Electronic Spectra, Resonance Raman Spectra and Solution Properties of Water-soluble Cu(II), Ni(II) and Co(III) Porphyrins

KAI BÜTJE and KAZUO NAKAMOTO*

Department of Chemistry, Marquette University, Milwaukee, WI 53233 (U.S.A.)

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Abstract

The Cu(II), Ni(II) and Co(III) complexes of the following water-soluble *meso*-substituted porphyrins were prepared: tetrakis(2-*N*), (3-*N*-) and (4-*N*-)methylpyridylporphyrin (**1, 2, 3**), tris(4-*N*-methylpyridyl)monophenylporphyrin (**4**), two isomeric bis(4-*N*-methylpyridyl)diphenylporphyrins (**5, 6**) and tetrakis(4-*N,N,N*-trimethylanilinium)porphyrin (**7**). The metalloporphyrins were characterized by UV–Vis and resonance Raman spectroscopy. The Cu(II) porphyrins are always four-coordinate; the complexes of more basic porphyrins, **4** through **7**, have a marked tendency to aggregate in aqueous and electrolyte solutions. In aqueous solution, the Ni(II) complexes of more acidic porphyrins, **1** through **4**, exist as equilibrium mixtures of four-coordinate square-planar species and six-coordinate diaquo forms; the relative amount of the latter increases with the acidity of the porphyrin. The Ni(II) derivatives of other porphyrins, **5** through **7**, are four-coordinate in water, but tend to form aggregates. The Co(III) complexes are six-coordinate in all cases. Resonance Raman spectra have been recorded for all complexes, band assignments are given, and structure-sensitive bands are discussed.

Introduction

meso-Substituted porphyrins with ionic substituents and their metal derivatives are currently under extensive study. They are easily accessible, they display a wide variety of interesting chemical and physical properties, and their solubility in water makes them very suitable model compounds for research related to biological phenomena. *meso*-Tetrakis(4-*N*-methylpyridyl)porphyrin (**3**, Fig. 1) and its metal compounds were shown to interact with nucleic acids in a complex and surprisingly specific manner [1–8]. Since the action of electrophilic agents on DNA plays a key role in chemical carcinogenesis as well as in cancer chemotherapy,
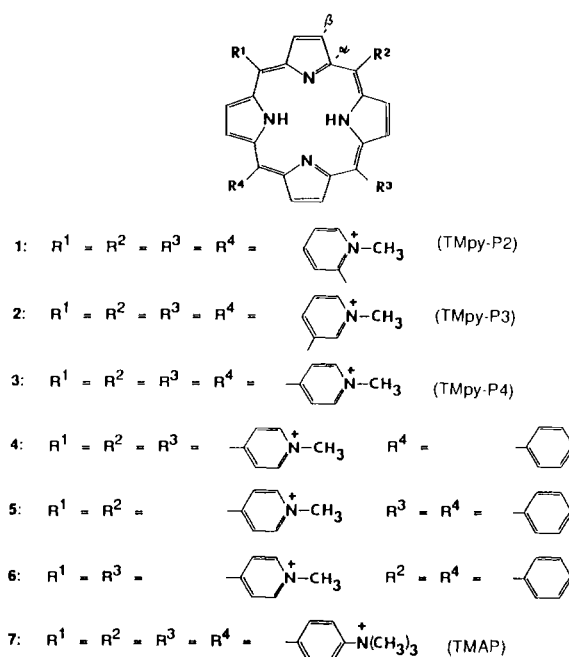

Fig. 1. Structure of water-soluble porphyrins.

and since haematoporphyrin derivatives are widely used in the photodynamic treatment of malignant tumors [9–11], the porphyrin–nucleic acid interaction continues to be of high interest. In order to design new, clinically useful drugs, it is desirable to know all the factors which make such interaction possible and which influence its mechanism. For example, a seemingly small modification such as the replacement of the central Cu(II) ion by Co(III) causes the mode of bonding of **3** to DNA to switch from intercalation at G–C sites to groove-binding at A–T sites [5].

In the present work, we attempt to further elucidate the nature of the interaction of metalloporphyrins with nucleic acids by UV–Vis and resonance Raman (RR) spectroscopy, keeping the central ion constant while varying the porphyrin ligand itself. This paper reports some chemical and spectroscopic properties of the Cu(II), Ni(II) and Co(III)

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*Author to whom correspondence should be addressed.

complexes of the following *meso*-substituted water-soluble porphyrins (Fig. 1): tetrakis(2-*N*-methylpyridyl)porphyrin (**1**); tetrakis(3-*N*-methylpyridyl)porphyrin (**2**); tetrakis(4-*N*,*N*,*N*-trimethylanilinium)-porphyrin (**7**); and three mixed (phenyl)(4-*N*-methylpyridyl)porphyrins (**4, 5, 6**) [12]. In the following paper, we will investigate the interaction of these metalloporphyrins with nucleic acids by the spectroscopic methods mentioned above.

