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Biophysical applications

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Nuclear forward scattering (NFS) of synchrotron radiation was applied to investigate the electronic and magnetic properties of (i) the diamagnetic "picket-fence" porphyrin $FeO_2(SC_6HF_4)(TP_{piv}P)$, which is a model for oxygenated hemoglobin and myoglobin and (ii) the paramagnetic "picket-fence" porphyrin $[Fe(CH_3COOH)(TP_{piv}P)]^-$, which is a model for the ferrous state of the prosthetic group, termed P460, of the multi-heme enzyme hydroxylamine oxidoreductase from the bacterium *Nitrosomonas europeae*.

1. Introduction

Transition metal ions are indispensable for the integrity of biological structures and the catalysis of biological reactions. Even the fact that these metal ions are often only trace elements in a living system in no way diminishes their significance. Whenever nature has to perform a difficult task it can be expected that a metal ion or sometimes a cluster of such ions is employed.

When searching for reasons and for an understanding of this behaviour it inevitably comes to the study of the electronic (and magnetic) structure of mono- or polynuclear metal centers in biomolecules and their biomimetic model compounds. Hence it is appealing to apply and to develop spectroscopies which are specific in the sense that they are metal-centered and even metal-selective. Mössbauer spectroscopy with the isotope ^{57}Fe is such a method, which has been extensively and successfully applied in the biosciences ([1,2] and references therein). With the advent of nuclear resonant forward scattering (NFS) of synchrotron radiation we have started testing the first biophysical Mössbauer applications in the time domain.

2. Diamagnetic Fe-porphyrin

In the oxygenated form of hemoglobin (HbO_2) and myoglobin (MbO_2), which represent the oxygen-transport and the oxygen-storage system, respectively, in many living systems, the O_2-molecule is covalently bound to the heme iron. The stereochemistry of the heme moiety and the reversibility of O_2-binding has been modelled by synthetic analogues, e.g., the "picket-fence" porphyrin $FeO_2(SC_6HF_4)(TP_{piv}P)$ (figure 1).

In a first set of NFS experiments we have investigated a powder sample of ^{57}Fe-enriched $FeO_2(SC_6HF_4)(TP_{piv}P)$ in the temperature range 4.2–200 K with zero

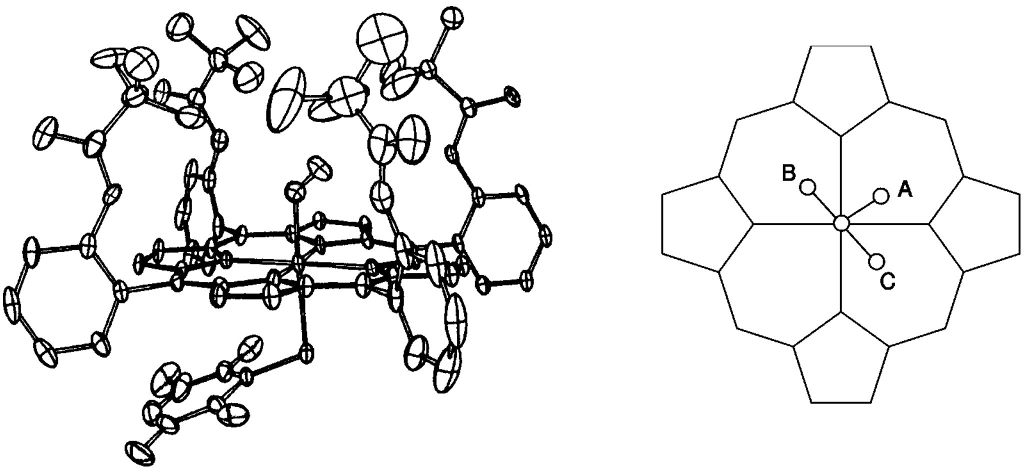

Figure 1. Molecular structure of the “picket-fence” porphyrin $FeO_2(SC_6HF_4)$ $(TP_{piv}P)$ [4]. The terminal oxygen atom of O_2 exhibits dynamic disorder among A, B and C sites affecting the $\Delta E_Q(T)$ behaviour (see text).

