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Structure of Porphyrin TPPS4 and Its Interaction with Metal Ions as Elucidated by 1H NMR and UV-Visible Spectra

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STRUCTURE OF PORPHYRIN $TPPS_4$ AND ITS INTERACTION WITH METAL IONS AS ELUCIDATED BY ^1H NMR AND UV-VISIBLE SPECTRA

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Running Title: $TPPS_4$ structure and interaction

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ABSTRACT

Porphyrins are a group of tetrapyrrole pigments. Physical and chemical properties of porphyrins are often related to their compositions and structures. We conducted ^1H solution NMR and UV-visible spectral analysis to characterize the structural feature of a water-soluble, synthetic porphyrin *i.e.* tetrakis (p-sulfonatophenyl) porphyrin, $TPPS_4$, and its interaction with different metal ions in aqueous solutions. The results indicate that tetrapyrrole and tetraphenyl rings in $TPPS_4$ molecule form a co-planar electron conjugation system; transition-metal ions show stronger binding capacity than alkali and alkali-earth metal ions; the relative stabilities of $TPPS_4$-metal ion complexes can be well assessed by NMR and UV-visible spectral data.

Key words: Porphyrin; NMR; $TPPS_4$

INTRODUCTION

Porphyrins and their derivatives are a large family of aromatic pigments. A porphyrin molecule consists of heterocyclic tetrapyrrole unit, called porphine, and meso-substituents. The complexes of porphine-metal ions exist as pivotal components in many native proteins such as chlorophyll and hemoglobin. For synthetic porphyrins, different meso-substituents can be incorporated into the tetrapyrrole unit, so that structures and properties of porphyrins can be significantly changed.

Different types of synthetic porphyrins have a broad range of applications in biological/biomedical field. For instance, some synthetic porphyrins were used as best catalysts for the bio-oxidation of certain drugs such as acetaminophen and ellipticine, so these porphyrins may have a great future in the study of in vivo drug oxidative metabolite pathways (1). The complexes of porphyrin–nuclease were used to investigate the DNA cleavage and to get

insight into its mechanism of action (2). More importantly, synthetic porphyrins can potentially serve as therapeutic drugs (called photosensitizers) for the photodynamic therapy of cancers (3-5), in which the uptake porphyrins are irradiated by light of certain wavelength; and the absorbed energy is transferred to oxygen, converting the regular triplet oxygen to singlet oxygen - an extremely reactive species that has the power to destroy the cells. Also, porphyrins can be used as contrast agents or tumor localizers in the magnetic resonance (MR) imaging (6-10).

In a variety of synthetic porphyrins, the water-soluble porphyrins are of particular interest. The higher aqueous solubility of a porphyrin is often desirable, and this can be achieved by preparing a porphyrin containing positively or negatively charged meso-groups (11-18). The water-soluble meso-tetrakis (*p*-sulfonatophenyl) porphyrin, $TPPS_4$, is an important member in this category. Because of its higher aqueous solubility and uniquely symmetric structure, $TPPS_4$ molecule has become an important target in many recent porphyrin studies (19-23). The water-soluble $TPPS_4$ is also found capable of binding to serum albumin, a rich transport protein in blood plasma, suggesting that $TPPS_4$ can be delivered in blood stream (14). In spite of these research developments, however, some fundamental issues regarding $TPPS_4$ structure and $TPPS_4$-metal ion interaction have not yet been clearly addressed.

To characterize structure of $TPPS_4$ in aqueous solutions, we synthesized $TPPS_4$ (see Fig. 1), and conducted ^1H NMR and UV-visible spectral analysis for $TPPS_4$ samples under varied pH or metal ion bindings. Our results revealed that tetrapyrrole and tetraphenyl rings in $TPPS_4$ maintain a co-planar structure to fulfill the p-π electron conjugation over the rings, and such configuration may further stabilize the entire molecule. This $TPPS_4$ structural characterization is of significance to the further investigation and elucidation of $TPPS_4$ interaction in biological systems, because structures (planar or non-planar) of porphyrins may strongly impact their interaction with other biomolecules. For instance, it has been suggested that when a planar porphyrin interacts with nucleic acid (DNA or RNA), the porphyrin ring is intercalated into the G-C base pair to form intercalating complex; in contrast, a non-planar porphyrin is simply bound onto the major/minor groove of nucleic acid (15). From the ^1H NMR and UV-visible spectral data, we also determined the relative strengths of $TPPS_4$ interaction with different metal ions, which can be used to assess the stabilities of these $TPPS_4$- metal complexes.