Experimental

Reagents

The metal-free ligands, 5,10,15,20-tetrakis(2-*N*-methylpyridyl)porphyrin (Fig. 1, **1**); its 3-*N*- and 4-*N*-methylpyridyl isomers (**2, 3**); 5,10,15-tris(4-*N*-methylpyridyl)-20-monophenylporphyrin (**4**); 5,10-bis(4-*N*-methylpyridyl)-15,20-diphenylporphyrin (**5**), 5,15-bis(4-*N*-methylpyridyl)-10,20-diphenylporphyrin (**6**) and 5,10,15,20-tetrakis(4-*N*,*N*,*N*-trimethylanilinium)porphyrin (**7**) were purchased as chlorides from Midcentury, Posen, IL, and used without further purification. All other chemicals were commercial products of analytical grade.

Preparation of Metalloporphyrins

The porphyrins were metalated by the method of Pasternack *et al.* [5] with slight modifications. In general, the free base was refluxed with a large excess of the corresponding metal(II) chloride in water, and the progress of the metal insertion was monitored by UV–Vis spectroscopy. When the reaction was completed, the metalloporphyrin was precipitated from the cooled solution by addition of solid NaClO₄. The perchlorate was isolated by centrifugation and washed several times with small amounts of ice-cold 0.2 M HClO₄. (**Caution:** due to the explosive nature of metalloporphyrin perchlorates [13], it should not be attempted to isolate them in dry form. However, while kept moist, they were found safe to handle.) The metalloporphyrin was then transformed into the chloride by anion exchange (Bio-Rad AG1-X8, 20–50 mesh, Cl⁻ form) and isolated by freeze-drying.

Co-**1**. Since the perchlorate is highly soluble, NaI was used to precipitate this metalloporphyrin. The iodide was washed with small amounts of cold water and transformed into the chloride by ion exchange.

Cu-, Ni-**4**, Co-**5**. After cooling to room temperature, the chlorides partly precipitated from the reaction mixture. However, for best yields they were converted into the perchlorates by adding NaClO₄ and gentle heating for 15–30 min. Then, the perchlorates were treated as described above.

Cu-, Ni-**5**, Cu-, Ni-, Co-**6**. Instead of water, aqueous acetone (30–40% acetone, vol./vol.) was used as reaction medium for the metal insertion. After metalation was completed, the acetone was distilled off and the solution slowly cooled to room temperature. The fine crystals of the metalloporphyrin chlorides were centrifuged off, washed several times with small amounts of ice-cold 0.2 M HCl and dried *in vacuo*.

Depending on the individual solubilities, yields ranged from 40 to 85%. For the metal complexes of a given porphyrin, the solubility of the chlorides, iodides and perchlorates generally increases in the order Cu < Ni < Co; solubilities are highest for complexes of **1** and lowest for complexes of **6**.

Solutions

For resonance Raman studies, aqueous solutions were used which contained the metalloporphyrin at concentrations of 5×10^{-6} to 5×10^{-5} M. To prepare a sodium phosphate buffer solution of pH 6.8 and ionic strength $\mu = 2.0$, 6 mM Na₂HPO₄, 20 mM NaH₂PO₄, 10 mM EDTA and 145 mM NaCl were dissolved in 100 ml of deionized water. This stock solution was diluted with deionized water to prepare buffers of lower ionic strength.

Spectral Measurements

UV–Vis spectra were measured on a Perkin-Elmer Model 320 UV–Vis spectrophotometer, using 0.1 cm quartz cells. Resonance Raman spectra of solutions were recorded on a Spex Model 1403 double monochromator coupled with a Spex DM1B data station, using excitation lines of a Coherent Model 100 Kr⁺ (406.7 and 413.1 nm), a Liconix He–Cd (441.6 nm) and a Spectra-Physics Model 2025 Ar⁺ laser (457.9 nm). Laser powers were 15–30 mW at the sample. To record spectra of two solutions simultaneously, a divided rotating cell was used, and difference spectra were calculated as described previously [8, 14].

