EPR SPECTROSCOPY OF IRON AZAPORPHYRINS

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EPR study of electronic structure of iron porphyrin complexes containing Fe(III) and Fe(I) ions are considered in this paper. It was found that the increase of a number of nitrogen atoms at *meso* positions of Fe(III)-azaporphyrins changes the character of the quantum-mechanically mixed spin state of Fe(III) ions (S = 5/2,3/2) by the increase of the intermediate-spin (S=3/2) contribution. Electron configuration of Fe(I)-porphyrins corresponds to the low-spin state (S=1/2) and remains unchanged during the *aza*-substitution and interaction with THF solvent.

INTRODUCTION

Metalloporphyrins, in particular iron complexes, perform important functions in many biological processes. Iron porphyrins participate in the biological cycles as the heme prosthetic groups such hemoproteins as hemoglobin and myoglobin as well as some cytochromes, peroxidases and catalases (Kadish, Smith & Guilard, 2003). Iron ions, placed at the centre of the prosthetic groups, play a key role in biological processes which involve hemoproteins. Sufficient background information on the relationship between a porphyrin ligand structure and an electronic configuration of the iron ions is provided to form a basis for discussion on the complex bioprocesses. Apart from the biological aspects, the molecular structure and physical properties of iron porphyrins promote these complexes for application in such contemporary technologies as non-linear optics, molecular semiconductors, liquid crystals and so on (Kadish, Smith & Guilard, 2000).

It is well known that electron configuration of Fe ion, which is the main reaction center of the iron porphyrins, is sensitive to molecular structure of the porphyrin and axial ligands. In this paper we report the results obtained from EPR spectroscopy for Fe(III)-porphyrins (on the basis of a Fe(III)-octaethylporphyrin {Fe(III)(OEP)Cl} complex) and their *aza*-substituted analogues such as Fe(III)Cl-monoazaetioporphyrin {Fe(III)(MAEP)Cl} Fe(III)(Cl)-diazaoctaethyl-porphyrin {Fe(III)(DAOEP)Cl} and Fe(III)(Cl)-teraaza-octaethylporphyrin {Fe(III(TAOEP)Cl} complexes. The reduced forms of the above complexes, containing a Fe(I) ion at the centre of the porphyrin ring instead of Fe(III) ion, are also presented. The *aza*-substitution is one of the modifications of the porphyrin macrocycle in which *CH* methine bridges are replaced with nitrogen atoms. Chemical properties of azaporphyrins and their X-ray, NMR and photoelectron spectroscopies are reviewed by Stuzhin and Khelevina (Stuzhin & Khelevina, 1996). The nearest surrounding of the Fe(III) as well as Fe(I) ions is the same in the complexes studied in this paper and the effect of *meso*-nitrogen atoms on their electron configuration is achieved through the delocalized π-electrons interaction with *d*-orbitals of the iron. Interaction between iron porphyrins and structurally smaller molecules like tetrahydrofuran (THF), which is one of the solvents commonly used in spectroscopic study of porphyrins and metallopor-phyrins is also discussed. It has been observed that THF is not an inert solvent in the case of Fe(I) porphyrins.

EXPERIMENTAL DETAILS

The Fe(III)(OEP)Cl complex was synthesized in the same way as it was described by Dolphin (Dolphin *et al*., 1976). Fe(MAEP)Cl and Fe(DAOEP)Cl were synthesized according to the literature procedures (Adler *et al*., 1970; Engel, Gossauer & Johnson 1978). Molecular structure of the above complexes is shown Fig.1. The reduced complexes were prepared chemically by contact of THF or dimethoxyethane (DME) solution with a sodium mirror. THF and DME solvents were carefully degassed by freeze-thow cycles. Stop wise reduction was controlled by means of electronic absorption spectra. The reduction products are air sensitive and the reaction was carried out on a vacuum line. A special cuvette which contained a vessel, where

