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EPR PROPERTIES OF FREE PORPHYRIN BASES

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Experiments have been carried out with free porphyrins bases belonging to two groups: (1) α, β, γ, δ *meso*-tetraphenylporphyrin (TPP) and (2) octaethylporphyrin (OEP). Group (1) included (TMPP) – *meso*-tetra(4-methyl)phenylporphyrin, (TDPP) – *meso*-tetra(4-deutero)phenylporphyrin, (TMXPP) – *meso*-tetra(methoxy)phenyl-porphyrin, (T4H3MXPP) – *meso*-tetra(4-hydroxy-3-methoxy)phenylporphyrin, (TPIPP) – *meso*-tetra(piperonyl) phenylporphyrin while Group (2) was represented by (OEP) – 2,3,7,8,12,13,17,18- octaethylporphyrin.

For synthesized compounds electron paramagnetic resonance (EPR) spectra have been measured. Linewidths ΔH, *g*-factors and EPR amplitudes (*A*) in arbitrary units were calculated. Deuteration of phenyl groups in *para* position of TDPP increases *g*-factor to 2.0028 but linewidth change is negligible. *g*-Factors all other porphyrins are in the range of 2.0024–2.0026. Linewidths of EPR spectra are between 0.39 mT and 0.67 mT. This indicates that unpaired electron is delocalized over more than one porphyrin ring. Partial deuteration (TDPP) of the porphyrin does not change the linewidth of the spectrum because deuterons are not localized close to the porphyrin ring. Methoxy and hydroxy peripheral substituents in porphyrins increase the intensity of EPR signal by one to two orders of magnitude in comparison to methyl, ethyl and piperonyl substituents.

INTRODUCTION

It is generally accepted that photodynamic therapy represents a promising approach to cancer treatment. The technique involves the systemic administration of a photosensitizer displaying a preferential affinity for tumors and absorbing light wavelengths in the 600 nm region (Wilson & Jeeves, 1987). Such wavelengths are not absorbed by the endogenous chromophores of animal tissues and are endowed with a maximal penetration power into most biological tissues. After a suitable time interval following administration of the drug, the neoplastic area is illuminated with light specifically activating the photosensitizer, thus inducing selective damage of the tumor. Most clinical photodynamic therapy protocols involve the use of a chemical derivative of porphyrin (Kessel & Cheng, 1985). In spite of the favorable results obtained, especially in the treatment of superficial and early stage neoplasias, some important factors still limit the efficiency of photodynamic therapy. These are: partially unknown photophysical reactions, low extinction coefficient in red spectral region and the relatively poor selectivity of tumor targeting as compared with most peritumoral tissues or some normal tissues (e.g. skin). Desirable features of a photosensitizer to be used for therapeutical applications include: chemical purity, stability under physiological conditions, lack of cytotoxicity in the dark and superior spectroscopic properties as compared with porphyrin. The requirement for efficient light absorption in the 600 –900 nm range restricts the choice of photodynamic therapy agents to photosensitizers characterized by extended electronic delocalizations. This goal can be achieved by a rational design of the chemical structure of the photosensitizer, although one must keep in mind that the structural features can profoundly influence both the hydrophobic/hydrophilic properties of the dye (hence its affinity for tumor tissues and its distribution among the intratumoral compartments) and the degree of dye aggregation in a variety of media (hence photosensitizing activity) (Dougherty, 1987; Frąckowiak, Waszkowiak, Ion, Wiktorowicz, Cofta & Manikowski, 2000). The optimal compromise is being sought through the interplay of selected factors, including the expansion of the conjugated electron system, the presence and type of the metal ion coordinated with the porphyrin-type macrocycle (Manikowski, Łożyński & Kubaszewski, 1998; Manikowski, 1999), and the nature of the functional groups possibly replacing the peripheral hydrogen atoms.