Results and Discussion

UV–Vis Spectra and Solution Properties

The Cu(II), Ni(II) and Co(III) complexes of the water-soluble porphyrins **1** through **7** (Fig. 1, Table 1) are readily obtained by refluxing the free ligand with an excess of the corresponding metal ion in water or a water–acetone mixture. As expected, the solubility in water decreases when the ionic *N*-methylpyridyl groups are replaced by phenyl groups. The solubility of the derivatives of **5** ('*cis*' isomer) is higher than that of complexes of **6** ('*trans*' isomer) because only the former has a net dipole moment. In order to identify and characterize the metalloporphyrins we employed UV–Vis and RR spectroscopy.

TABLE 1. Properties of water-soluble porphyrins

Porphyrin	λ_{max} (nm)	$\epsilon \times 10^{-5}$ $(M^{-1} cm^{-1})$	$pK_a{}^a$
1	414[b]	2.31[b]	-0.9
2	417[b]	3.32[b]	1.8
3	424[b]	2.26[b]	1.4
4	422	1.85[c]	2.4
5	421	1.4[d]	
6	418	2.4[d]	
7	413[e]	4.16[e]	3.6

[a]For the reaction $H_3P \rightarrow H_2P + H^+$, ref. 15. [b]Ref. 16. [c]This work. [d]Ref. 17. [e]Ref. 18.

The absorption maxima and extinction coefficients of these complexes in aqueous solution ($\sim 10^{-5}$ to 10^{-4} M) are listed in Table 2. During our studies on interactions of these metalloporphyrins with nucleic acids in buffered solutions, marked spectral differences between the aqueous and the buffer solutions were noticed in several cases. For a systematic study of this solvent effect, the UV–Vis spectra of dilute aqueous solutions of the metalloporphyrins ($\sim 5 \times 10^{-5}$ M) were compared with those containing the same concentrations of the metalloporphyrins in sodium phosphate buffer of pH 6.8 and ionic strength $\mu = 0.18$ (Table 2). Since these spectral changes are related

TABLE 2. Soret bands of water-soluble metalloporphyrins

Compound	Water (this work)		Water (literature data)		Buffer[a]	
	λ (nm)	$\epsilon \times 10^{-5}$ $(M^{-1} cm^{-1})$	λ (nm)	$\epsilon \times 10^{-5}$ $(M^{-1} cm^{-1})$	λ (nm)	Relative intensity (water = 1)
Cu-1	414.5	2.21	415	1.34[b]	414.5	0.97
Ni-1	{ 407[c]				407	0.98 }
	427	1.34[d]	428	2.0[e]	427	0.98 }
Co-1	426	1.95			426.5	0.88
Cu-2	419.5	2.57	420	2.7[f]	419.5	1.01
Ni-2	{ 411	1.17[g]			411	1.04 }
	432[h]				432	0.99 }
Co-2	430	2.18	432	1.31[i]	432	0.90
Cu-3			424	2.31[j]	424	0.98
Ni-3	{ 419	0.97[g,k]	420	1.49[m]	420	1.04 }
	441[l]		440		440	0.99 }
Co-3			437	2.15[n]	437.5	0.94
Cu-4	422	1.19			421	0.86
Ni-4	417	1.35			417.5	0.84
Co-4	435	1.42			437	0.96
Cu-5	{				397[o]	0.41 }
	418	1.21			412.5	0.47 }
Ni-5	415	1.17			415	0.68
Co-5	434	1.68			435	0.95
Cu-6	{ 418	p			380[o]	0.23 }
	450	q			444.5	0.48 }
Ni-6	416	p			445	0.41
Co-6	432	q			433	0.98
Cu-7	{ 414	3.46	411	4.0[r]	401[e]	0.56 }
			410.5	4.0[s]	413	0.66 }
Ni-7	408	2.05			407	0.89
Co-7	426	2.07	427	2.78[t]	427	0.96

[a]Phosphate buffer ($\mu = 0.18$, pH 6.8), see text. [b]Ref. 13. [c]Shoulder due to 4-coord. species, relative intensity (427 nm band = 1) $\cong 0.57$. [d]Main band, of 6-coord. species; apparent ϵ value, calculated under the assumption that conc. of 6-coord. form is equal to total porphyrin conc. [e]Ref. 7. [f]Ref. 19. [g]Main band, of 4-coord. species; apparent ϵ value, calculated under the assumption that conc. of 4-coord. form is equal to total porphyrin conc. [h]Shoulder due to 6-coord. species, relative intensity (441 nm band = 1) $\cong 0.55$. [i]Ref. 20. [j]Ref. 21. [k]Ref. 42. [l]Shoulder due to 6-coord. species, relative intensity (419 nm band = 1) $\cong 0.77$. [m]In 30% acetone, 4-coord. form alone, ref. 22. [n]Ref. 23. [o]Additional new band. [p]The amounts isolated were too small to determine ϵ with accuracy. [q]Shoulder, relative intensity (418 nm band = 1) = 0.22 (see Fig. 3). [r]Ref. 24. [s]Ref. 25. [t]Ref. 26.