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reduction reaction took place, a cell for absorption and a quartz tube for the EPR spectra, allowed to carry out the reaction and spectroscopic measurements in anaerobic conditions. The EPR measurements were performed at liquid-nitrogen temperature using a conventional X-band spectrometer, equipped with an IBM PC data acquisition system. The EPR parameters such as diagonal values of the effective **g** tensor and Gaussian or Lorentzian linewidths were found by fitting the experimental
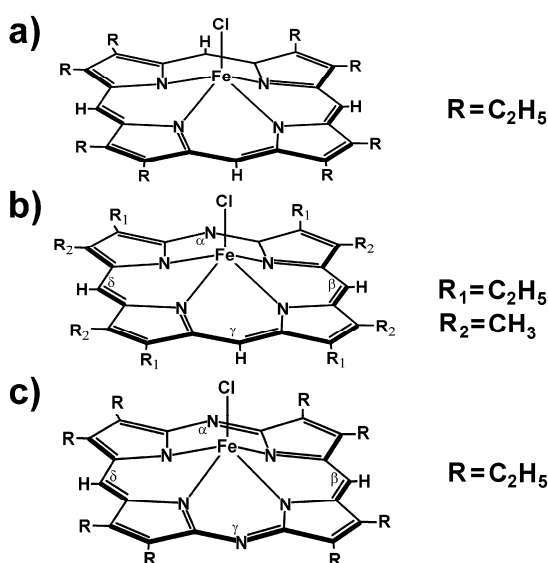

Fig.1. Molecular structure of Fe(III)(OEP)Cl (a), Fe(III)(MAEP)Cl (b) and Fe(III)(DAOEP)Cl (c).

spectra to the theoretical ones using effective spin Hamiltonian (Pilbrow, 1990) and the least-squares procedure on the basis of the simplex and Marquardt optimization methods. Microelemental analysis for carbon, hydrogen and nitrogen of the complexes studied in this paper was performed at the Laboratory of Microanalysis, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, in Łódź. It was obtained the following results: for {Fe(III)(OEP)Cl} ($C_{36}H_{44}N_4FeCl$) (Found: C, 68.92; H, 7.01; N, 9.15%; Calculated: C, 69.29; H, 7.11; N, 8.98%), for {Fe(III)(MAEP)Cl} ($C_{31}H_{35}N_5FeCl$) (Found: C, 66.01; H, 6.60; N, 12.43%; Calculated: C, 65.44; H, 6.20; N, 12.31%), and for {Fe(III)(DAOEP)Cl} ($C_{34}H_{42}N_6FeCl$) (Found: C, 65.58; H, 6.90; N, 14.97%; Calculated: C, 65.23; H, 6.76; N, 13.42%). In the case of the Fe(III)(DAOEP)Cl complex the nitrogen content in this molecule, obtained from the elemental analysis, exceeds significantly the calculated value. An analysis of the procedure of synthesis and the above result of the microanalysis indicate that a dimethyamine radical $(CH_3)_2N^{\bullet}$ could be bonded with

this complex. This radical could appear in the solution as a result of the breakdown of some molecules of dimethylformamide solvent (DMF) which was used at the final stage of the synthesis. The DMF solvent is not stable in the presence of strong bases, strong acids or light and is split into formic acid and dimethylamine, especially at elevated temperature (Weissermel, 2003).

RESULTS AND DISCUSSION

Azaporphyrins may be considered to be structural intermediates between relatively well-studied porphyrins and their structural analogues - phthalocyanines. The azaporphyrins provide excellent examples to explore the subtle effects of ligand structure on the electron configuration of coordinated iron ions in porphyrin complexes. Much can be learned from a comparison of the azaporphyrin spectroscopic properties with those characteristic for their unsubstituted analogue.

Let us consider Fe(III)-complexes at first. The electron configuration of Fe(III) ion in chloroiron porphyrins with unsubstituted methine *CH* bridges corresponds to the pure high spin state ($S=5/2$) (Pamler , 1983; Lexa, Momenteau & Mispelter, 1974) while in the case of chloroiron tetraazaporphyrin (four methine bridges substituted by nitrogen atoms) the pure intermediate spin state ($S=3/2$) of the Fe(III) ion has been found (Fitzgerald *et al*., 1992). It is interesting to know how the electron configuration of Fe-ions changes during a successive substitution of the methine bridges by nitrogen atoms.