applied field (figure 2), at 4.2 K and 160 K with a field of 6.01 T applied parallel to the $\vec{\sigma}$ polarization of the synchrotron beam (figure 3), at 4.2 K with a field of 4 T applied perpendicular to $\vec{k}_\gamma$ and rotated by an angle Θ around $\vec{k}_\gamma$ from $\Theta = 0$ to 90° $(\|\vec{\sigma})$ in steps of 15° (figure 4), and at 4.2 K with zero applied field and with $K_4Fe(CN)_6$ as additional reference scatterer at 300 K (figure 5). All spectra were fitted with the CONUSS program [3], as indicated by the solid lines in figures 2–5.

The advantage of using a powder sample is that it provides a higher effective thickness and therefore a higher count rate than frozen solutions. However, the disadvantage of powder samples in a NFS experiment is that it is difficult to prepare them without thickness inhomogeneity over the sample volume. NFS spectra sensitively reflect – via dynamical beats – the magnitude of the effective thickness t_{eff} of the sample and, hence, also inhomogeneities of t_{eff}. The fits of the spectra shown in figures 2–5 were performed adopting a rectangular distribution of t_{eff} by $\pm 25\%$. The quadrupole splitting of $FeO_2(SC_6HF_4)(TP_{piv}P)$ exhibits a strong temperature dependence, as can be seen from the shift of the first minimum of the quantum beat spectrum with respect to the vertical line drawn in figure 2, and from the ΔE_Q vs. T plot in figure 6. In view of the fact that all “magnetic spectra”, even at 160 K, could be fitted by keeping the magnetic hyperfine field zero and taking into consideration the applied field only (figures 3 and 4), it is concluded that the ground state of $FeO_2(SC_6HF_4)(TP_{piv}P)$ is diamagnetic $(S = 0)$ and that the energy of excited paramagnetic states is much higher than kT. The strong temperature dependence of ΔE_Q therefore cannot be ascribed to this electronic configuration; it is, instead, attributed to the dynamical disorder of O_2 molecules among various structural sites (figure 1) [4]. Since such a “jump behaviour” is a stochastic process it should also