TABLE 3. Soret bands of water-soluble Cu(II) and Ni(II) porphyrins in 50% ethanol

Compound	λ (nm)	Relative intensity (in water = 1, see Table 2)
Cu-4	420	1.01
Ni-4	418	0.95
Cu-5	418	1.65
Ni-5	418	1.15
Cu-6	415.5	1.81
Ni-6	419	1.02
Cu-7	413	1.17
Ni-7	410	1.07

to association equilibria (see below), we also recorded spectra of some of the metalloporphyrins in 50% ethanol, i.e. under conditions where association is minimal [27] (Table 3).

It has been shown previously [16] that the Soret bands of the isomeric meso-tetrakis(N-methylpyridyl)porphyrins as well as those of their Zn(II) complexes are blue-shifted when the N^+–CH_3 group is moved from the 4 to 3 to 2 position. A comparison of Tables 1 and 2 shows that the Cu(II), Ni(II) and Co(III) complexes of these porphyrins follow the same rule. For the Ni porphyrins, the Soret region consists of two bands which are assigned to a four- and a six-coordinate species (see below),
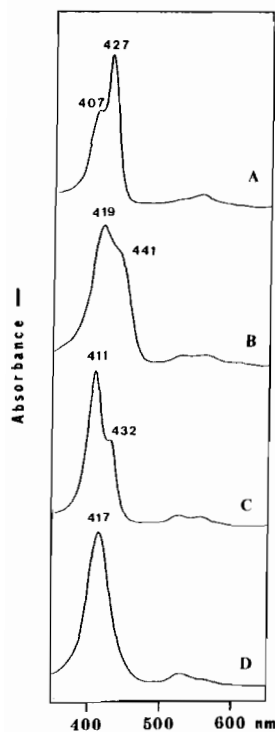
both of which exhibit this blue shift (Fig. 2, traces B, C, A). A hypsochromic shift is also observed for porphyrins and metalloporphyrins when the N-methylpyridyl groups are successively replaced by the phenyl groups.

Cu porphyrins: effect of aggregation

In the Soret region, all the water-soluble Cu(II) porphyrins exhibit only one band which shows no or only a little shift relative to the metal-free bases (cf. Tables 1 and 2). The extensively studied Cu-3 is four-coordinate [5], and thus the similarity of the spectra of the other water-soluble Cu porphyrins suggests that they are four-coordinate as well, bearing no axial water ligands. This is important with respect to possible intercalation properties since only those metalloporphyrins without or with only loosely bound axial ligands are able to intercalate into DNA [5].

When water is replaced by sodium phosphate buffer of pH 6.8, $\mu = 0.18$, there is practically no change in the UV–Vis spectra of the isomers Cu-1, 2, 3. However, Cu-4 displays a marked and reproducible hypochromicity, and this effect becomes much more pronounced when the second N-methylpyridyl group is replaced by the phenyl group (Table 2). The Soret bands also show large shifts and considerable broadening for Cu-5 and Cu-6. On the other
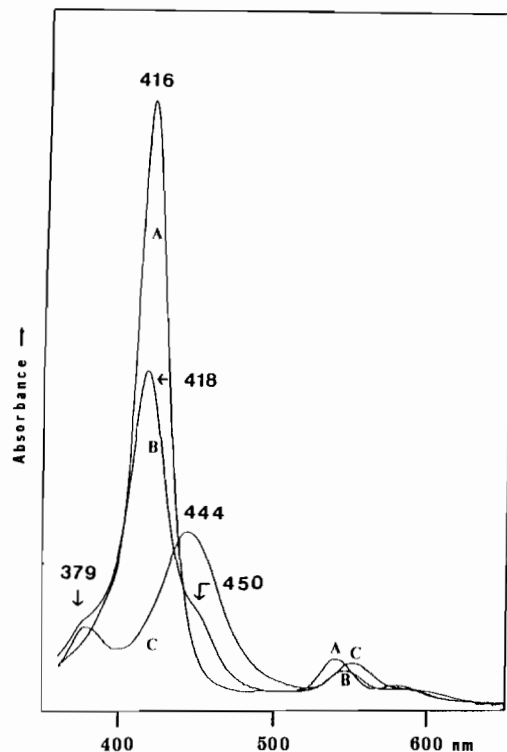

Fig. 2. UV–Vis spectra of four- and six-coordinate Ni(II) porphyrins in water: (A) Ni-1, (B) Ni-3, (C) Ni-2, (D) Ni-4.