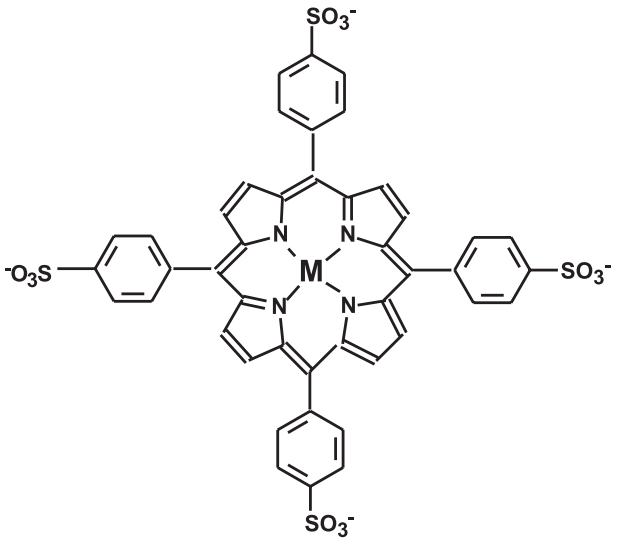

Figure 1. Metal ion (M)-bound tetra (*p*-sulfonatophenyl) porphyrin, $TPPS_4$.

MATERIALS AND METHODS

$TPPS_4$ synthesis: Analytical grade chemicals from Sigma-Aldrich were used without further purification. Following the preparation method established earlier (24), typically 0.1 mole pyrrole and 0.1 mole benzaldehyde were reacted for five hours in 50 ml of refluxing propionic acid. After cooling down, the product, meso-tetraphenyl porphyrin, was precipitated in saturated sodium acetate solution, and was washed with methanol-water solution and dried using an oven. For further purification, the crude porphyrin was dissolved in chloroform, and the solution was passed through alumina column, then the solvent was slowly evaporated. The *p*-sulfonation on phenyl rings was achieved by reacting 0.5 g tetraphenyl porphyrin with 15 ml fuming sulfuric acid (20% free SO_3) in a closed vessel, and it was kept in an oven (80°C) overnight. The product was neutralized with 4 M NaOH solution, and treated by Soxhlet extraction using methanol as solvent. Solid $TPPS_4$ was obtained after methanol evaporation, and the high purity of $TPPS_4$ was verified by its characteristic UV-visible spectrum, as shown in Fig. 2.

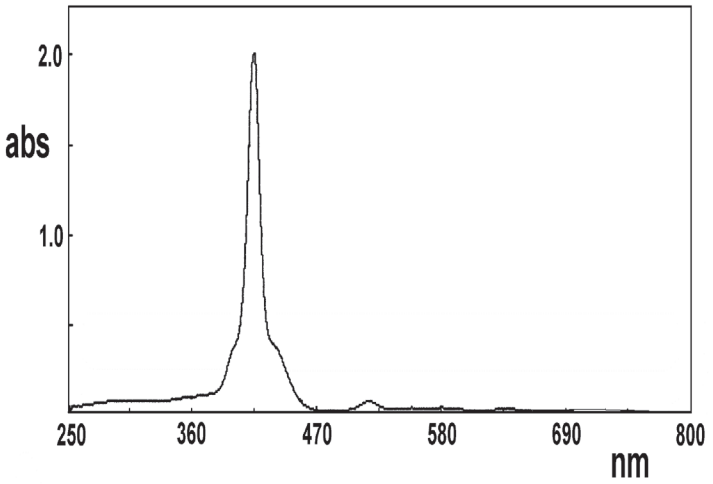

Figure 2. UV-visible absorbance of free $TPPS_4$.