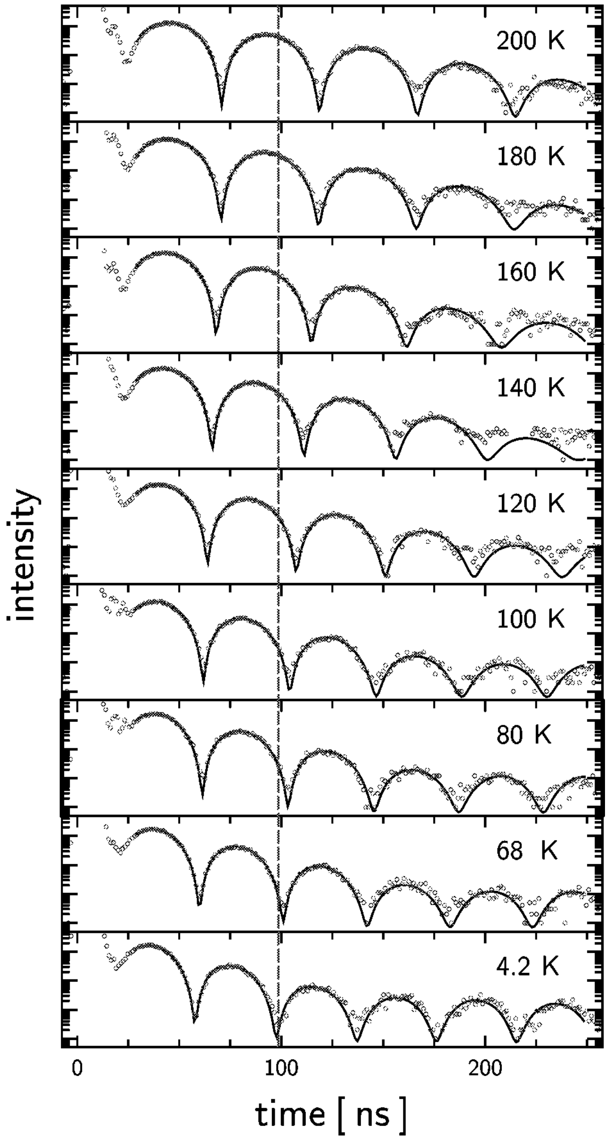

Figure 2. Temperature-dependent NFS spectra of $FeO_2(SC_6HF_4)(TP_{piv}P)$ recorded in zero applied field (HASYLAB, BW4). With the vertical line as reference it is obvious that the beat frequency and hence the quadrupole splitting is significantly temperature dependent. Fits were performed with CONUSS [3].

contribute to the dephasing of originally coherently emitted radiation. Indeed we observe a significant, though small, discontinuity of the effective thickness t_{eff} around 120 K (figure 7).

We want to extend these studies to the oxygenated form (MbO_2) and to the deoxygenated form (Mb) of ^{57}Fe-enriched myoglobin which both show similar $\Delta E_Q(T)$

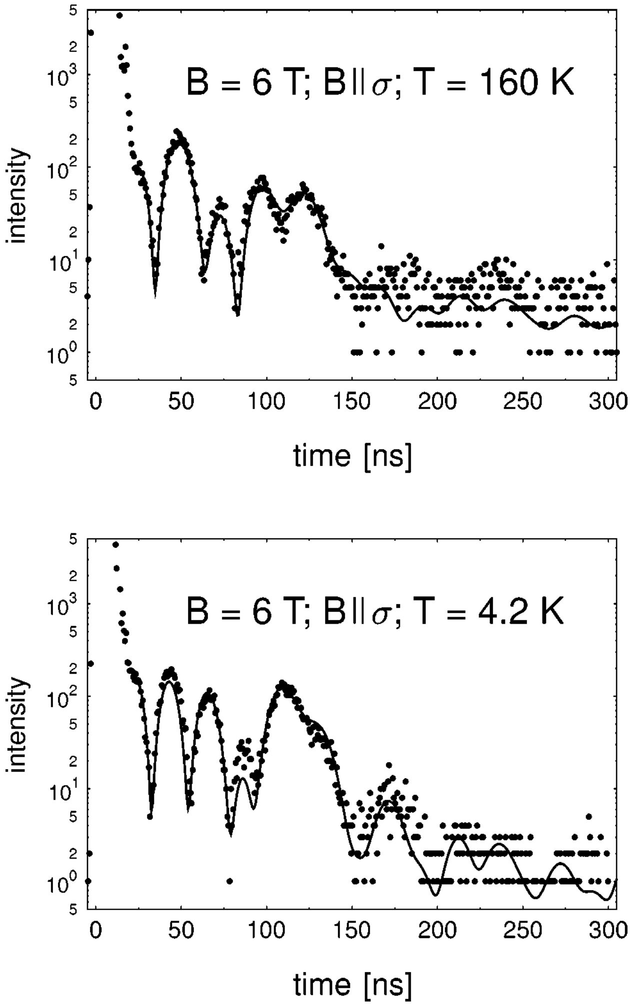

Figure 3. NFS spectra of $FeO_2(SC_6HF_4)(TP_{piv}P)$ recorded at 4.2 K and 160 K in a field of 6.01 T applied parallel to the polarization of the synchrotron radiation (HASYLAB). Fits were performed with CONUSS [3], using the quadrupole splittings from the corresponding zero-field spectra and additionally the applied field (i.e., magnetic hyperfine field is zero). The asymmetry parameter is taken $\eta = 0$ at 4.2 K, and $\eta = 0.5$ at 160 K.

behaviour. The diamagnetic MbO_2 should exhibit – if O_2 is dynamically disordered – a discontinuity in t_{eff}, while the paramagnetic Mb should not.