Fig. 3. UV–Vis spectra of Cu-6 in (A) 50% aqueous ethanol, (B) water, (C) sodium phosphate–sodium chloride buffer (pH 6.8, $\mu = 0.18$).
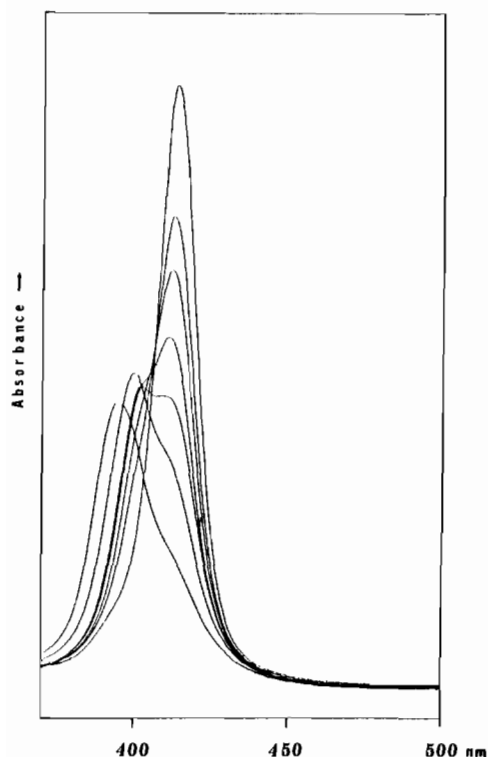
Fig. 4. UV–Vis spectra of Cu-7 in water and sodium chloride–sodium phosphate buffers (pH 6.8) at increasing ionic strength ($\mu \sim 0$; 0.05; 0.1; 0.2; 0.4; 0.8; 1.6 in the order of decreasing intensity at 414 nm).

hand, if water is replaced by 50% aqueous ethanol as solvent, the Soret band becomes narrower and stronger (Table 3). This is shown in Fig. 3 for Cu-6.

Similarly, Cu-7 shows only one strong and narrow Soret band at 414 nm in dilute aqueous solution. When the ionic strength is increased by adding phosphate buffer or other electrolytes, new bands appear which are blue-shifted relative to the original peak. In a series of experiments, the total concentration of Cu-7 was maintained constant at $\sim 4.4 \times 10^{-5}$ M, and the ionic strength was varied from ~ 0 to 1.6 (Fig. 4). It is clearly seen that these high-energy bands become more prominent at the expense of the original 414 nm band as the salt concentration is increased. In the low concentration range, up to $\mu \sim 0.1$, a shoulder appears at ~ 402 nm, and an isosbestic point is observed at ~ 407 nm. When the ionic strength is increased to $\mu \sim 0.4$, this shoulder develops into a separate maximum, but the spectral curves do not show isosbestic behavior anymore. At even higher salt concentrations, the maxima are further blue-shifted ($\lambda_{max} \sim 399$ nm for $\mu = 0.8$, 395 nm for $\mu = 1.6$), with significant band broadening. Bettelheim et al. [28] found very similar behavior of aqueous solutions of Cu-7 when the total porphyrin concentration was increased. They report a Soret band at 412 nm in very dilute

solution ($< 8 \times 10^{-7}$ M) and a second band at 393–398 nm at higher concentrations ($> 4 \times 10^{-4}$ M) and suggest an equilibrium involving an aggregated species. Similar to Cu-5 and Cu-6, the Soret band of Cu-7 becomes more intense when ethanol is added (Table 3).

