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Emission Mössbauer spectroscopy: novel applications for probing structural organisation of metalloenzyme active centres

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Test object:

• glutamine synthetase (GS; doped with $^{57}$Co$^{2+}$),

  *a key enzyme of nitrogen metabolism in many organisms*

  (isolated from *Azospirillum brasilense*, a plant-growth-promoting N$_2$-fixing rhizobacterium)

Methodology:

• $^{57}$Co emission Mössbauer spectroscopy

  *(in rapidly frozen aqueous solutions)*
Emission ($^{57}\text{Co}$) Mössbauer spectroscopic study of $^{57}\text{Co}^{2+}$-doped GS active centres

Sample with $^{57}\text{Co}$ (source of $\gamma$-radiation) $\rightarrow$ $\gamma$-quanta $\rightarrow$ Absorber of $\gamma$-radiation (with $^{57}\text{Fe}$) vibrating with velocities up to $\pm 10$ mm/s $\rightarrow$ Detector (with PC-operated multichannel analyser)

Characterisation of bacterial GSs

One of two hexameric rings located face-to-face, with total 12 subunits
(D. Eisenberg e.a., 2000)

Location of one of the 12 active centres (between subunits)
Characterisation of bacterial GSs

Distance between the cation-binding sites:

\[ n_1 \leftrightarrow 6 \text{ Å} \rightarrow n_2 \] (no bridging residues): the two sites are ‘spectroscopically independent’
Emission ($^{57}\text{Co}$) Mössbauer spectroscopic study of $^{57}\text{Co}^{2+}$-doped GS active centres

**PREREQUISITES:**

1. Possibility to remove strongly bound cations from the native enzyme
   
   (treatment with 5 mM EDTA $\rightarrow$ reversible loss of activity)

2. Possibility to insert $\text{Co}^{2+}$ into the active centres
   
   (addition of $\text{Co}^{2+}$ $\rightarrow$ regain of activity)

3. Specific $[^{57}\text{Co}^{2+}]:[\text{GS}]$ molar ratio ($12 \leq x \leq 24$)
   
   (to avoid multiple binding of $^{57}\text{Co}^{2+}$ beyond active centres)
57Co Emission Mössbauer Spectroscopy:

Probing the structure of cation-binding sites at the active centres

Macromolecule (side and top views)

Glutamine synthetase from *Azospirillum brasilense*

Active centre: 2 cations in sites 1 & 2

Spectrogram


Basic conclusions:

• EMS allows different cation-binding sites in $^{57}$Co-doped metalloproteins to be characterised.

• EMS data on $^{57}$Co$^{2+}$-doped bacterial glutamine synthetase (GS) reveal two different cation-binding sites at each GS active centre.

• Isostructural substitution of $^{57}$Co$^{2+}$ for other cations (e.g. for Zn$^{2+}$) expands the EMS applicability and importance.
Thank you