3. Paramagnetic Fe-porphyrin

The investigation of the electronic structure of paramagnetic molecules with mono- or oligo-nuclear iron centers is expected to benefit from NFS studies because additional external parameters (compared to conventional Mössbauer spectroscopy),

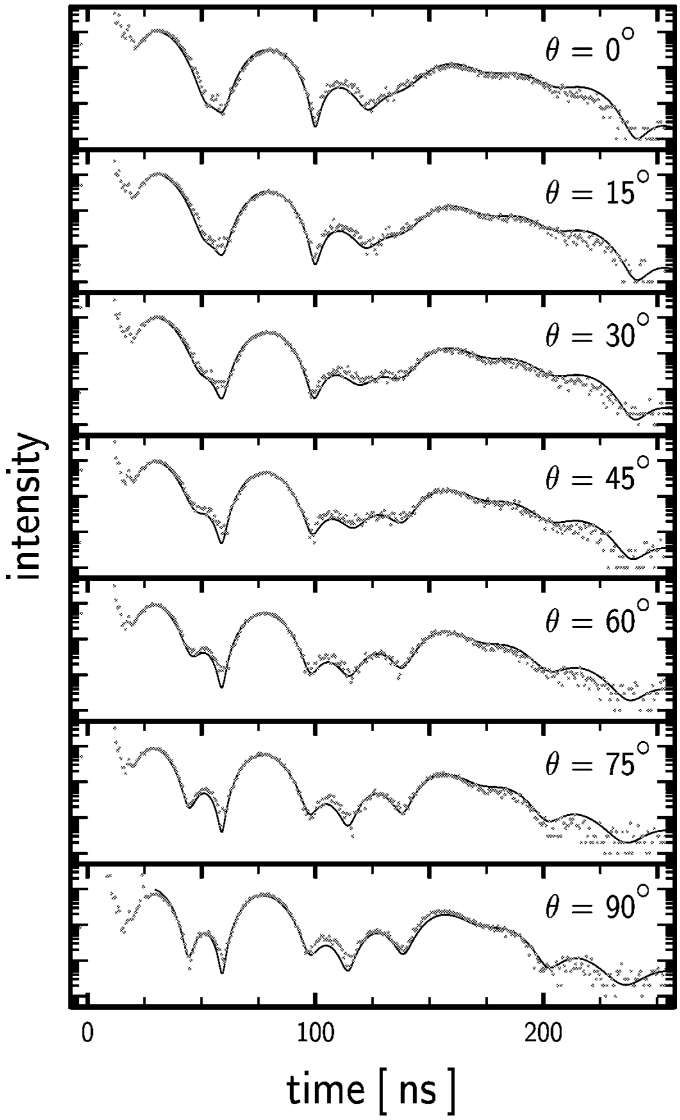

Figure 4. NFS spectra of $FeO_2(SC_6HF_4)(TP_{piv}P)$ recorded at 4.2 K and at different orientations of the applied field (4 T); $\Theta = 0^\circ$ corresponds to $B \perp k_\gamma \perp \sigma$ and $\Theta = 90^\circ$ to $B \| k_\gamma$ (HASYLAB). Fits were performed with CONUSS [3].

such as polarization and time structure of the probing radiation, can be introduced into the protocol of experimental conditions. In order to gain access to the wealth of information that can be obtained frome time-resolved nuclear scattering experiments, especially on randomly oriented scatterers (frozen solutions or polycrystals of Fe-proteins), a theoretical approach is needed by which the experimental NFS spectra can be simulated. The evaluation of NFS spectra for this general case numerically