NMR measurements: NMR samples were prepared by dissolving solid $TPPS_4$ in D_2O in absence or presence of metal chloride salt (KCl, $CaCl_2$, $NiCl_2$ or $CuCl_2$). $TPPS_4$ and salt concentrations were typically 0.1 M. After taking account of possible salt hydrolysis effect on sample pH, the final pH was adjusted in 6.1-10.3 range using NaOH and HCl (accurate to pH 0.1). No other pH buffer substances were used to avoid the interference of impurities. ^1H spectra were acquired on Varian mercury-200 spectrometer at room temperature, with 90° pulse-width of 14.5 μs. The chemical shift values were referenced to TMS.

UV-visible spectra: UV-visible absorbance (in 250-800 nm wavelength) were recorded on a Beckman DU-7500 spectrophotometer, using samples of free $TPPS_4$ and K^+-, Ca^{2+}-, Zn^{2+}-, Co^{2+}-, Mn^{3+}- or Fe^{2+}-bound $TPPS_4$ (1:1 molar ratio) at pH 7.0.

RESULTS

1. ^1H NMR spectra

$TPPS_4$ at neutral pH: Fig. 3 shows a representative ^1H spectrum of $TPPS_4$ acquired at pH 7.0. The two major peaks, peak **a** around 7.59 ppm and peak **b** around 6.54 ppm, were assigned to tetraphenyl-H and tetrapyrrole-H, respectively. The *p*-sulfonate groups and nitrogens in porphyrin core were deprotonated at pH 7.0, therefore no proton signals were detected for these sites.

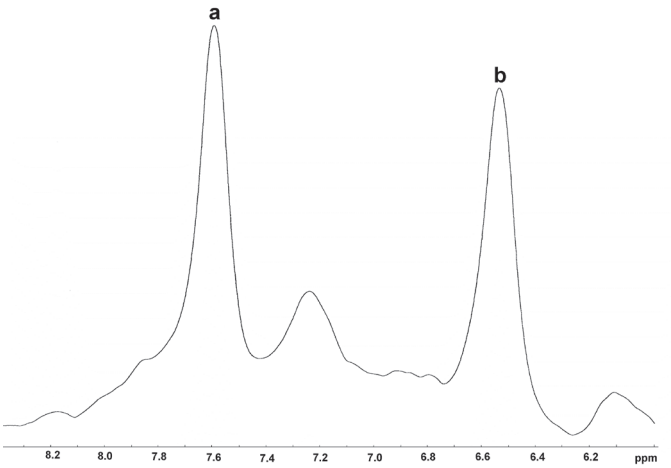

Figure 3. ^{1}H spectrum of $TPPS_4$ at pH 7.0, with peak **a** assigned to tetraphenyl-H and peak **b** assigned to tetrapyrrole-H.

Effects of pH variation: Because of low solubility of protonated $TPPS_4$ at low pH range, it was not possible to acquire solution NMR spectra at sample pH below 5.0. When pH was increased in pH ~ 6-10 range, however, we observed that both tetraphenyl-H and tetrapyrrole-H of $TPPS_4$ were somewhat down-field shifted, as shown in Fig. 4.