MATERIALS AND METHODS

Porphyrins were synthesized and purified according the modified procedure described previously (Manikowski, Kubaszewski, Łożyński & Wróblewski, 1994). Experiments have been carried out with free base porphyrins in powder state belonging to two groups: (1) α, β, γ, δ *meso*-tetraphenylporphyrin (TPP) and (2) octaethylporphyrin (OEP).

Group (1) (TMPP) – *meso*-tetra(4-methyl)phenylporphyrin
(TDPP) – *meso*-tetra(4-deutero)phenylporhyrin
(TMXPP) – *meso*-tetra(methoxy)phenylporphyrin
(T4H3MXPP) – *meso*-tetra(4-hydroxy-3-methoxy)phenylporphyrin
(TPIPP) – *meso*-tetra(piperonyl)phenylporphyrin

Group (2) (OEP) – 2,3,7,8,12,13,17,18-octaethylporphyrin

Electron paramagnetic resonance (EPR) spectra were measured at room temperature using a Radiopan SE/X-2540 spectrometer with RCX 660 microwave cavity operating in TM_{110} mode with unloaded Q factor 7000. In all measurements modulation amplitude was 0.05 mT and microwave power attenuation 10 dB.

RESULTS AND DISCUSSION

The porphyrin macrocycle with 26 π-electrons is highly conjugated. At room temperature the porphyrin free base exhibits four-fold symmetry, indicating rapid exchanges of the inner protons among the four pyrrole nitrogens. The phenyl rings are considered to rotate in several porphyrins of group (1). If the rotation is restricted to some extent, the phenyl groups are noncoplanar with the plane of porphyrin ring. Hence, they participate negligibly in the porphyrin π-electron system. OEP has been used as a highly symmetric compound in the study of porphyrins. The g-values of π-radical systems are very close to that of the free-electron spin value, since in a delocalized system the orbital angular momenta are completely quenched. Consequently one observes EPR spectra of such radical systems in solutions even at room temperature and above. For synthesized compounds electron paramagnetic resonance (EPR) spectra have been measured. Linewidths ΔH, *g*-factors and EPR amplitudes (A) in arbitrary units are listed in Table 1.

Table 1. EPR parameters: linewidth ΔH, *g*-factors and EPR amplitudes A of measured porphyrins

Compound	ΔH [mT]	*g*	A
TMPP	0.39	2.0026	4
TDPP	0.51	2.0028	24
TMXPP	0.56	2.0026	76
T4H3MXPP	0.47	2.0025	200
TPIPP	0.49	2.0024	6
OEP	0.67	2.0025	3

Deuteration of phenyl groups in *para* position of TDPP increases *g*-factor to 2.0028 but the linewidth change is negligible. *g*-Factors all other porphyrins are in the range of 2.0024–2.0026. Linewidths of EPR spectra are between 0.39 mT and 0.67 mT. This indicates that unpaired electron is delocalized over more than one porphyrin ring. Partial deuteration (TDPP) of the porphyrin does not change the linewidth of the spectrum because deuterons are not localized close to the porphyrin ring. Methoxy and hydroxy peripheral substituents in porphyrins increase the intensity of EPR signal by one to two orders of magnitude in comparison to methyl, ethyl and piperonyl substituents.

The applications of EPR to biological systems containing natural or artificial porphyrin chromophore are mainly based on the following:

1. Identification of paramagnetic centers.
2. Distinctions between a free-radical type system and a transition metal ion, identification of more than one paramagnetic center and also aggregation (through *g*-values, linewidths and temperature dependence).
3. Identification of the electronic ground state (through hyperfine couplings in a free radical, ZFS in triplet states of π-systems and ZFS or the *g*- and the A-tensors for transition metal ions).
4. Characterization of the symmetry of the ligand field and the nature of the metal-ligand bond (through *g*-values, the A-tensor and the ZFS parameters).
5. Estimation of the concentration of the paramagnetic center (through integration of the signal).

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