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Aggregation of copper(II) derivatives of *meso*-substituted porphyrins in frozen aqueous media

Geoffrey Dougherty*
Oxconsult, P.O. Box 79, Abingdon, Oxon. OX14 1YS (UK)

and Robert F. Pasternack
Department of Chemistry, Swarthmore College, Swarthmore, PA 19081 (USA)

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Abstract

The degree of aggregation of three *meso*-substituted Cu(II) porphyrin derivatives has been studied in frozen aqueous solutions to which polar organic molecules were progressively added. Electron paramagnetic resonance spectra show that the water-soluble porphyrins give rise to solute–solute interactions in their solutions. The relative amounts of each species present depend on the composition of the solutions, with organic solvents such as glycerol shifting the equilibria markedly towards the monomeric species. No specific dimeric species were detected for the copper(II) chelates of tetrakis(4-*N*-methylpyridyl)porphine (Cu(II)TMpyP-4) and tetrakis(2-*N*-methylpyridyl)porphine (Cu(II)TMpyP-2) under any of the solution conditions reported, whilst there was evidence to suggest the presence of a relatively low concentration of the dimeric species of the copper(II) chelate of tetrakis(4-sulfonatophenyl)porphine (Cu(II)TPPS). The tendency to aggregate is found to be Cu(II)TPPS > Cu(II)TMpyP-4 > Cu(II)TMpyP-2. The spectra of Cu(II)TMpyP-2 are particularly interesting in that two distinct species — presumed to reflect the presence of atropisomers — are progressively distinguishable on addition of a polar organic solvent.

Introduction

Porphyrins and their metallo derivatives play a vital role in biological processes, but their study in aqueous and non-aqueous solution is often complicated by the presence of dimers or higher aggregates [1]. The nature and kinetics of μ-oxo type dimerization as found in Fe(III) porphyrins is fairly well characterized [2], but the conditions that promote association in compounds where μ-oxo bonding is absent are less clear. The stabilization for such non-covalently linked aggregates has been proposed to arise primarily from π–π interactions [3–6] although, more recently, the importance of π–σ interactions has been discussed [7].

Derivatives of the cationic water-soluble porphyrin tetrakis(4-*N*-methylpyridyl)porphine (H_2TMpyP-4) have been investigated for a large number of applications including superoxide dismutase mimics [8], DNA binding reagents [9, 10] and sensitizers for photodynamic therapy [11, 12]. An important advantage of working with H_2TMpyP-4 and many of its metal derivatives is their putative limited tendency to aggregate [3, 4]. Absorbance, NMR and relaxation data have been offered as evidence for the monomeric nature of H_2TMpyP-4, CuTMpyP-4, NiTMpyP-4 and ZnTMpy-P-4 in solution upto about 0.1 mM [3, 4, 13].

However, a recent claim has been made based largely on fluorescence data that H_2TMpyP-4 forms a stable dimer, even below micromolar concentrations [14, 15]. We describe here electron paramagnetic resonance (EPR) studies which were undertaken to probe the aggregation tendencies of a number of *meso*-substituted porphyrins further. EPR spectroscopy has been used previously to study the aggregation status and dynamic properties of micellar solutions of surfactants [16, 17] and, recently, it has been used to help characterize a number of tetranionic porphyrins [18] and tetracationic porphyrins [19] in different micellar media. We report here on the copper(II) derivatives of two tetracationic porphyrins (Cu(II)TMpyP-4 and Cu(II)TMpyP-2) and a *meso*-substituted tetranionic porphyrin, tetrakis(4-sulfonatophenyl)porphine (CuTPPS), in aqueous buffers containing varying amounts of glycerol, ethanol or dmso (dimethyl sulfoxide). Copper(II) porphyrins are usually square coplanar with little tendency to add axial ligands and, therefore, their aggregation properties generally reflect those of the free-base porphyrins from which

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*Author to whom correspondence should be addressed.

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they have been derived [4, 20]. A counter-example to this correlation is the copper(II) derivative of tetrakis(4-trimethylaminophenyl)porphine which dimerizes under conditions at which the free-base porphyrin remains monomeric [21] although the free-base porphyrin does aggregate on anionic surfaces [22]. Therefore, we conclude that these copper derivatives may be used as probes of the relative tendencies for the free-base porphyrins to aggregate.