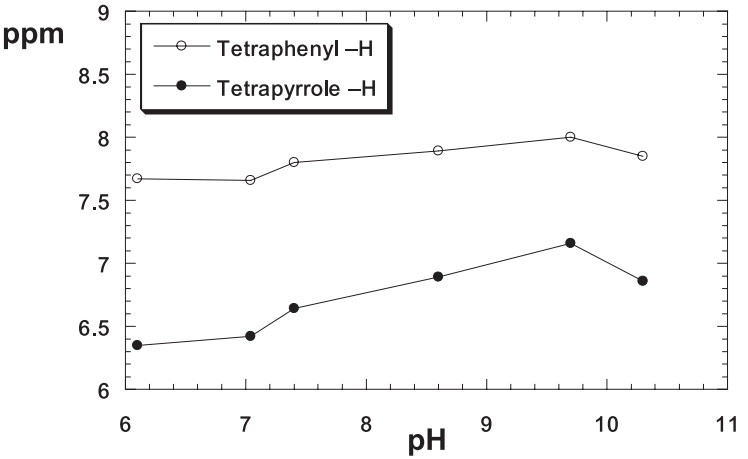

Figure 4. ^{1}H chemical shifts of $TPPS_4$ under varied pH.

Effects of metal ions: Effects of metal-ions on $TPPS_4$ are described in Fig. 5. From bottom up, the ^{1}H spectra were obtained for $TPPS_4$ samples

in absence or presence of K^+, Ca^{2+}, Ni^{2+}, Cu^{2+}, respectively. Relative to free $TPPS_4$, interaction of K^+ or Ca^{2+} with $TPPS_4$ induced about 0.10-0.30 ppm up-filed shifts, with slightly greater effect on tetraphenyl-H than on tetrapyrrole-H and greater effect of Ca^{2+} than K^+. In contrast, interaction of transition-metal ion Ni^{2+} with $TPPS_4$ caused about 0.10–0.50 ppm down-filed shifts, with greater effect on tetrapyrrole-H than on tetraphenyl-H; while interaction of Cu^{2+} with $TPPS_4$ resulted in a very broad, irresolvable peak.

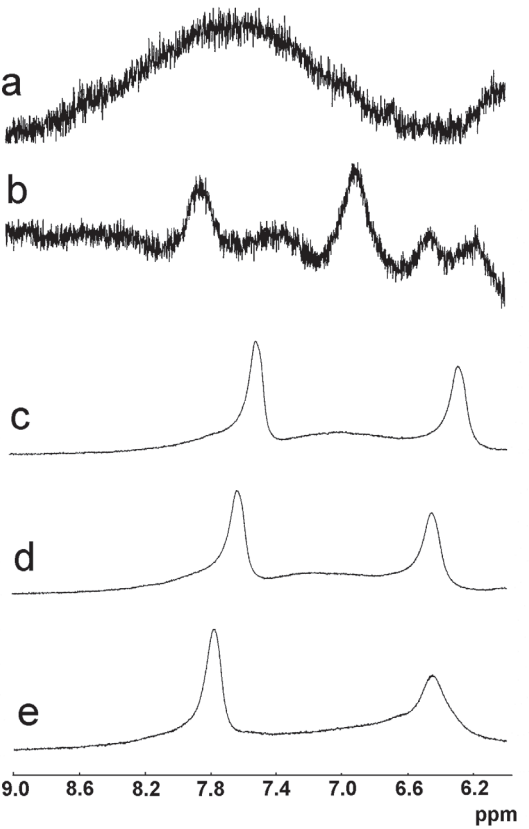

Figure 5. Effect of metal ions on ^1H spectra of $TPPS_4$. (a) Cu^{2+} -$TPPS_4$; (b) Ni^{2+}-$TPPS_4$; (c) Ca^{2+}-$TPPS_4$; (d) K^+ -$TPPS_4$; (e) free $TPPS_4$.

2. UV-visible absorbance

Intense Soret-band: The UV-visible spectrum of our free $TPPS_4$ sample at neutral pH was characterized by an intense Soret-band centered at 414 nm, as shown earlier in Fig. 2. This peak characterizes the monomeric, deprotonated form of porphyrin (25, 26). But other peaks (Q-band) at longer wavelengths were found rather week and insignificant in this case.