A typical feature of the EPR spectrum of Fe(III) ions in axial symmetry is an intensive low-field component close to $g_{\perp} = 6$ and a less prominent absorption derivative near $g_{//} = 2$ (Fig. 2a). The difference in the intensity of the $g_{\perp}$ and $g_{//}$ components is due to a larger number of molecules with symmetry axes nearly perpendicular to the applied magnetic-field direction ($g_{\perp}$), that is, the molecules with axes approximately in the equatorial plane about the field direction, in comparison with a very few molecules with the symmetry axis aligned close to the single field direction ($g_{//}$). An EPR spectrum of the Fe(III)(MAEP)Cl complex (Fig. 2b) is similar in shape to the spectrum characteristic for high-spin state of the Fe(III)(OEP)Cl one. Numerical analysis of the EPR spectra has shown some splitting of the intensive component with $g_{\perp}$-factors deviated from the value equal to 6.00 and equal to $g_x = 5.93$, $g_y = 5.86$ for the Fe(III)(MAEP)Cl complex and $g_x = 5.93$, $g_y = 5.76$ for the Fe(III)(DAOEP)Cl one. The splitting of the $g_{\perp}$ component observed for Fe(III)(MAEP)Cl and Fe(III)(DAOEP)Cl complexes indicates slight distortion of axial symmetry in the nearest surrounding of iron(III) ions caused by substitution of one *CH* group and two *CH* groups (at α and γ positions), respectively, by
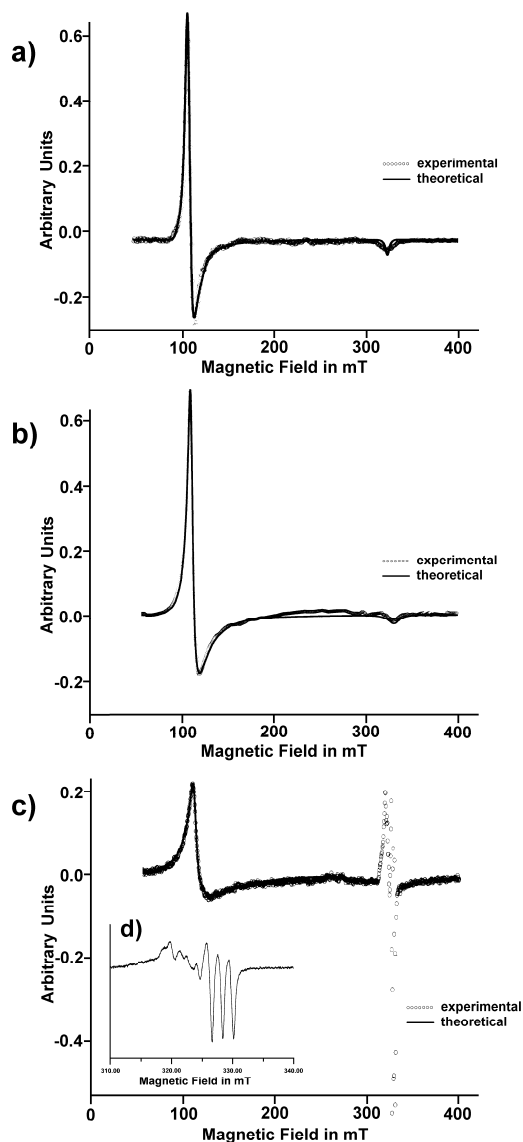
nitrogen atoms. The distortion

Fig.2. EPR spectra of Fe(III)(OEP)Cl (a), Fe(III)(MAEP)Cl (b), Fe(III)(DAOEP)Cl (c), hyperfine structure in the spectrum of Fe(III)(DAOEP)Cl (d) in frozen DME solution at 80 K.

of axial symmetry estimated by the *E/D* ratio on the basis of the simple expression $g_x - g_y = 48E/D$ (Pamler, 1983) was found to be marginal and equal to 0.0015 for the Fe(III)(MAEP)Cl complex and 0,0035 for the Fe(III)(DAOEP)Cl (maximum *E/D* value equal to 0.33 corresponds to 'pure' rhombic symmetry).