Experimental

The two non-metalloporphyrins, H_2TMpyP-4 and H_2TMpyP-2, were purchased as the tosylate salts from Midcentury Chemicals and converted to their copper(II) derivatives, as chloride forms, as previously described [10]. Cu(II)TPPS was prepared as described previously [23]. Concentrations were determined spectrophotometrically using ϵ (424 nm) $= 2.31 \times 10^5$ M^{-1} cm^{-1} for Cu(II)TMpyP-4 [4], ϵ (415 nm) $= 2.21 \times 10^5$ M^{-1} cm^{-1} for Cu(II)TMpyP-2 and ϵ (412 nm) $= 4.16 \times 10^5$ M^{-1} cm^{-1} for Cu(II)TPPS in distilled water. Absorbance data were obtained on a Varian 2200 spectrophotometer with a thermostated cell compartment.

Some experiments used a phosphate buffer, at pH 6.8, containing 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM ethylenediamine tetraacetic acid (EDTA) and either NaCl or $NaNO_3$ to give a final ionic strength of $\mu = 0.2$ M. Reagent grade glycerol, ethanol and dmso were used as received.

EPR spectra of the porphyrins were recorded at ~110 K using a Bruker ER200D EPR spectrometer at X-band (9–10 GHz). Temperature was controlled using a Bruker ST100/700 variable temperature accessory. The typical metalloporphyrin concentration in the quartz sample tube was ~0.5 mM.

Results

Figure 1 shows the EPR spectra of Cu(II)TMpyP-4, at an ionic strength of 0.2 M, with increasing proportions of glycerol. With no glycerol present, the spectrum is dominated by a single isotropic resonance at $g = 2.07$ with a peak-to-peak derivative width of 3.7 mT and with no resolvable structure such as would be expected from a mixture of species. With progressive addition of glycerol prior to freezing the spectra become better resolved. At around 10% vol./vol. glycerol the spectrum is characteristic of the monomeric square-planar Cu(II) corresponding to the $\Delta M_s = 1$ transition, associated with an axial g tensor ($g_{\parallel} = 2.22$; $g_{\perp} = 2.07$) and with rich hyperfine splitting in both $g_{\parallel}$ and $g_{\perp}$ regions ($A_{\parallel} = 210 \times 10^{-4}$ cm^{-1}; $A_{\perp} = 30 \times 10^{-4}$ cm^{-1}).

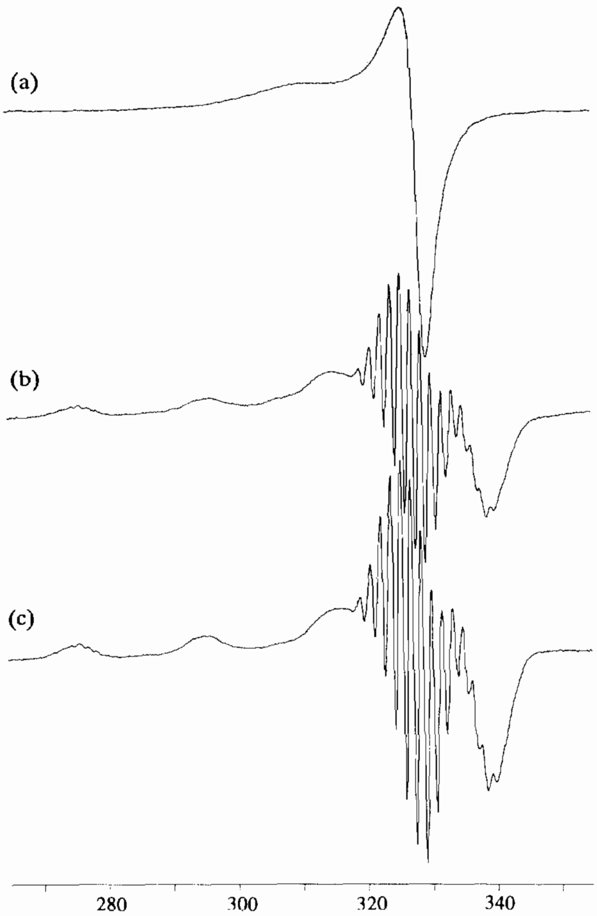

Fig. 1. First-derivative EPR spectra of Cu(II)TMpyP-4, at an ionic concentration of 0.2 M and with various proportions of glycerol. Microwave power 10 mW, frequency 9.50 GHz, modulation amplitude 1.0 mT, temperature 110 K. (a) No glycerol, (b) 10% glycerol, (c) 20% glycerol.