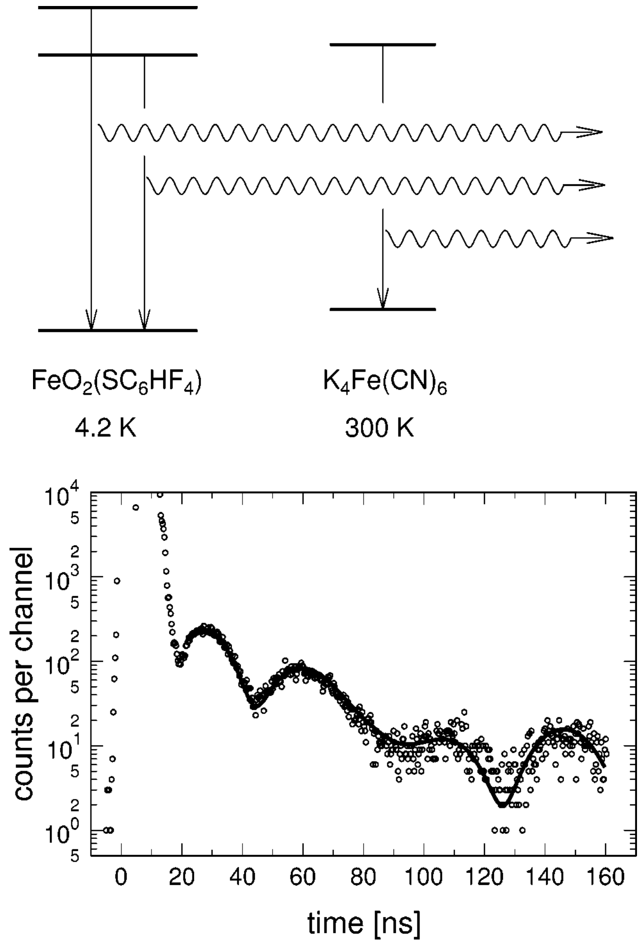

Figure 5. NFS spectrum of $FeO_2(SC_6HF_4)(TP_{piv}P)$ recorded at 4.2 K together with $K_4Fe(CN)_6$ at 300 K as reference scatterer which is placed outsite the cryostate (HASYLAB). Fit is performed with CONUSS [3]; the relative isomer shift of the two compounds is $\Delta\delta = 0.30\ \mathrm{mms}^{-1}$.

by the SYNFOS program has been outlined elsewhere ([5] and section VIII-3 of this issue). Here we present as a test case the measured and calculated NFS spectra of a powder sample of the paramagnetic "picket-fence" porphyrin $[Fe(CH_3COO)(TP_{piv}P)]^-$ in applied fields.

Application of a field $\vec{B}$ to a ferrous high-spin complex induces spin-expectation values according to the spin Hamiltonian [1]

$$H = D\left[\widehat{S}_z^2 - \frac{1}{3}S(S+1) + \frac{E}{D}\left(\widehat{S}_x^2 - \widehat{S}_y^2\right)\right] + \beta_e\widehat{\vec{S}}\cdot\tilde{g}\cdot\widetilde{B}, \tag{3.1}$$

where D, E/D and the tensor $\tilde{g}$ describe the influence of the ligand field via electronic spin–orbit and Zeeman interaction, respectively, $\widehat{\vec{S}}$ represents the effective spin