Effects of metal ions: By adding different metal ions to $TPPS_4$ solutions, we found that the Soret-band of $TPPS_4$ was more or less red-shifted. Fig. 6 summarizes the wavelength of Soret-band for K^+-, Ca^{2+}-, Zn^{2+}-, Co^{2+}-, Mn^{3+}-, or Fe^{2+}-bound $TPPS_4$. We found that increases of absorption wavelength for these $TPPS_4$-metal ion complexes can be correlated to the decreases of the metal ion radii, with a "best" fitting to a polynomial curve ($Y = 546.1 - 2.86X + 0.022\ X^2 - 5.8x10^{-5}\ X^3$). In particular, the K^+, Ca^{2+}, Co^{2+} and Fe^{2+} data are well fit to the curve.

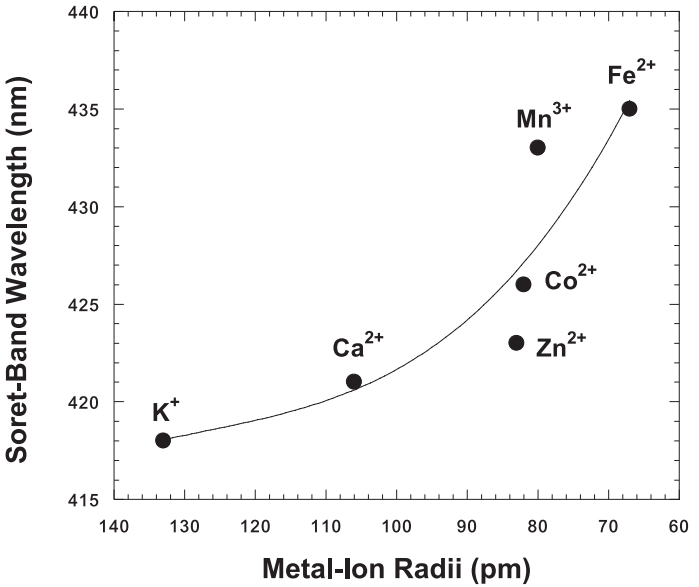

Figure 6. The red-shift of UV-visible Soret-band (in nanometer) of $TPPS_4$-metal complexes, in correlation with metal-ion radii (in picometer).

DISCUSSION

1. The co-planar structure of $TPPS_4$

^1H chemical shifts of two raw materials used for our $TPPS_4$ synthesis can be referenced from the on-line spectral database (SDBS), where phenyl-H of benzaldehyde has 7.56 ppm (3, 5 positions) and 7.87 ppm (2, 6 positions); and pyrrole-H has 6.74 ppm (2, 5 positions) and 6.24 ppm (3, 4 positions), respectively. When $TPPS_4$ is formed, the protons at 3, 4 positions of pyrrole remain in tetrapyrrole ring but the protons at 2, 5 positions are eliminated. Comparing the SDBS results with our $TPPS_4$ spectral data (Fig. 3), it can be found that the value of 7.59 ppm (peak **a**) for tetraphenyl-H is in between the two resonance values for parent benzaldehyde. However, the value of 6.54 ppm (peak **b**) for tetrapyrrole-H is 0.26 ppm down-field shifted from the parent pyrrole-H at 3, 4 positions.

It should be understood that the tetrapyrrole unit is a highly conjugated, nearly planar macrocycle with 22 delocalized bonding π-electrons, obeying the well-known Hückel's 4n+2 rule (where n is an integer number) for stability or aromaticity of ring structure. Relative to pyrrole, the down-field shift of peak **b** in $TPPS_4$ spectrum (Fig. 3) suggests that formation of a large π-conjugation in porphyrin macrocycle enhances the "ring-current" effect on nuclear desheilding of tetrapyrrole protons.

Fig. 4 shows that effects of pH variation on chemical shifts of tetrapyrrole-H and tetraphenyl-H are nearly identical. Over the entire pH range of 6.1-10.3, $TPPS_4$ should be fully deprotonated. However, pH changes appear to have similar nuclear shielding/deshielding at tratrapyrrole and tetraphenyl sites, suggesting a common "ring current" effect. We believe that this is indicative of an important structural feature of $TPPS_4$, *i.e.* the tetrapyrrole and tetraphenyl rings are co-planar with a large p-π electron conjugation over the entire $TPPS_4$ molecule.