It was found (Dzilinski *et al.*, 2001; Dzilinski *et al.*, 2003) that the increase of a number of nitrogen atoms at *meso*-positions leads to the increase of the intermediate S=3/2 contribution to the quantum-mechanically mixed

spin states (S=5/2,3/2) of Fe(III) ions. This is reflected in the EPR spectra by the shift of the $g_\perp$ values from $g_\perp = 6.0$, characteristic for the pure high-spin state (S=5/2), towards the value $g_\perp \cong 4$, characteristic for the pure intermediate (S=3/2) spin state. A contribution of that intermediate spin was estimated on the basis of the Maltempo model (Maltempo & Moss,1976). According to this model the wave functions of the two states ([6]A and [4]A) are mixed by spin-orbit coupling and the resultant ground state has the form: $|\Psi\rangle = a|^6A\rangle + b|^4A\rangle$, with $a^2+b^2=1$ and a/b decreasing with increase of the intermediate-spin component. The $g_\perp$ value for the quantum-mechanically mixed state is expressed by $g_\perp = 6a^2+4b^2$. Calculation of the a and b parameters gives the contribution of the intermediate components to the ground states equal to about 5% for Fe(III)(MAEP)Cl and 8% for Fe(III)(DAOEP)Cl. Comparing the $g_\perp$ value for Fe(III)(OEP)Cl with those for Fe(III)(MAEP)Cl, Fe(III)(DAOEP)Cl and Fe(III)(TAOEP)Cl one can conclude that there is no quantitative relationship between mixed spin-state character estimated on the basis of the $g_\perp$ values and a number of methine *CH* groups replaced by nitrogen atoms. In general, one can say that the change of Fe(III) ions electron configuration is connected with the Fe-N_p bond lengths. If these bonds become shorter the contribution of the intermediate spin component (S = 3/2) to the quantum mechanically mixed spin state is increasing (Nakamura M. et al., 2002). The C-N bonds in *meso*-positions of the *aza*-substituted porphyrin ring are shorter in comparison with the C-C bonds of the unsubstituted porphyrin. The shorter bonds of the bridges the shorter Fe-N_p bonds at the centre of the complex. So, the increasing number of the methine *CH* bridges substituted by nitrogen atoms leads to increasing contribution of the intermediate spin to the ground state of the Fe(III)-porphyrins.

It should be noted that an additional hyperfine structure in the form of three intensive lines and a complex hyperfine structure in the form of many low-intensity lines (Fig. 1d) were observed in EPR spectrum of Fe(III)(DAOEP)Cl (Fig. 1c) instead of the single weak-intensity line near $g_{//} =2$ in EPR spectrum of Fe(III)(MAEP)Cl (Fig. 1b). On the basis of the elemental analysis of the complex expected as Fe(III)(DAOEP)Cl one can suppose that the three intensive lines (Fig. 1d) belong to the nitrogen atom (hyperfine splitting constant A_{iso}=1,758 mT) of the dimethylamine radical $(CH_3)_2N^{\bullet}$ attached to the complex. The A_{iso} value suggests that an unpaired electron is localized on the nitrogen atom. These lines are detectable at room temperature in contrary to the hyperfine lines of the relatively low intensity which appear in the EPR spectrum at about 220 K. The low-intensity hyperfine structure can be detected to the moment when the sample is frozen. When the sample is thaw and next again frozen the relative intensity of the