Dimerization and even higher aggregation in aqueous solution are common phenomena among porphyrins and metalloporphyrins [27] and often lead to spectral changes such as those observed for Cu-4, 5, 6, 7 (Table 2), e.g. red or blue shift, hypochromicity and broadening of the Soret band. High ionic strength promotes association because the positive charges which would prevent a close approach are shielded by counterions. Organic solvents such as ethanol, acetone or ethylene glycol are well known for their ability to break up porphyrin aggregates into monomers. Thus, only the spectra reported in Table 3 (50% ethanol) for Cu-4, 5, 6, 7 correspond to the monomers; these compounds are already considerably aggregated in water (Cu-4 presumably less than the others), and even more in the buffer. For example, the aqueous solution of Cu-6 shows an absorption maximum at 418 nm and shoulders at 450 and 379 nm (Fig. 3, trace B). Comparison with traces A and C shows that the maximum is due to the monomer whereas the shoulders are due to the aggregate. The behavior of Cu-7 suggests an equilibrium between the monomer ($\lambda = 414$ nm) and one other species, probably a dimer ($\lambda = 402$ nm), in aqueous solutions containing not too high porphyrin and electrolyte concentrations (up to $\mu \sim 0.1$). Formation of several higher aggregates ($\lambda < 400$ nm) occurs at higher salt or total porphyrin concentrations. The absence of isosbestic points at $\mu > 0.1$ indicates that more than two species are present in such solutions.

Recently, Gibbs et al. [17] found that the metal-free porphyrins 5 and 6 in aqueous solution, like their Cu complexes discussed here, form aggregates upon increasing salt concentration, probably dimers in a rapid first step and higher oligomers in a second slower step. The observed spectral changes upon association were very similar to those found in this study. These workers [5] also noted that metal-free 7 in aqueous solution is aggregated where the metal-free 3 is not (although the latter is still subject to some controversy in the literature [29–32]).

It is well established that Cu-3 has a negligible tendency to dimerize or oligomerize in dilute aqueous solutions [21]. Neither Cu-3 nor its positional isomers, Cu-1 and Cu-2, shows marked spectral changes upon increase of the ionic strength (Table 2) or addition of alcohol (not included in Table 3). Thus, Cu-1 and Cu-2 also do not aggregate under our experimental conditions.

Replacement of the charged N-methylpyridyl by the phenyl groups strongly promotes the aggregation

tendency, as stated above. However, the decrease of coulombic repulsion between the charged groups is only one of the reasons for this behavior since Cu-7 also aggregates; 7 carries the same number of positive charges as 1, 2 and 3, which do not associate. The basicity of the porphyrin ligand is probably the more important factor; it seems that the tendency to form di- or oligomers increases along with the pK_a of the porphyrin (Table 1). This is in good agreement with the results of Pasternack et al. [21, 29] who found that tris(4-sulfonatophenyl)mono-phenylporphyrin, tetrakis(4-carboxylatophenyl)porphyrin (TCPP) and its Ni(II) and Cu(II) complexes have a high tendency to dimerize since both porphyrins are rather basic ($pK_a \sim 5-6$).

It is interesting to note that the spectral changes of the 'cis' and 'trans' isomers Cu-5 and Cu-6 upon addition of buffer are quite different (Table 2). The reason may be that di- and/or oligomers with different types of stacking are formed due to the different charge distribution. Unfortunately, very little information is available so far concerning the structure of porphyrin aggregates in solution.

Finally, we note that the behavior of the visible bands (500—600 nm) upon aggregation closely parallels that of the Soret bands, i.e. both Soret and visible bands are broadened and red-shifted. This is demonstrated in Fig. 3 for Cu-6.

Ni porphyrins: four- versus six-coordination

In aqueous solution, the four-coordinate, diamagnetic Ni-3 exists in equilibrium with a six-coordinate, paramagnetic species with two axially bound water ligands [22]. Whereas the former is characterized by a Soret band at 419 nm, i.e. at somewhat higher energy than the metal-free porphyrin (424 nm), the latter gives rise to a Soret absorption at 441 nm. It has been estimated from magnetic data that the equilibrium mixture contains about 45% four-coordinate and 55% diaquo form at room temperature [22]. An analogous splitting of the Soret band, one slightly blue-shifted and the other strongly red-shifted with respect to the free ligand, is also found for Ni-1 and Ni-2 (Fig. 2, traces A, B, C; Table 2). The former band (407/411 nm) is assigned to the square-planar and the latter band (427/432 nm) to the six-coordinate form of these metalloporphyrins. The intensity ratios of the Soret bands and hence the concentration ratios of both species vary with the porphyrin. Due to lack of magnetic data, it is at present not possible to give exact values for the relative concentrations for four- and six-coordinate species and, hence, exact ϵ values for both forms. However, by comparing the spectra of Ni-1 and Ni-2 with that of Ni-3 (Fig. 2) and assuming, as a first approximation, that the ratios of ϵ(four-coordinate):ϵ(six-coordinate) are similar for all three Ni porphyrins, we estimate

the following concentration ratios of four- to six-coordinate forms: Ni-1, 30:70; Ni-2, 55:45. It has been reported previously that Ni-1 is almost exclusively six-coordinate in water [13]; however, no spectral data were published.