To understand this structure feature, it is crucial to know that both parent materials, pyrrole and benzaldehyde, are also planar molecules; the carbonyl carbon in benzaldehyde takes sp^2 hybrid and keeps in-plane with phenyl ring. During the formation of tetraphenyl porphyrin, it is four carbonyl carbons that transform into methine bridges (=C-) to interconnect pyrroles and phenyls. Therefore, it is very likely that all parent molecules of $TPPS_4$ preferably maintain a co-planar structure throughout the entire synthesis process, unless there are other significant steric factors to force phenyl rings out of tetrapyrrole plane; but that appears not happened here. Such co-planar structure would extend the p-π electron delocalization to further stabilize $TPPS_4$ molecule. As the result, all carbons and nitrogens in $TPPS_4$ keep in-plane with totally 50 delocalized p-π electrons over the entire conjugation system.

This analysis can be further justified by UV-visible absorbance data. For the parent materials, the UV absorptions occur at ~210 nm (pyrrole) and ~250 nm (benzaldehyde), corresponding to π-electronic transitions on pyrrole ring and phenyl ring, respectively (27, 28). If the tetrapyrrole and tetraphenyl rings in $TPPS_4$ were still two separated conjugation systems, we could observe two absorption peaks at different wavelengths, or at least a much broader peak due to peak overlap. However, our $TPPS_4$ free-base has only one sharp band at ~414 nm, as shown in Fig. 2. The result also agrees with some earlier measurements (14, 26). This strongly suggests that the pyrroles and phenyls may indeed form a large, co-planar p-π conjugation, leading to a single absorption peak in visible-light range. Besides, it was found by Raman and infrared studies that the *p*-sulfonation on phenyl groups of $TPPS_4$ may alter the vibrations of C-C bonds between tetrapyrrole and phenyls and, to some extent, affect the π-electron system on porphyrin ring (29). This result implies an extended electron conjugation in $TPPS_4$, in consistence with our conclusion.

The co-planar structure of $TPPS_4$ meso-tetraphenyl rings and porphyrin-core is novel, and its finding is somewhat unexpected to us. Such unique

structural feature may have its inherent significance to the stability and interaction of $TPPS_4$, as mentioned in Introduction section. By comparing $TPPS_4$ with other porphyrin- or corrin-ring structures, several interesting points can be further made here. First, the co-plane of side-rings and porphyrin-core ring in $TPPS_4$ is not the same as that in some synthetic bis-porphyrins, in which only the space-separated porphyrin-core rings are nearly co-planar (30). Second, the structures of porphyrins may strongly depend on their substituents and sample conditions. For instance, X-ray study showed that the crystalline meso-tetrakis (pentafluorophenyl) porphyrin (TF_5PP) has its phenyl rings twisted by ~75°–88°, making them almost perpendicular to the tetrapyrrole plane (31). This sharp difference from our $TPPS_4$ sample can be attributed to the crystallographic packing forces between neighboring molecules in crystalline TF_5PP. Third, it should also be recognized that $TPPS_4$ structure is significantly distinctive from certain corrin systems such as Vitamin-B12. The $TPPS_4$ ring is more rigid and more flat when viewed from the side, due to its larger conjugation system consisting of porphyrin-core ring and tetraphenyl rings, as we justified above; whereas Vitamin-B12 contains a much smaller conjugated chain within part of the ring system, and thus its side groups are surely not in-plane with the corrin-ring.