lines was significantly changed while the intensity of the three former intensive lines remains the same. It suggests that the hyperfine structure with the three intensive lines can be assigned to one paramagnetic centre while the low-intensity lines have to be attributed to many centres. The changeable form of this part of the EPR spectrum indicates that the hyperfine-interaction lines correspond to some statistically average orientation of CH_3 groups of the $(CH_3)_2N^\bullet$ radical with respect to d-orbitals of the Fe(III) ion. We suppose that the observed hyperfine structure is a result of the overlapping of a great number of spectra with somewhat different hyperfine splittings from local orientations of CH_3 groups in relation to d_{xz} and d_{yz} orbitals of the Fe(III) ion containing unpaired electrons. In such a case numerical simulation of this hyperfine structure is rather impossible. Our quantum chemical calculations of the Fe(III)(DAOEP)Cl molecular structure, performed on the basis of the Density Functional Theory (DFT), using the Amsterdam Density Functional (ADF) computer programme (te Velde, 1992), indicate the ruffled shape of the porphyrin ring and orientation of the $(CH_3)_2N^\bullet$ group, bonded to one of the two N atoms in *meso*-positions, nearly perpendicular to the porphyrin ring. In our opinion, such orientation of the $(CH_3)_2N^\bullet$ radicals makes possible the overlapping of the CH_3 groups protons with d_π-orbitals of iron ion which can lead to the observed hyperfine structure. This hyperfine structure appears in the frozen solution when the rotation of the CH_3 groups around the symmetry axis of the radical and protons inside the CH_3 groups is stopped. The binding of a $CH_2Cl_2N_2$ group to a nitrogen atom in *meso*-positions of Fe(III)Cl-monoazaoctaethylporphyrin complexes {Fe(III)(MAOEP)Cl} was observed and discussed by Balch at al. (Balch et al., 1993).

Let us consider now the Fe(I)-porphyrins. At the first stage of the reduction process divalent Fe(II)-porphyrins are generated, which can be diamagnetic or paramagnetic with integer spin system. Because of large magnitude of the resulting zero-field splitting in the integer-spin Fe(II) ions, there are no allowed EPR transitions in the field range and microwave frequency available to conventional spectrometers and Fe(II) complexes will not be considered in this paper. It should be noted that gamma irradiation of frozen solutions of Fe(II) hemoproteins at 77 K leads to reduced or oxidized products which show EPR spectra (Davydov R., 2008). These spectra exhibit features characteristic of Fe(I) species with S=3/2 spin state produced by antiferromagnetic coupling between a Fe(II)-complex (S=2) and porphyrin anion radical (S=1/2), as well as of Fe(III)-complex with quantum mechanically mixed (S=3/2, 5/2) ground state generated by irradiation of the frozen six-coordinate Fe(II)-complexes. The mentioned above products are stable only at low temperature. The chemical reduction of Fe(II)- complexes leads to Fe(I)-

porphyrins, stable at wide range of temperature. The spectrum of Fe(I)-porphyrin EPR exhibits characteristic relatively intensive $g_{//}$ component (Fig. 3). The EPR spectra of the Fe(I)-azaporphyrins are similar in shape and parameters to the spectrum of the unsubsituted Fe(I)(OEP) (Table 1). It means that the electron configuration of Fe(I) ions coordinated to the mono- and diazaporphyrins correspond to the low-spin state (S=1/2) as it was established for unsubstituted Fe(I)-porphyrins (Teraoka *et al.*, 1987). In the low-spin Fe(I) systems the deviation of $g_\perp$ factor from 2.0 is dependent on the inverse of separation between ^2A ground state and the
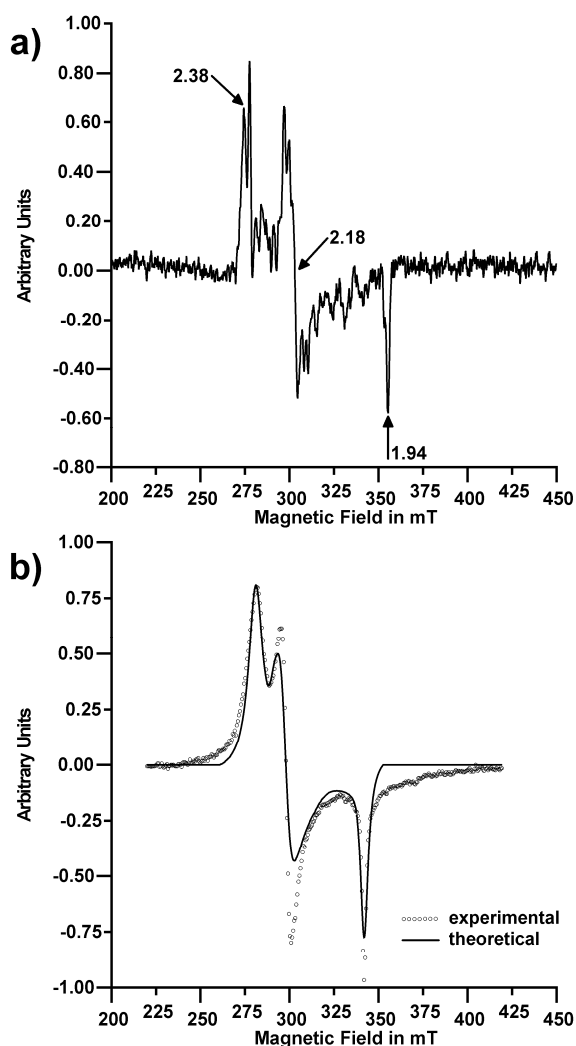