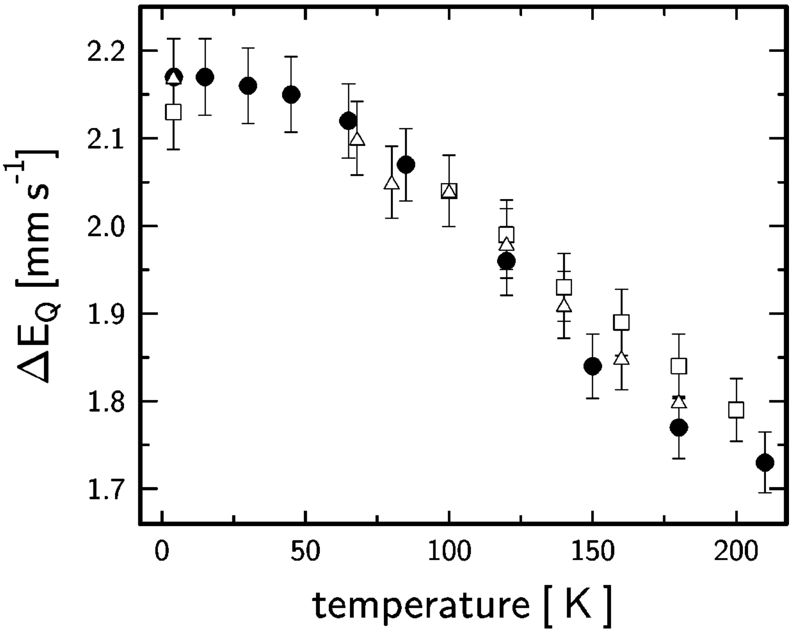

Figure 6. Temperature dependence of the quadrupole splitting ΔE_Q of $FeO_2(SC_6HF_4)(TP_{piv}P)$, derived from conventional Mössbauer studies (●) [4] and from two different NFS studies (□, △).

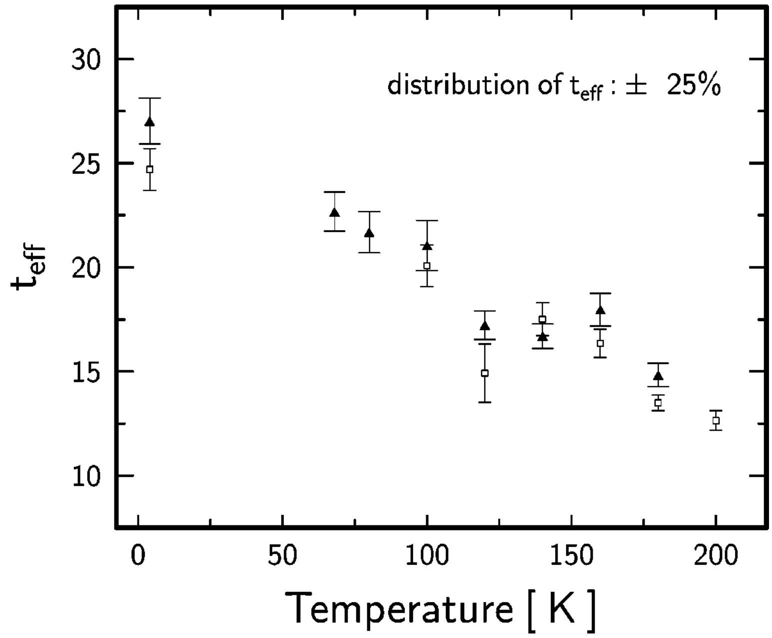

Figure 7. Temperature dependence of the effective sample thickness t_{eff} of $FeO_2(SC_6HF_4)(TP_{piv}P)$ as derived from two different sets (▲, □) of NFS spectra.