Increased proportions of glycerol have little effect, save for some increased resolution of the nitrogen superhyperfine structure ($A_{\parallel}^{N} = 14 \times 10^{-4}$ cm^{-1}). A similar trend is apparent in the spectra of Fig. 2, with Cu(II)TMpyP-4 in distilled water and increasing proportions of glycerol. *Indeed in distilled water the monomeric species is predominant even in the absence of glycerol*: it is only at an ionic strength of 0.2 M, with no glycerol, that the broad spectrum – indicative of a mixture of species – is obtained.

The spectra of Cu(II)TMpyP-2 show the features typical of monomeric square-planar Cu(II) (Figs. 3 and 4), although the values of $A_{\parallel}$ (about 150×10^{-4} cm^{-1}) are somewhat smaller than expected. At $\mu = 0.2$ M, the inclusion of increased proportions of glycerol resulted in an additional set of parallel features (centered around $g_{\parallel} = 2.30$) which increased in strength as the proportion of glycerol increased. The same trend occurred with ethanol or dmso replacing glycerol, although it was less dramatic, and with NO_3^- replacing Cl^- as the coun-

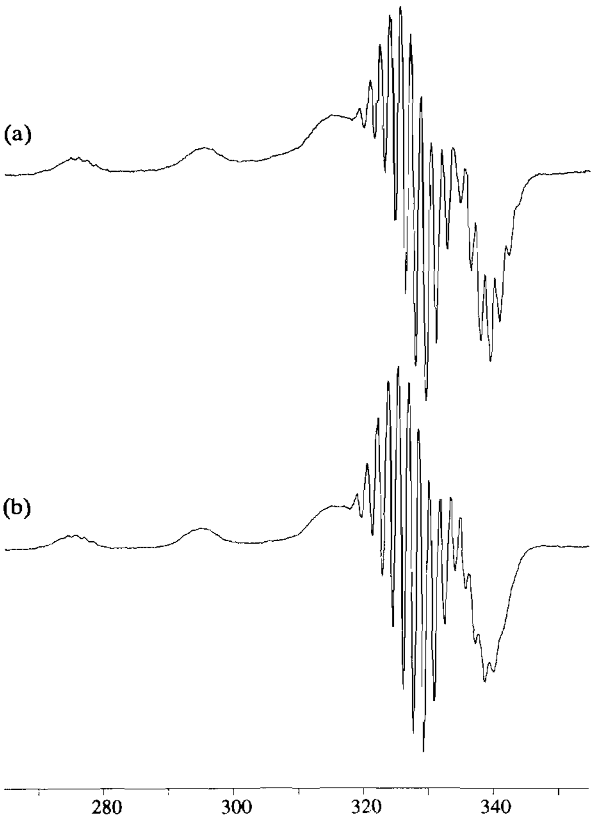

Fig. 2. First-derivative EPR spectra of Cu(II)TMpyP-4, in distilled water and with various proportions of glycerol. Microwave power 10 mW, frequency 9.50 GHz, modulation amplitude 1.0 mT, temperature 110 K. (a) No glycerol, (b) 10% glycerol.

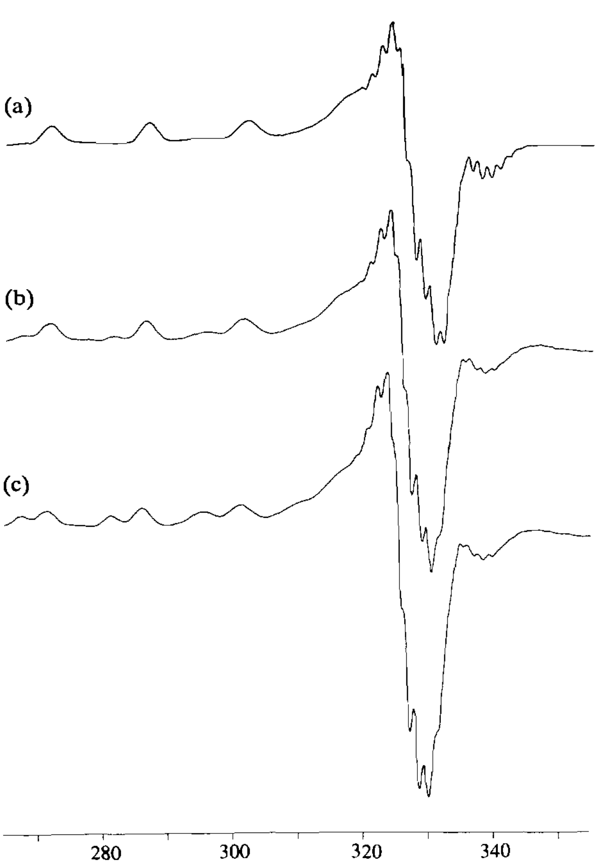

Fig. 3. First-derivative EPR spectra of Cu(II)TMpyP-2, at $\mu = 0.2$ M and with various proportions of glycerol. Microwave power 10 mW, frequency 9.50 GHz, modulation amplitude 1.0 mT, temperature 110 K. (a) No glycerol, (b) 20% glycerol, (c) 40% glycerol.

terion (not shown). These additional features were not apparent in mixtures of distilled water with glycerol.

