Mössbauer spectroscopy of Iron Containing Proteins and Related Model Complexes

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50 Years After - The Mössbauer Effect Today and in Future
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Research focus: Conventional and Synchrotron based Mössbauer spectroscopy (ESRF, Grenoble; and planning: 2010 Petra III DESY, Hamburg)
Closed Cycle Mössbauer Cryostat in Kaiserslautern

CRYO – Industries of America

Temperature range: 2–300K
Max. Magnetic Field: 5 T
Cool down time: 48 h
1. High-Valent Iron Spezies in Physiologically Relevant Enzymatic Reactions
   - Cytochrome P450
   - Nitric Oxide Synthase (NOS)

2. New Iron-Sulfur-Proteins
   - The radical SAM enzyme coproporphyrinogen III oxidase HemN
   - The ATP-binding cassette protein ABCE1

3. Outlook
Regulation of cell function
- a very complex phenomenon

1. High-valent iron oxidation states and protein radicals in NO synthases and cytochrome P450

Compounds I: High-valent $[Fe^{IV}=O]^{2+}$-species + radical on the porphyrin ring

neuronal disorder, cardiovascular diseases, inflammatory cancer, oxidative stress... etc
The bacterium *Pseudomonas Putida* metabolizes Camphor ($C_{10}H_{16}O$).

Cytochrom P450$_{cam}$ introduces oxygen $O$ at position 5 of Camphor (Monooxygenation in 5-Position).
Preparation of cpd I using the „shunt“- reaction:

- Addition of peracetic acid to native P450cam

\[
\text{HO-O-C-CH}_3
\]

- Cryo-freezing of the reaction mixture within milliseconds

Investigation by

- Mössbauer spectroscopy
- EPR spectroscopy
Preparation of intermediates by rapid freeze quench

Rapid Freeze Quench set-up.

packing factor: ½

Isopentane (-110 °C)

sample for EPR experiments

Mössbauer
**Mössbauer spectroscopy**

- Doublet with $\delta=0.13$ mms$^{-1}$ and $\Delta E_Q=1.94$ mms$^{-1}$ characteristic for Fe$^{IV}$ ($S=1$) (13 ± 2% relative contribution)

**EPR spectroscopy**

- Creation of a Tyrosylradical?

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P450cam: Radical assignment by high-field EPR (94 GHz)

Heller-McConnel-relation:
\[ A(H_{\beta 1,2}) = \rho_{\pi}(C_1)\{B^1 + B^{1'}\cos^2\theta_{1,2}\} \]

Calculated dihedral angles:
\[ \theta_1 = +40.50^\circ; \theta_2 = -79.50^\circ \]

⇒ Tyr96 is the radical

Crystal structure:

\[ \theta_1 = 26^\circ \]
\[ \theta_1 = 41^\circ \]

Crystal structure:

Edge on view:

94 GHz EPR

\[ g_x, g_y, g_z \]

Splitting of \( g_z \) and \( g_x \)

Splitting of \( g_x \), \( g_y \) and \( g_z \)

To detect Compound I the electron transfer must be disrupted!
**Comparison**

**Chloroperoxidase (CPO)**

Chloroperoxidase (CPO) contains iron in the Fe(IV) state, which is involved in the reaction with a porphyrin-π-cation radical. The oxidation state of iron is Fe(IV) and the porphyrin radical is reduced by electron transfer from Tyr96.

- Fe(IV) S=1
- exchange coupling between Fe(IV) and porphyrin radical

**P450cam**

P450cam also contains iron in the Fe(IV) state. The iron oxidation state is Fe(IV) S=1 with a porphyrin-π-cation radical reduced by electron transfer from Tyr96.

Nitric oxide synthases (NOS) belong to a physiologically highly important heme enzyme family which catalyzes the synthesis of the messenger molecule NO in all forms of life.

