce low field (H_2O) the symmetry is axial.
- b) Rhombic signal of methemoglobin
- c) If z ligand produce high field (CN^-) the symmetry is rhombic



Caratterizzazione di specie paramagnetiche Transition metal ions

- EPR spectrum obtained from a solution of Fe(I)(NO)₂ and N-acetylcysteine
- at pH=7.0 , 298 °K and physiological conditions

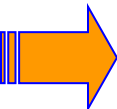




Il problema della larghezza di riga: l'inclusione dei termini superiperfini



- La forma di riga di uno spettro EPR dipende dai seguenti fattori:
 - differenza di popolazione dei livelli energetici tra i quali avviene la transizione;
 - fenomeni di rilassamento;
 - dinamica molecolare (moto lento e moto veloce).
- In particolare nella regione di moto lento la forma della riga dello spettro EPR è fortemente influenzata dalla dinamica molecolare e le interazioni anisotrope diventano particolarmente evidenti e gli spettri non possono più essere descritti come semplici lorentziane (come nel caso di spettri ESR di moto veloce), in tal caso è necessario considerare i termini di interazione superiperfine.
 - Gli studi di Freed et al., sui nitrossidi in liquidi, hanno permesso di ottenere informazioni sulla dinamica di rotazione.
 - La loro analisi si basa su programmi destinati all'elaboratore per simulazione degli spettri di ESR basati sull'equazione stocastica di Liouville (SLE).
- Tuttavia, l'applicazione del metodo di Freed a complessi di metalli di transizione di interesse biologico è stata a lungo preclusa per la mancanza di un programma completo in grado di simulare gli spettri ESR che contengono più di un'interazione iperfine.



L'algoritmo di calcolo sviluppato, per includere i termini della interazione superiperfine in un programma di simulazione, si basa sul formalismo della Equazione Stocastica di Liouville (SLE) la quale è una equazione del moto per l'operatore magnetizzazione per un insieme di molecole paramagnetiche non interagenti che subiscono delle riorentazioni casuali descritte da un processo stocastico



Cu(II) level splitting diagram

