Inter- and inframolecular dynamics of iron porphyrins

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Abstract. The temperature dependent Mössbauer spectroscopy and EXAFS analysis of the dynamical properties of selected iron-porphyrin derivatives: FeTPPCl and FePPIXCl has been presented. It has been shown that these iron--porphyrin properties may be modified by the outer ligands, but they are also strongly influenced by the intermolecular interactions, which are reduced in frozen solutions of the studied complexes.

Key words: EXAFS • Fe-porphyrins • molecular interactions • Mössbauer spectroscopy

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Introduction

The Mössbauer spectroscopy offers a unique opportunity for the investigation of the local properties of iron ions which often, like for instance in iron porphyrins, are the reaction centers as well as the charge and energy transport centers which is the essence of all life processes [2, 4]. In particular, the convolution of the dynamical and electronic properties of those biologically active molecules focuses attention of researchers. There have been already performed extension studies on both the solid state and solution studies on the electronic properties of iron porphyrins, what has been summarized in [5, 9, 10]. Silver *et al*. have presented studies in the aqueous solution of the iron-porphyrin complexes [10]. However, in this paper we attempt to gain some new information on two problems. First one, how variation in the outer ligands of the heme ring in iron-porphyrins influences the strength of the iron. Secondly, how the porphyrin-porphyrin interactions may block the flexibility of the entire molecule. For this purpose, electronic and molecular properties of two selected types of iron porphyrins in a powder form as well as in DMF solutions have been examined and compared.

Materials and methods

Two types of the iron-porphyrin compounds have been selected for the studies: ferriprotoporphyrin IX chloride (FePPIXCl) and *meso*-tetraphenyloporphyrin iron(III) chloride, (FeTPPCl) purchased by Alfa Aesar. From the structural point of view, the first compound FePPIXCl is the native porphyrin sub-unit of the haemoglobin, which
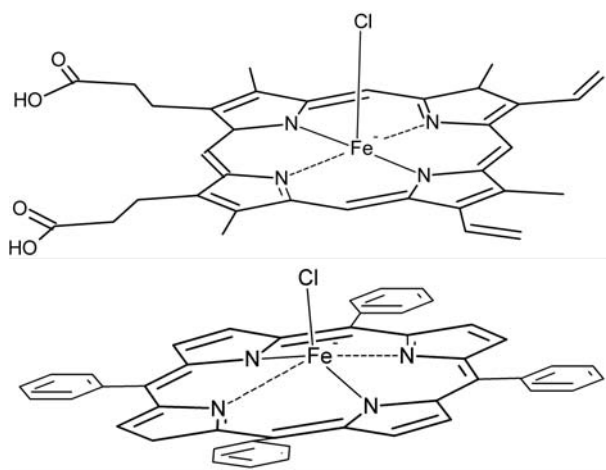
Fig. 1. Molecular structure of the studied compounds: FePPIXCl (top), FeTPPCl (bottom).

for the experimental research is often modelled by the less chemically reactive FeTPPCl. Both complexes consist of the macro-cyclic ring, where four phenyl groups are connected through the methane bridges. The structural difference appears out of the ring, where in case of FePPIXCl four methyl, two propionic and two vinyl chains are positioned and in case of FeTPPCl four phenyl rings. Molecular structures of the studied compounds are presented in Fig. 1. In both cases iron Fe(III) is nested in the centre of the ring, however due to dimensional misfit it is pushed out of the plane. In case of the heme group in haemoglobin, the iron ion is divalent.

The Mössbauer spectra of the powder samples were recorded in the transmission mode between 25 K and 306 K. The thickness of the powder samples with natural abundance of Fe has been optimized to 203 mg/cm^2, according to the Long procedure [3]. A bath cryostat, with a 25 mCi ^{57}Co(Rh) source at room temperature has been used.