The spectra of Cu(II)TPPS (Figs. 5 and 6) show a single broad isotropic resonance at $g = 2.08$ with no glycerol present. As with Cu(II)TMpyP-4, increasing proportions of glycerol result in resolvable hyperfine structure especially in the $g_\perp$ region. However, the spectral assignment of the parallel structure is difficult to determine. The small hyperfine splitting ($A_\parallel \sim 110 \times 10^{-4}$ cm^{-1}) is about half the normal splitting for a monomer ($\sim 210 \times 10^{-4}$ cm^{-1}), as would be expected for a dimer spectrum. Comparing the Cu(II)TPPS spectra (Figs. 5 and 6) with those for Cu(II)TMpyP-4 (Figs. 1 and 2), it is clear that larger proportions of glycerol are required to cause disaggregation in the former compound: 50% vol./vol. for an ionic strength of 0.2 M, and 5–10% in distilled water.

Interestingly, no half-field spectrum associated with $\Delta M_s = 2$ was detected with any of the three compounds under any of the conditions reported.

Discussion

The properties of water-soluble *meso*-substituted porphyrins depend on the position and charge type of the periphery of the molecule. Anionic porphyrins, such as H_2TPPS, are considerably more basic and have been reported as having a greater tendency to aggregate than cationic porphyrins, such as H_2TMpyP-4 and H_2TMpyP-2 [3]. Spectrophotometric studies indicate that the metal-free porphyrin, H_2TPPS, aggregates significantly at concentrations of about 10 μM and this was shown to result almost exclusively in dimers [3]. Cu(II)TPPS was also reported as dimerizing, whereas Zn(II)TPPS aggregated only at considerably higher concentrations [24, 25]. ^{1}H NMR studies conducted at concentrations of about 0.3 mM confirmed the aggregation of H_2TPPS [24] to form dimers, tetramers etc., but even H_2TMpyP-4 was shown to self-associate under these conditions [13].

Spectral and relaxation studies indicate that Cu(II)TMpyP-4 shows no tendency to aggregate at concentrations up to 0.1 mM [4, 19]. Our results indicate

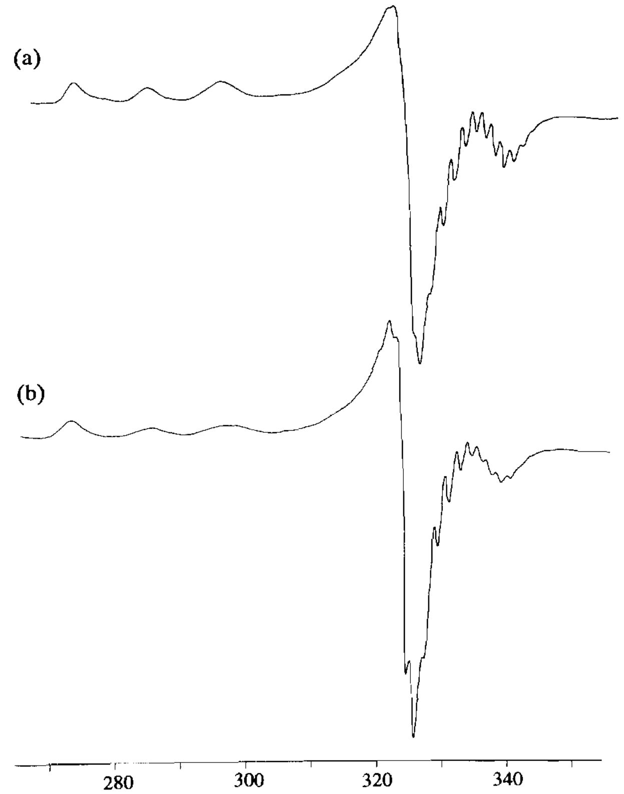

Fig. 4. First-derivative EPR spectra of Cu(II)TMpyP-2, in distilled water and with various proportions of glycerol. Microwave power 10 mW, frequency 9.50 GHz, modulation amplitude 1.0 mT, temperature 110 K. (a) No glycerol, (b) 20% glycerol.

