



ESRF

	Experiment title: Reactive intermediates of cytochrome P450 prepared by the freeze-quench method	Experiment number: LS-728
Beamline: ID 18	Date of Experiment: from 29. Sept. 7 ⁰⁰ to: 1. Oct. 7 ⁰⁰	Date of Report: 24. Nov. 97
Shifts: 6	Local contact(s): GRÜNSTEUDEL Hermann	<i>Received at ESRF :</i>

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Report:

Reactive Intermediates Studied by Nuclear Forward Scattering

According to the preparation procedure described in the application we have prepared transient intermediates, obtained 25 ns and 200 ns after the mixing of ⁵⁷Fe-enriched cytochrome P450_{cam} (low-spin form) with m-CPBA. The reaction product (compound I) was freeze-quenched at -78°C in an isopentane cooling bath. The resulting frozen material was collected as "snow" (50 - 100µl) and packed into sample holders which are appropriate for nuclear resonant forward scattering (NFS) experiments using Synchrotron radiation.

Parallel to this procedure we have also prepared the compound I analog of ⁵⁷Fe-enriched iron-tetramesityl porphyrin (FeTMP; -50 µl).

After setting up the experiment (installation of cryostate and counters) we have performed an NFS experiment at 4.2K with the enzyme as- prepared (compound I). After several hours of measuring time we were unable to detect a quantum beat spectrum in single bunch mode. (Note that we have applied for 16 bunch mode). Therefore we have continued our experiment with the ⁵⁷FeTMP compound I analog at 4.2K. Within - 2h we were able to record a NFS spectrum which exhibits reasonable statistics up to - 150 ns (Fig. 1). Beyond this delay time the obtained statistics are not satisfactory within the experimental conditions available (50µl sample volume, - 2h measuring time, single bunch mode). Nevertheless, we were able to show that the experimental time spectrum between - 20 and 150 ns exhibits remarkable similarities compared to the theoretical time spectrum which results from a simulation using the SYNFOSS program package [1]. This simulation represents (parallel) spin coupling between the ferryl iron (S=1) and the porphyrin cation radical (S = 1/2), as expected for a compound I analog.

In summary we have shown with a compound I analog that the proposed experiment with the compound I enzyme is promising, however, we need 16 bunch mode (instead of single bunch

mode) and more beam time for the successful study of at least 2 enzyme samples (12 instead of 6 shifts).

II. Angular Resolved Molecular Vibrations Studied by Nuclear Inelastic Scattering

Within the remaining beam time (3 shifts) we have investigated the time-integrated nuclear inelastic scattering (NIS) intensity at energies from -100meV to $+150\text{meV}$ around the resonance energy E_0 of the ^{57}Fe Mössbauer transition in $[\text{CN}_3\text{H}_6]_2[\text{Fe}(\text{CN})_5\text{NO}]$ at room temperature. Two different orientations of single crystals were investigated, i.e. c-axis and a-axis parallel to the Synchrotron beam. The obtained NIS spectra clearly indicate the presence of angular resolved molecular modes (Fig. 2). We are presently analyzing these modes on the basis of molecular orbital calculations in the local density approach.

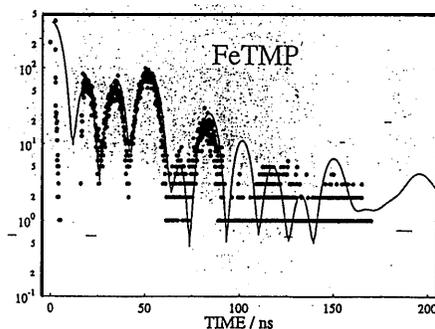


Fig. 1: Mössbauer time spectrum of $^{57}\text{FeTMP}$ (compound I analog) with SYNFOS simulation [1], indicating parallel spin coupling between ferryl iron ($S=1$) and porphyrin cation radical ($S'=1/2$). Spin-Hamiltonian parameters are: $B^{\text{appl}}=15\text{ mT}\perp\gamma$, $T=4.2\text{K}$, $J=-(41, 41, 45)\text{T}$, $D=25\text{ cm}^{-1}$, $E/D=0.05$, $\Delta E_Q=1,4\text{ mms}^{-1}$, $\eta=0$, $A_i=-(27,5, 27,5, 10)\text{T}$.

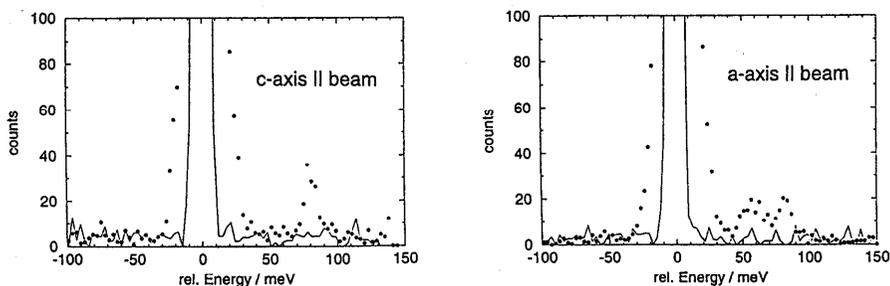


Fig. 2: Nuclear inelastic scattering of single crystals of $[\text{Fe}(\text{CN})_5\text{NO}]$ in different orientations at room temperature, together with the scattering intensity in forward direction (solid line).

[1] M. Haas, E. Realo, H. Winler, W. Meyer-Klaucke, A.X. Trautwein, O. Leupold, and H. D. Rüter. Nuclear resonant forward scattering of synchrotron radiation by randomly oriented iron complexes which exhibit nuclear Zeeman interaction. Phys. Rev. B56, 1-7 (1997)