Next, both samples were dissolved in DMF (*N,N*--dimethylformamide) (Sigma-Aldrich), using 40 mg of the powder sample per 2 ml of the solvent. The saturated solutions were inserted into a tight container, transparent for 14.4 keV radiation and resistant against low temperature and placed into a liquid nitrogen bath cryostat (Wissel), as in the case of the powder samples. The spectra were recorded at temperatures 80 K and 180 K (melting point of DMF is 212 K), then, the temperature of the solution was set up to 250 K for 24 h and further the spectra at 80 K and 130 K temperatures were taken again. Due to the solvent properties, the resonance effect was less than 1%. However, the compact geometry of the cryostat and the fats electronic enabled to reach the count rate of 15 000 counts/s in the Mössbauer line for the 25 mCi source. The measurement was typically performed for 48 h with the final statistics of 10^7 counts, with a relative statistical error of 3*10^{-4}, 10 times smaller than the observed resonance effect. No resonant absorption signal at 250 K after 48 h measurement proves that there was no detectable iron pollution in the counter and/or the cryostat windows. Data have been numerically analyzed with the use of the MOSMOD program [7].

The XAFS data were collected at the beamline A1 at Hasylab-DESY, operated by the synchrotron radiation storage ring DORIS III. In the studied case, the double crystal Si(111) monochromator, covering the energy range (2.4) 5–18.5 keV was used to tune the synchrotron energy. Energy resolution was 0.5 eV. The measurements were performed at selected temperatures out of the (5–300) K range. A PIPS-detector http://hasylab.desy.de/e70/e207/e674/e6092/e30091/detectors/pips_detector/index_eng.html and a He-OXFORD cryostat were applied. Porphyrin samples were examined in a powder form (prepared in the cellulose pellets). For data analysis, the ATHENA program have been used [8].

Results

The mean square displacement of the resonance nucleus $<x^2>$ may be approximated by a sum of two components: $<x^2>_i$ arising from internal vibrations of the Mössbauer probe and $<x^2>_m$ from the motion of the entire molecule. If these movements are not correlated, a case where $<x^2> = <x^2>_i + <x^2>_m$ should be considered. The $<x^2>_i$ factor is related to the strength of the chemical bond of the Fe atom inside the molecule, while $<x^2>_m$ carries information of the intermolecular interactions. From the temperature dependence of the intensity of the Mössbauer absorption line, only the factor of the total $<x^2>$ may be determined; the separation of its components is a difficult task. An attempt of elucidation of this problem is based on the concept that the comparison of the temperature dependence of the $<x^2>$ for the powders of FePPIXCl and FeTPPCl is related to the influence of the outer ligands on the Fe ion dynamics. The influence of the intermolecular interaction on the dynamics of the entire molecule may be concluded from the comparison of the $<x^2>$ in powder and in frozen solution for each specimen separately.

The temperature dependence of the $<x^2>$ factor for FePPIXCl and FeTPPCl is shown in Fig. 2. It has been found that for the powder samples replacing the outer chains in Fe-PPIX-Cl by the phenyl rings in FeTPPCl increases the Debye temperature (estimated for the temperature range: 80–220 K), from Θ_D (FePPIXCl) = 150(2) K up to Θ_D (FeTPPCl) = 164(2) K, respectively. Above 220 K, a specific anharmonicity causes the Debye