that whilst some aggregation occurs at high porphyrin concentration (~ 0.5 mM) in an aqueous buffer at $\mu = 0.2$ M, these higher-order aggregates are readily broken down by the addition of moderate amounts (~ 10% vol./vol.) of glycerol. Furthermore this disaggregation proceeds from polymeric to monomeric species, with scant evidence for the presence of a distinct dimeric species, similar to that observed with the copper(II) chelate of tetracarboxyphenylporphyrin [26]. *No aggregation was apparent with Cu(II)TMpyP-4 in distilled water at a porphyrin concentration of ~ 0.5 mM.*

The copper(II) chelate of H_2TMpyP-2 shows no tendency to form higher aggregates under any of the conditions studied. This may be related to its inability to intercalate into DNA, a process which also depends on the establishment of sufficiently strong π–π interactions. During this current study, we used the technique of monitoring the EPR spectrum of a thin film of metalloporphyrin–DNA under different orientations [27–29] to show that Cu(II)TMpyP-2 cannot intercalate (results not shown): an earlier study reported that the metal-free H_2TMpyP-2 is also unable to intercalate into DNA [30].

The values for $A_{\parallel}$ (~ 150×10^{-4} cm^{-1}) are quite small for square-planar copper(II), although values of around 175×10^{-4} cm^{-1} have been reported previously [31] for the spectra of a copper porphyrin interacting with nucleic acid bases in a water–glycerol mixture.

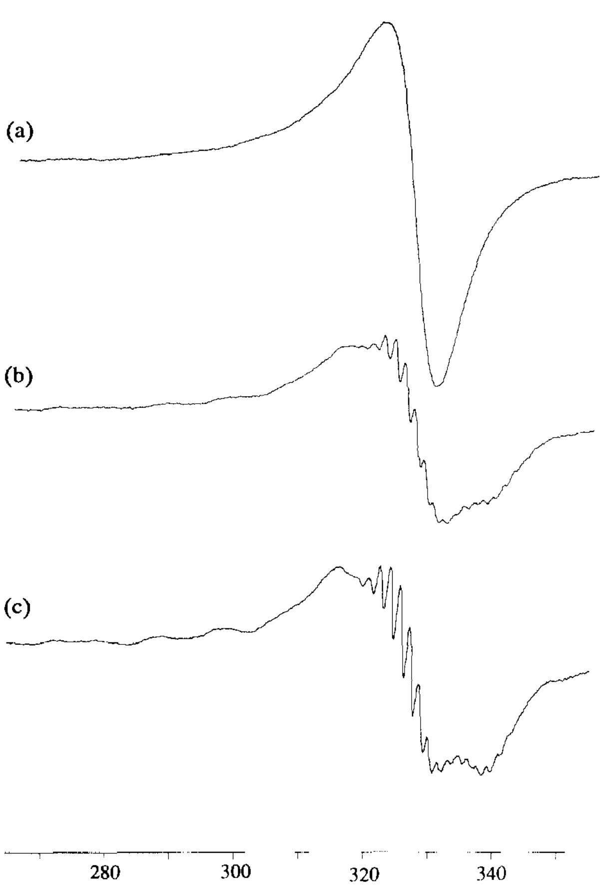

Fig. 5. First-derivative EPR spectra of Cu(II)TPPS, at $\mu = 0.2$ M and with various proportions of glycerol. Microwave power 10 mW, frequency 9.50 GHz, modulation amplitude 1.0 mT, temperature 110 K. (a) No glycerol, (b) 25% glycerol, (c) 50% glycerol.

As glycerol is progressively added to Cu(II)TMpyP-2 (Fig. 3), a second spectrally distinct species is resolved with $g_{\parallel} = 2.30$ and $A_{\parallel} = 150 \times 10^{-4}$ cm^{-1}. This effect is not dependent on the electrolyte, since similar results were obtained replacing NaCl by $NaNO_3$, and does not appear to be related to aggregation phenomena. It does not occur with the Cu(II)TMpyP-4 spectra.