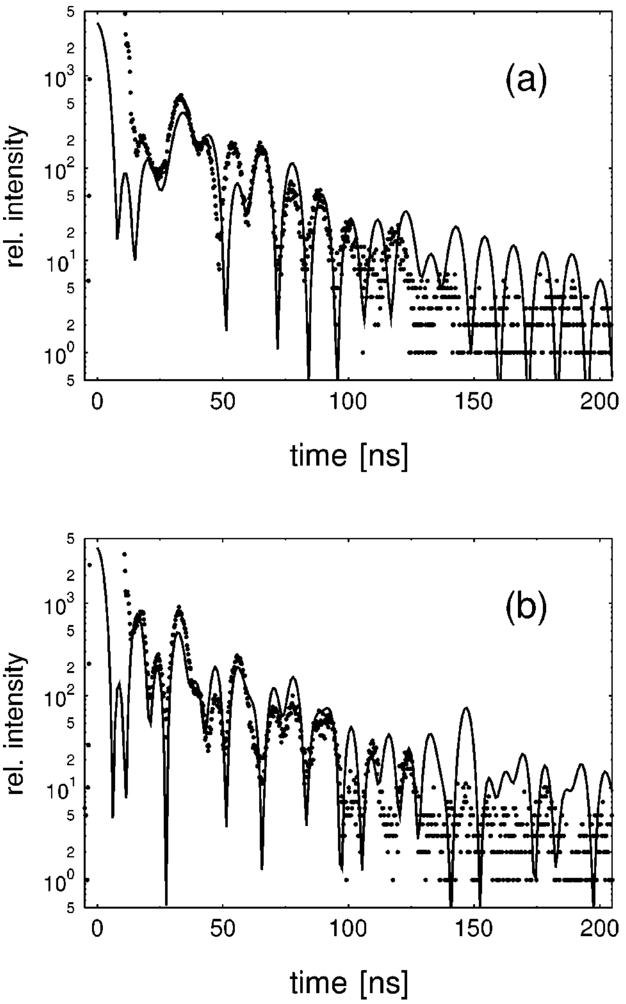

Figure 8. NFS spectra of [Fe(CH$_3$COOH)(TP$_{\text{piv}}$P)]$^-$ recorded at 3.3 K in a field of 6.0 T applied (a) perpendicular to $\vec{k}_\gamma$ and $\vec{\sigma}$ and (b) perpendicular to $\vec{k}_\gamma$ and parallel to $\vec{\sigma}$. The solid lines are calculations with the SYNFOS program (section VIII-3 of this issue) using $\Delta E_Q = 4.25$ mms^{-1} and $\eta = 0$, and the spin-Hamiltonian parameters given in the text.

operator, and β_e is the Bohr magneton. The spin-expectation values $\langle\vec{S}\rangle$ depend on the orientation of the applied field with respect to the molecular frame of reference. Accordingly, the magnetic hyperfine splitting of the nuclear levels, $\langle\vec{S}\rangle\widetilde{A}\vec{I}$, is accompanied for the randomly oriented paramagnetic sample by complicated bands of resonant absorption. The specificity of these bands in the present case is defined by the spin-Hamiltonian parameters of [Fe(CH$_3$COO)(TP$_{\text{piv}}$P)]$^-$, which are $D = -0.8$ cm^{-1}, $E/D = 0$ for the zero-field splitting, and $A_{x,y}/g_{\text{n}}\beta_{\text{n}} = -17$ T, $A_z/g_{\text{n}}\beta_{\text{n}} = -12$ T for the magnetic hyperfine coupling parameters [6], with g_{n} and β_{n} representing the nuclear g-factor and the nuclear magneton. In figure 8 we present NFS spectra of the