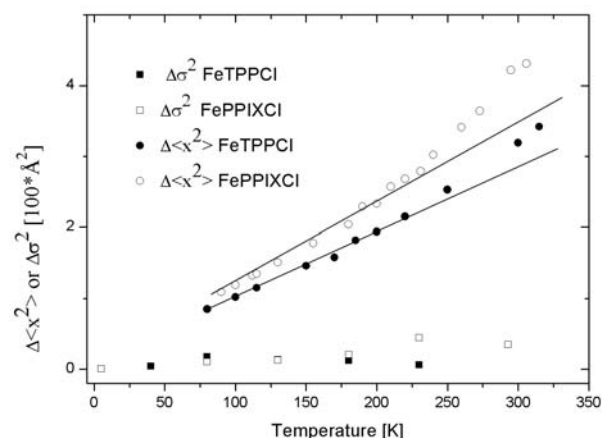**Fig. 2.** Temperature dependence of the mean square displacement of iron atoms $\Delta<x^2>$ (circles) compared with the mean square distance $\Delta<\sigma>^2$ (squares) between Fe and N for powder forms of FePPIXCl (full symbols) and FeTPPCl (open symbols).
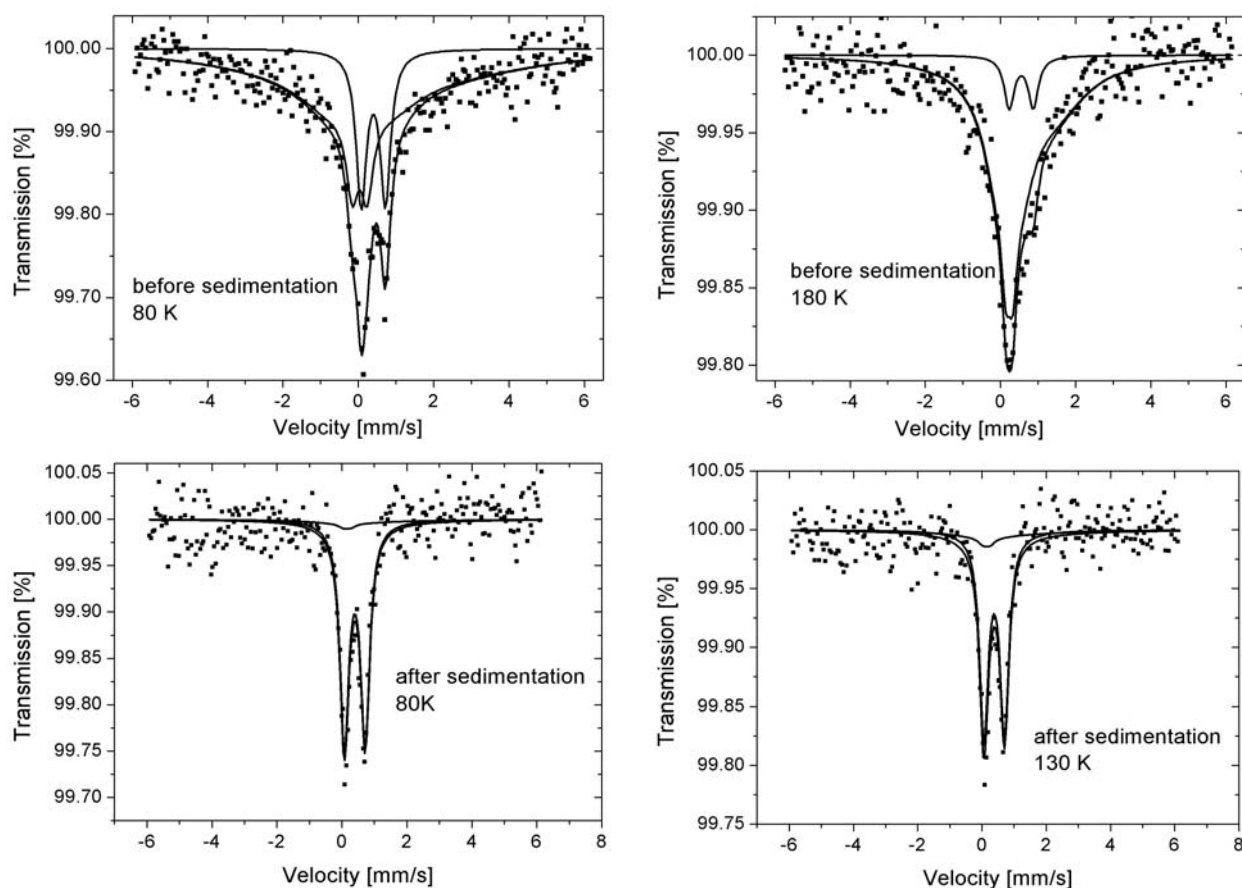
Fig. 3. Mössbauer spectra of the FeTPPCl solution in DMF measured at 80 K (upper left) and 180 K (upper right). The spectra measured after sedimentation are shown in the second row. Fitted subspectra are assigned to isolated molecules (symmetric doublet), aggregates (relaxation pattern).