The tendency of Ni porphyrins to add axial water ligands is closely related with the basicity of the porphyrin. A highly acidic porphyrin strongly withdraws electron density from the central ion, making it more electrophilic, i.e. more susceptible to a nucleophilic attack [21]. Thus, the relative amount of the Ni diaquo species decreases as the basicity of the free porphyrin increases in the order $1 < 3 < 2$ (Fig. 2, traces A, B, C). Consequently, in the spectrum of the Ni complex of a more basic porphyrin, 4, only one Soret band is found in the region of four-coordinate species (Fig. 2, trace D). However, it can be shown by RR spectroscopy that a small amount of the diaquo complex is still present in aqueous solutions of Ni-4 (see below). Obviously, the Soret band of six-coordinate Ni-4 is not resolved because it is too weak and hidden under the band of the four-coordinate form. For the Ni complex of the very basic porphyrin, 7, not even a trace of the six-coordinate species can be detected by RR spectroscopy. This is also true for Ni-5 and Ni-6; although the pK_a values of these porphyrins have not yet been determined, they are probably more basic than 4 because one more of the strongly electron-withdrawing N-methylpyridyl groups has been replaced by a phenyl group. In going from 3 to 4, such a replacement leads to an increase of the pK_a value by 1 unit (Table 1); therefore, 5 and 6 are expected to be about as basic as 7.

All six-coordinate Ni(II) porphyrins can be stripped of their axial water ligands by adding acetone, as first noted for Ni-3 [22]. In all cases, the high-energy band grows at the expense of the low-energy band. As expected, Ni-1 loses its water ligands less easily than Ni-2 and Ni-3; for a nearly quantitative conversion into the square-planar form, a 90:10 mixture of acetone and water is needed for Ni-1, whereas in the other cases a 30:70 mixture is sufficient. The addition of electron donors stronger than water, such as pyridine and imidazole, causes red shifts of the Soret bands of those Ni porphyrins which are normally four-coordinate in water. At a ratio of imidazole:porphyrin = 1000, the Soret bands of Ni-4, Ni-5 and Ni-6 are red-shifted by about 20, 7 and 4 nm, respectively, whereas no shift is shown by Ni-7. Evidently, equilibria involving the six-coordinate species $NiP(Im)_2$ and $NiP(Im)$-(H_2O) (P: porphyrin) exist, and the tendency to form these species decreases in the order $4 > 5 > 6 > 7$, as the basicity increases.

Not only the Soret bands, but also the visible bands of the four-coordinate Ni porphyrins are red-shifted upon addition of axial ligands. For Ni-1

through Ni-3 and water as axial ligand, this shift is approximately 1000 cm^{-1} for both the Soret and the visible bands.

Table 2 shows that the Ni complexes of the more basic porphyrins, 4, 5, 6 and 7, exhibit changes similar to those of the corresponding Cu porphyrins upon increasing ionic strength, thus suggesting aggregation. It is interesting to note that Ni-3 which is monomeric in water [22] undergoes dimerization in dimethylformamide, according to recent electrochemical studies by Kadish et al. [33].

Co porphyrins

Since the reaction products of Co(II) salts with the porphyrins 1, 2, 3 and 7 have been characterized as containing Co(III) and two axial water ligands [5, 23, 34, 35], it is reasonable to assume the same for Co-4, 5 and 6 because the UV–Vis spectra of these complexes are very similar.

The UV–Vis spectra of most of the Co(III) porphyrins show no or little dependence on electrolyte (Table 2) or ethanol concentration. Probably the axial water ligands prevent association. For a similar reason, the five-coordinate Zn(TCPP)(H$_2$O) does not dimerize, in contrast to the corresponding four-coordinate Ni and Cu porphyrins [21]. Thus, some small hypochromicity of the Soret bands of Co-1, 2 and 3 upon addition of buffer is more likely due to the well-known deprotonation equilibria [23] of the type CoP(H$_2$O)$_2$ ⇌ CoP(H$_2$O)(OH) + H$^+$.

In contrast to the observations of Ozer et al. [36] who noted precipitation at pH > 2, we found that Co-7 is soluble in water over the entire pH range. This may suggest that the sample used by these workers was not or only partially quaternized.