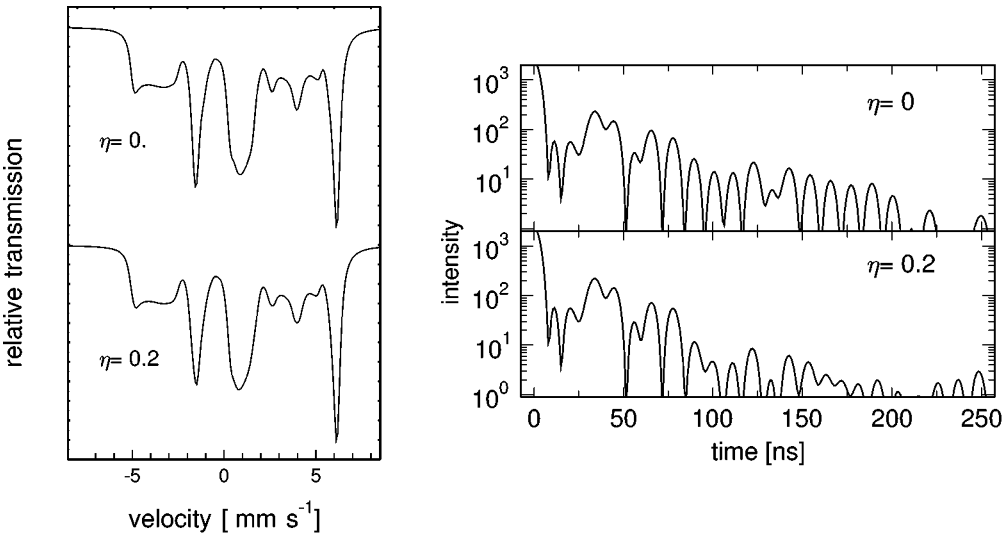

Figure 9. Mössbauer spectra in the energy domain and in the time domain, calculated with the spin-Hamiltonian parameters of the ferrous high-spin iron porphyrin $[\mathrm{Fe(CH_3COOH)(TP_{piv}P)}]^-$ for temperature 4.2 K, slow relaxation and applied field of 6.0 T perpendicular to $\vec{k}_\gamma$ and $\vec{\sigma}$ of the incoming beam. The energy-domain spectra were calculated according to the procedure described in [6], and the time-domain spectra result from SYNFOS calculations described in [5] and in section VIII-3 of this issue.

paramagnetic porphyrin complex $[\mathrm{Fe(CH_3COO)(TP_{piv}P)}]^-$, recorded at 3.3 K in a field of 6 T applied perpendicular to $\vec{k}_\gamma$ and $\vec{\sigma}$ (a), and parallel to $\vec{\sigma}$ (b). The powder sample had very high effective thickness (t_{eff}) in order to guarantee reasonable statistics at least up to a delay time of $\sim$150 ns. The integrated count rate in the interval 20–150 ns of the delay time was $\sim$2 Hz. The solid lines in figure 8 represent SYNFOS simulations with the above given spin-Hamiltonian parameters and $t_{\mathrm{eff}} = 20$. Even without the further improvement taking into account the thickness distribution the calculations reflect the main features of the measured NFS spectra.

The sensitivity of the forward scattering intensity on any of the Mössbauer or spin-Hamiltonian parameters is improved over the sensitivity obtained with conventional Mössbauer spectra, provided the NFS spectrum extends to high enough delay times. Taking the asymmetry parameter η of the electric field gradient tensor as an example, this is demonstrated by comparing Mössbauer spectra of $[\mathrm{Fe(CH_3COOH)(TP_{piv}P)}]^-$ in the energy domain and in the time domain (figure 9). The energy-domain spectra were calculated according to the procedure described in [6], and the time-domain spectra result from SYNFOS calculations described in [5] and in section VIII-3 of this issue. Changing η from 0 to 0.2 yields well resolved changes in the NFS spectrum at delay times $\geqslant$70 ns, while the conventional Mössbauer spectrum is almost unaffected.

In an actual attempt to unambiguously derive Mössbauer and spin-Hamiltonian parameters from a SYNFOS analysis of experimental NFS spectra measurements have to be carried out for a number of different experimental conditions such as temperature, magnitude and direction of applied field. In this respect Mössbauer spectroscopy in the energy domain and in the time domain are comparable.

4. Conclusions

First biophysical NFS applications demonstrate the usefulness of ^{57}Fe-Mössbauer spectroscopy with synchrotron radiation in biology and chemistry. The advantages of the time-domain compared to the energy-domain spectroscopy, namely

(i) the high sensitivity of the quantum-beat structure on Mössbauer and spin-Hamiltonian parameters, especially when the delayed count rate is detected up to $\sim$250 ns,

(ii) the high degree of collimation of the synchrotron beam for 3rd generation machines ($\sim$1 mm^2), allowing small sample volumes of < 50 μl, and

(iii) the time structure and polarization of the radiation,

make it possible to design experiments which were not possible before.

References

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