model may not be applied. That anharmonicity effect is stronger for the FePPIXCl compound where iron is more weakly bounded than in FeTPPCl. Assuming that in a powder form, the intermolecular interactions for both samples are similar one may conclude that, surprisingly, the remote ligands influence the dynamics of the iron located in the centre of the porphyrin ring.

Quite interesting results has been obtained for frozen solutions of the studied porphyrins. The spectrum of FeTPPCl recorded at 80 K of the quenched sample is shown in Fig. 3 (upper left). The spectrum re-measured after warming up the sample up to 250 K, well above the melting point of DMF, is shown in Fig. 3 (bottom left). Interpretation of that result is straightforward. In the cold solution the sedimentation of the porphyrin aggregates took place, leaving later in the frozen solution (exposed to the 14.4 keV beam) mainly isolated molecules. Thus, the spectrum may be decomposed into two components: the symmetric quadrupole doublet assigned to the paramagnetic isolated molecules and magnetic relaxation pattern typical for aggregates of the porphyrins in the powder samples. A comparison of the relative areas of these two fractions has made it possible an estimation of the influence of the intermolecular interaction on the total $<x^2>$. The difference in the recoil free fractions for the isolated molecules and aggregates is evident from the visual inspection of the spectra measured at 80 K and 180 K (before sedimentation), see Fig. 3. A decrease of the intensity of these two fractions with increasing temperature leads to the

Θ_D = 87(2) K for the isolated molecules and Θ_D = 133(2) K for aggregates, still lower than for aggregates in the powder sample, where Θ_D = 164(2) K. Almost a double increase of the mean square displacement of the iron when compare the isolated and aggregated molecules in the solution has been observed. This may suggest some influence of the solvent on the porphyrin bonds.

For the FePPIXCl compound the same experiment was performed, nevertheless the effect of sedimentation was not that strongly observable as in the previous case. The reason may be better solubility of FePPIXCl in such hydrophilic solvent as DMF in contrast to FeTPPCl which possesses hydrophobic phenyl groups. Due to the lack of sedimentation, it might be assumed that the concentrated solution consists mostly of very tiny aggregates of the porphyrin molecules and interpretation of the data is not straightforward as in the previous case. Nevertheless, the same tendency, namely the lower Debye temperature for the isolated molecules then for the aggregates has been observed, see Fig. 4.

Additionally, local dynamics of the Fe atoms has been examined by EXAFS technique which delivers a value of the standard deviation, σ^2, of the distances R between the central ion (in our case Fe) and the neighbouring atoms (here N atoms) [6].