In humans:
- iNOS: inducible (immune system response)
- nNOS: neuronal (neuro-signal transmission)
- eNOS: endothelial (blood pressure regulation)

Questions:
- How does this work?
- What are the reaction intermediates?

? Compound I ?
9.6 GHz EPR of nNOS after 8ms reaction with peracetic acid (8ms)

**Experiment**
- Start nNOS
  - 90% Fe$^{3+}$-low-spin (2.43; 2.28; 1.90)
  - 10% Fe$^{3+}$-high-spin (7.40; 4.20; 2.00)

**Simulation**
- 8ms nNOS intermediate
  - 80% radical (g=2.0)
  - 20% unknown species g=(2.23; 2.24; 1.96)

**Unknown S=1/2 species:**
- Fe(IV)=O porphyrin-π-cation radical with strong antiparallel exchange coupling (with $g_{\perp}$~2.2 and $g_{||}$=1.96, $J>>D$).

nNOS: Mössbauer spectroscopy identifies Fe(III)

**Start material**

- nNOS: $\delta = 0.38 \text{ mms}^{-1}$, $\Delta E_Q = 2.48 \text{ mms}^{-1}$ (42±7%)

- Clusters of iron hydroxide (artefact): $\delta = 0.518 \text{ mms}^{-1}$, $\Delta E_Q = 0.675 \text{ mms}^{-1}$ (57±3%)

**8 ms reaction time**

- Intermediate: Doublet with $\delta = 0.27 \text{ mms}^{-1}$ und $\Delta E_Q = 2.36 \text{ mms}^{-1}$ (29% ±5%)

- $\delta > 0.20 \text{ mms}^{-1}$:
  - no Fe(IV)
  - Fe(II)O$_2$ (unlikely)
  - Fe(III) ($S=1/2$) coupled to a radical ($S'=\frac{1}{2}$) possibly Trp 409?
Mössbauer spectroscopy of neuronal NOS

Perry & Marletta: PNAS 95, 11101 (1998)

1QW6

Mössbauer spectrum of nNOS purified in the presence of EDTA:

T=4.2 K
B=20 mT

relative transmission

velocity [mm/s]
2. The [4Fe-4S] cluster of the radical SAM enzyme coproporphyrinogen III oxidase HemN

The Radical SAM enzyme oxygen-independent coproporphyrinogen III oxidase HemN catalyzes the oxidative decarboxylation of coproporphyrinogen III to protoporphyrinogen IX during bacterial heme biosynthesis.
The [4Fe-4S] cluster of the radical SAM enzyme coproporphyrinogen III oxidase HemN

- Anaerobically purified HemN has a [4Fe-4S]$^{2+}$ cluster in which only three iron atoms were coordinated by cysteine residues (isomer shift of $\delta = 0.44(1)$ mm/s).
- The fourth non cysteine-ligated iron exhibits $\delta = 0.57(3)$ mm/s which shifts to $\delta = 0.68(3)$ mm/s upon addition of SAM

The ATP-binding cassette protein ABCE1

- Expressed in almost all organisms an essential for life
- Fundamental function in translation initiation and/or ribosome biosynthesis
- Molecular mechanisms not known

ABCE1 has two \([4\text{Fe}-4\text{S}]^{2+}\) clusters with different electronic environments, one ferredoxin-like \((\text{CPX}_n\text{CX}_2\text{CX}_2\text{C})\) and one unique ABCE1-type cluster \((\text{CXPX}_2\text{CX}_3\text{CX}_n\text{CP})\)

3. Overview and Outlook

- **New iron-sulfur proteins** have been and will be detected
- **Polynuclear iron-oxo-centers** are currently under investigation in our laboratory (in cooperation with P. Sadler, University of Warwick, GB)
- **Enzymatic reaction intermediates**
- **Biofunctionalized nanoparticles (Au-Fe-oxide nanoparticles)**
- **Highvalent Fe(IV)-, Fe(V), and Fe(VI) complexes**
- **Iron-uptake in plants and organic tissues**
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