Whilst Cu(II)TMpyP-2 and Cu(II)TMpyP-4 are both tetracationic, the distribution of charge within the porphyrin rings is different [32]. Cu(II)TMpyP-4 has all of the charged nitrogens located symmetrically within the porphyrin plane, and consequently has no net dipole moment. Cu(II)TMpyP-2 can exist as four atropisomers, with different charge distributions within the cyclic framework for each isomer. The most polar isomer has all of the charged nitrogens located on one side of the porphyrin plane, another has only three of its four charges on one side of the plane, and the other two are a *cis–trans* isomer pair with symmetrically placed

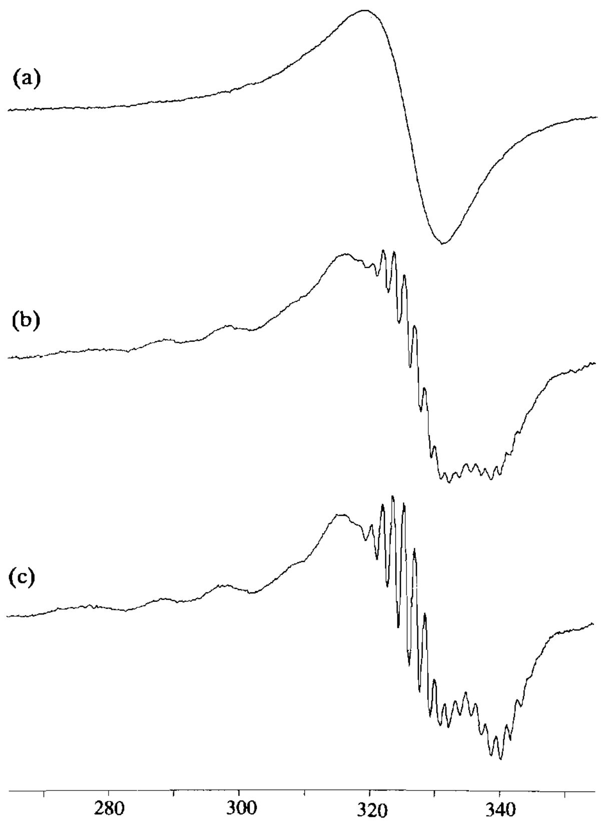

Fig. 6. First-derivative EPR spectra of Cu(II)TPPS, in distilled water with various proportions of glycerol. Microwave power 10 mW, frequency 9.50 GHz, modulation amplitude 1.0 mT, temperature 110 K. (a) No glycerol, (b) 5% glycerol, (c) 10% glycerol.

charges and no net dipole moment [32]. EPR is sensitive to small changes in electron distribution, which result in distinctive g and A values. Changing the solvent composition may lead to changes in the charge distribution amongst the atropisomers, resulting in distinct spectra.

The single isotropic absorption observed in EPR experiments with Cu(II)TPPS in frozen aqueous solution (either distilled water or 0.2 M phosphate buffer) has been reported previously, and has been explained in terms of an exchange 'narrowing' effect resulting from the cumulative interaction of the electron spins of a large number of Cu(II)-containing molecules stacked relatively close together [26, 33]. In the earlier EPR studies the hyperfine structure was not resolved until the solvent contained about 50% vol./vol. of dmso; we see complete resolution at 5% vol./vol of glycerol with distilled water (Fig. 6) or 50% vol./vol. of glycerol with 0.2 M NaCl/phosphate buffer (Fig. 5). This effect of ionic strength is expected, since higher ionic concentration stabilizes aggregate formation between species of like charge. The effect of adding glycerol or dmso is to break down the higher order aggregates with glycerol exhibiting somewhat greater effectiveness. Although no half-field spectrum associated with $\Delta M_s = 2$ was detected under any of the conditions reported here, we consider that the value of the hyperfine splitting in the $g_{\|}$ region observed as the proportion of glycerol was increased indicates the presence of a dimer species—albeit at a relatively low concentration.

The tendency of these three porphyrin derivatives to aggregate is in the order Cu(II)TPPS > Cu(II)TMpyP-4 > Cu(II)TMpyP-2. The greater tendency of anionic porphyrins to aggregate has been long recognized [3]. It has been suggested that the negative charges on the periphery lead to a partial localization of electron density near the center of the ring system, resulting in stronger van der Waals interactions for stacking/aggregation. The observation of the limited tendency of Cu(II)TMpyP-4 to aggregate at high concentration ($\sim$0.5 mM), apparent at $\mu = 0.2$ M but not in distilled water, is in agreement with the bulk of earlier evidence for both the copper-containing and free-base variants [3, 4, 13].

Clearly the degree of aggregation is influenced by the nature of the peripheral charges and their distribution in the exocyclic structure. For the tetracationic porphyrins used in this study, stacking seems to be a limited occurrence as previously suggested.

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