Fig.3. EPR spectra of Fe(I)(MAEP) in THF solution (a) and in DME solution (b) at 80 K.

Table 1. *g*-factors in EPR spectra of frozen Fe(I)-porphyrins at 80 K.

Molecule	Solvent	$g_\perp(g_x, g_y)$	$g_{||}(g_z)$
Fe(I)(OEP)	THF	2.26	1.93
Fe(I)(MAEP)	DME	2.35, 2.21	1.92
Fe(I)(DAOEP)	DME	2.40, 2.18	1.84

nearest ^{2}E excited state (Yamaguchi & Morishima, 1992). Comparing the $g_\perp$ values for unsubstituted Fe(I)(OEP) with the average value of g_x and g_y for the Fe(I)-azaporphyrins (Table 1) one can notice that this separation slightly decreases with an increasing number of nitrogens at *meso* positions. The EPR spectra of the Fe(I)-porphyrins in THF solution show a low-intensity hyperfine structure (Fig. 3a) similar to that observed in the case of the Fe(III)(DAOEP)Cl complex (Fig. 2c) but without the hyperfine structure from a nitrogen ion. We suggest that hyperfine structure in Fe(I)-porphyrin spectra can be connected with interaction of unpaired electrons localized on *d*-orbitals of Fe(I) ion with protons of one or two THF molecules, loosely bonded to the Fe(I)-complex through a THF oxygen atom. A hyperfine structure from the proximal histidine ligand bonded to Fe(I) hemes through a nitrogen atom was observed in EPR spectra (Davydov R., 2008). Pure form of the Fe(I) EPR spectrum (without this hyperfine structure), recorded for Fe(I)(MAEP) in DME solution (Fig. 3b), confirms the suggestion that the observed low-intensity hyperfine structure is derived from THF molecules. The A_{iso} constant cannot be determined because of unknown number of individual spectra which overlap one another giving resultant hyperfine structure with different shape after each freezing of the solvent. It should be noted that THF bonded to the Fe(I)-porphyrins does not change the electron configuration of the Fe(I) ion, in contrary to Fe(II) ions (Sinyakov & Shulga, 1993). The EPR signal exhibits noticeable rhombicity caused by distortion of axial symmetry in the *aza*-porphyrins.

CONCLUSIONS

In conclusion, it should be noted that Fe(III)-porphyrins are rather a unique example of organometallic complexes which show the quantum-mechanically mixed spin states. The quantum-mechanically mixed spin state of Fe(III) ions in the azaporphyrins changes with the increase of a number of nitrogen atoms at *meso* positions of the porphyrin ring. It suggests significant interaction between π-electrons of the porphyrin ligand

and *d*-orbital electrons of the iron ion. The electron configuration of Fe(I) ions in the unsubstituted porphyrins and the *aza*-substituted ones corresponds to the low-spin (S=1/2) state which is not sensitive to the interaction with THF molecules. The influence of the *aza*-substitution on the electron configuration of Fe(III) ions is more significant in comparison with the Fe(I) ones.

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