Including all of the factors that may influence the EXAFS oscillations, a final and detailed version of the EXAFS equation, for small variation in a distance within a shell, is given by:
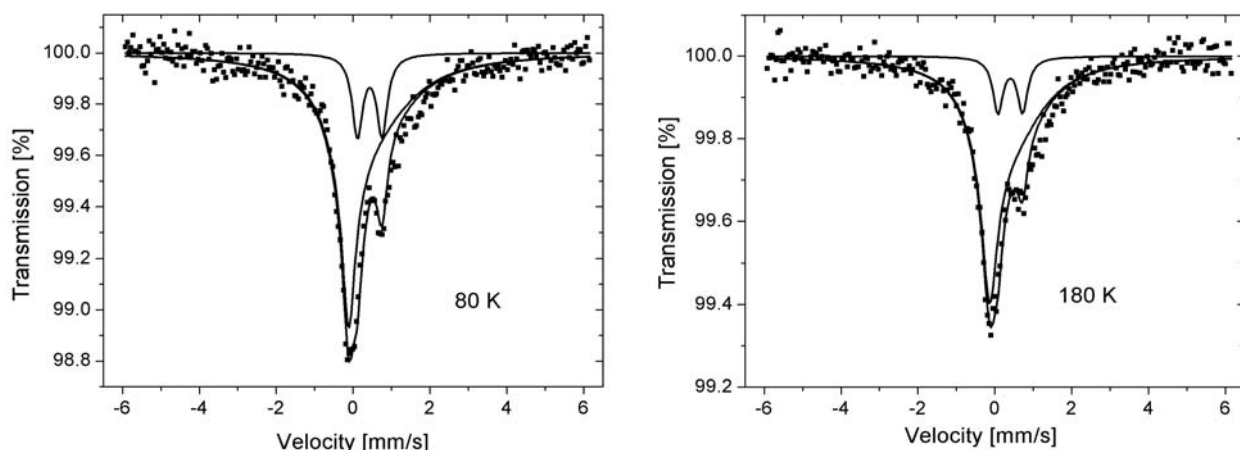
Fig. 4. Mössbauer spectra of the FePPIXCl solution in DMF measured at 80 K and 180 K. Fitted subspectra are assigned to the isolated molecules (symmetric doublet) and aggregates (relaxation pattern).

$$\chi(k) = \sum_j \frac{N_j e^{-2k^2\sigma_j^2} e^{-2R_j/\lambda(k)} f_j(k)}{kR_j^2} \sin[2kR_j + \delta_j(k)]$$

where j represents the individual coordination shell of identical atoms at approximately the same distance from the central atom, and N_j is the number of atoms in the coordination shell. Studying molecular dynamics of the porphyrin complex, we are focusing on the damping factor $\exp(-2k^2\cdot\sigma^2)$ which arises from an assumption of a small disorder in the absorber-scatter distances, where $\sigma^2 = \sigma^2_D + \sigma^2_T$ is the mean-square displacement in the bond distance R and k is the photoelectron wave vector $\sqrt{2m_e(E-E_0)/\hbar^2}$. It is an allowance for the back-scattered wave destructive interference due to the thermal motions of atoms (σ^2_T), but also includes contributions from what is termed 'static disorder' (σ^2_D), which refers to variations in the individual bonding distances that do not result in separable EXAFS frequencies. The σ^2 factor is commonly referred to as a Debye-Waller factor, but it should not be confused with the factor of the same name used in crystallography (referring to the mean-square motion of an atom about its mean position). Because (σ^2_D) is temperature independent, the temperature variation of σ^2, $\Delta\sigma^2 = \Delta\sigma^2_T(T)$, carries information of the thermal motion.

In case of the FePPIXCl powder compound $\Delta\sigma^2$ is much less than $\Delta<x^2>$ which points out the collective vibration of Fe and its N ligands [1]. The same behavior has been observed for FeTPPCl compound (Fig. 1). However, in case of the $\Delta\sigma^2$ factor compared between FePPIXCl and FeTPPCl no statistically different result has been achieved, what suggests that the outer ligands do not strongly influence the collective motion of iron and its closest neighbours.

Discussion

The presented results lead to two quite unexpected conclusions.
(i) The distant outer ligands influence the force constants between the central iron atom and its four nitrogen neighbours. In particular, replacing of the hydro carbonate chains by phenyl rings stiffens the molecule.

However, it might occur, that distant outer ligands have an impact on the bonding between the molecules in the powder form, finally influencing the residual $<x^2>_m$ contribution to the entire $<x^2>$ factor.
(ii) The intermolecular interaction in the solid state of the powder sample, blocks, at least in the case of FeTPPCl the vibration of the entire molecule, which becomes much more flexible in an isolated form in solid solution.

These results point out that the Mössbauer spectroscopy studies, which are practically restricted to the solid state specimen, should be taken with some care. Certainly, the data concerning the local dynamic state of the probe atom are valuable and reliable. However, the description of the conformational processes, gain from the study of the powder samples, may be misleading.

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