2. The binding strengths of metal-ions

From ^1H line-shapes of $TPPS_4$-metal ion complexes in Fig. 5, it can be generally concluded that binding strengths of metal ions are in a trend of $K^+ < Ca^{2+} < Ni^{2+} < Cu^{2+}$. The up-field shifts of both tetrapyrrole-H and tetraphenyl-H in K^+- or Ca- bound $TPPS_4$ are mainly due to electrostatic interaction between porphyrin-core and metal ion, and such interaction reduces the "ring current" on both tetrapyrrole and tetraphenyl (because of their co-planar conjugation), increasing the nuclear shielding of all these protons. In contrast, the down-field shifting of Ni^{2+}- bound $TPPS_4$ is probably caused by direct coordination between transition-metal ion and porphyrin-core. Unlike alkali and alkali-earth ions, transition-metal ions possess d-electrons, which can be delocalized through their direct coordination with porphyrin-core, increasing the "ring current" and proton deshielding. In a $TPPS_4$-metal ion complex, the metal ion coordinated to tetrapyrrole-core typically adopt sp^3d^2 hybrid with four orbitals in porphyrin plane and two orbitals in perpendicular ± z direction, giving rise to octahedral geometry. However, Cu^{2+} may experience the so-called "Jahn-Teller effect" because of its uneven 9 d-electron configuration, which results in geometry distortion and extra binding strength. The very broad peak of $TPPS_4$-Cu^{2+} in Fig. 5 is a clear evidence of strong interaction between Cu^{2+} ion and $TPPS_4$.

The direct coordination between metal ion and tetrapyrrole-core also somewhat extends the conjugation from porphyrin to metal ion. According to quantum theory, an electronic excitation involved in a larger conjugated system requires lower energy absorption, corresponding to lower radiation frequency or longer wavelength. The UV-visible absorption wavelength of

$TPPS_4$-metal complexes, i.e. red-shift of $TPPS_4$ Soret-band upon binding with different metal ions (Fig. 6), confirms such explanation. Clearly, the effect on red-shift is in a trend of $K^+ < Ca^{2+} < Zn^{2+} < Co^{2+} < Mn^{3+} < Fe^{2+}$, and such trend can be correlated with different metal ion sizes, i.e. the smaller is a metal ion, the more red-shifted is the Soret-band of its $TPPS_4$ complex. This is in agreement with the so-called "Irving-Williams series", which states that the higher is the charge density of a metal ion, the more stable is its ligand binding. Therefore, by comparing the red-shift of the Soret-band, we are able to assess the relative stabilities of $TPPS_4$-metal ion complexes.

It should be noticed that our results presented here are qualitative. In the future, we will extend our work to quantitatively determine the $TPPS_4$-metal ion bindings. The strength of porphyrin-metal ion interaction may depend on various factors, including the porphyrin (P) species and its charge state (such as H_2P or P^{2-}), the metal ions, solvents, temperature, etc. In fact, the binding constants (K) were obtained for some porphyrin-metal ion complexes (32). For instance, when binding to N-alkylated porphyrin HN-Me-TPPS, Cd^{2+} and Zn^{2+} have the binding constants $K=1.3x10^{-2}$ and $3.3x10^{1}$, respectively; and when binding to TMPyP[4], another water-soluble porphyrin, the stability constants are Zn^{2+} ($8.3x10^{25}$) > Mg^{2+}($7.5x10^{17}$) > Li^+ ($3.8x10^{-2}$) (32). The binding trends revealed in these quantitative data are somewhat consistent with our qualitative prediction, although the literature values are not totally comparable to ours because they involve fundamentally different materials and experimental methods.

In summary, our ^1H NMR and UV-visible spectral analysis suggests that the tetrapyrrole unit and tetraphenyl rings form a large co-planar conjugation system in water-soluble synthetic porphyrin $TPPS_4$. For deprotonated $TPPS_4$, pH effects on resonance frequencies of tetraphenyl-H and tetrapyrrole-H are nearly identical, but ^1H line-shapes of metal ion bound $TPPS_4$ strongly depend on metal ion species. In general, transition-metal ions show stronger binding affinity on porphyrin core than alkali and alkali-earth ions. The relative stabilities of $TPPS_4$-metal ion complexes can be well assessed by ^1H NMR and UV-visible data. Elucidation of these spectral and structure features will be helpful to a broad range of porphyrin syntheses and applications.

ACKNOWLEDGEMENT

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