Resonance Raman Spectra

Typical resonance Raman (RR) spectra of the water-soluble metalloporphyrins (Soret excitation) are shown in Figs. 5 and 6. The RR frequencies of
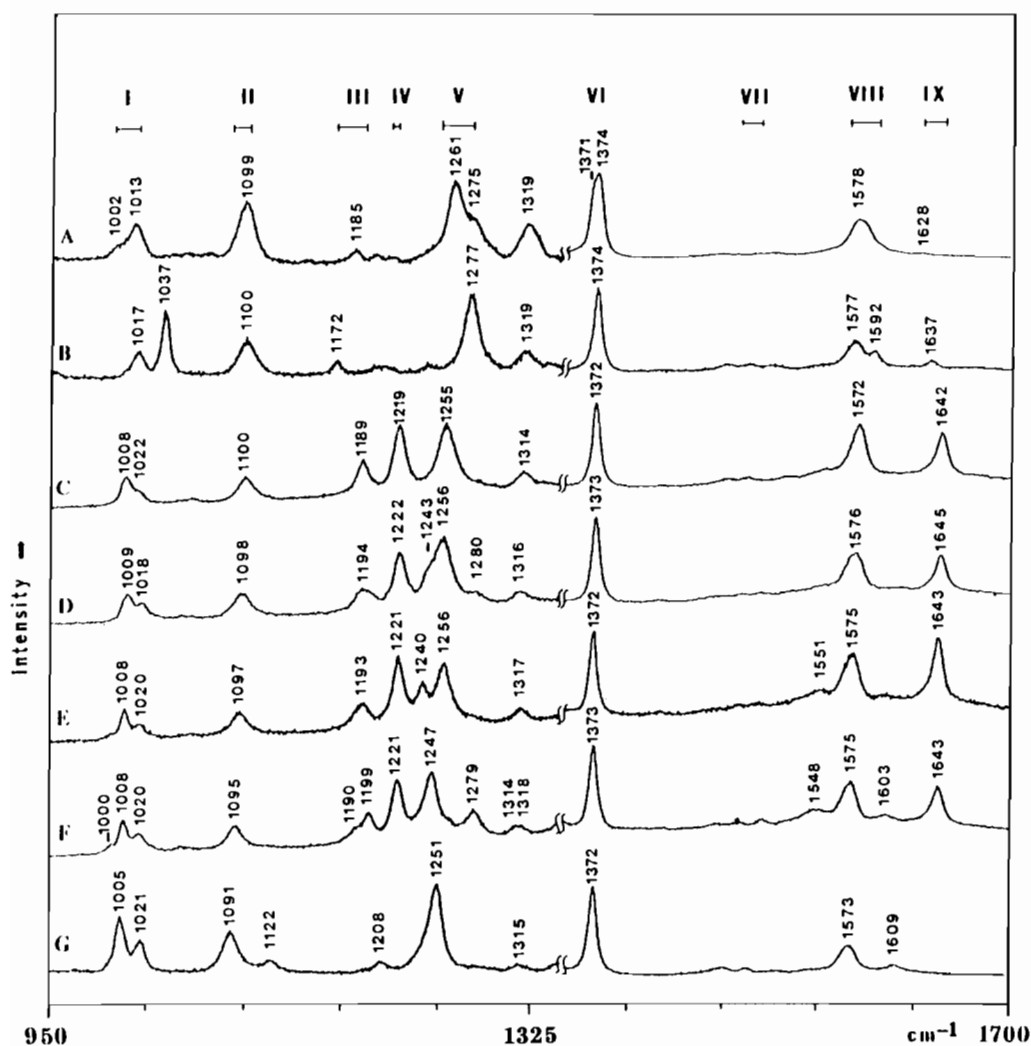

Fig. 5. RR spectra of water-soluble Co(III) porphyrins (aqueous solutions, ~2 × 10^{-5} M): (A) Co-1, (B) Co-2, (C) Co-3, (D) Co-4, (E) Co-5, (F) Co-6, (G) Co-7; λ_{exc} = 413.1 nm.

TABLE 4. RR frequencies (cm^{-1}) and band assignments of four-coordinate Ni(II) porphyrins

Ni-1	Ni-2	Ni-4	Ni-5	Ni-6	Ni-7	Assignment
			999m,sh			ph ring breathing
1010m	1014m	1007m	1008m	1006m	1007m	$\nu_s(C_\alpha-C_m)$, A_{1g} (I)
					1019w,sh	$\delta_{as}(C_\beta-H)$, B_{2g} or δ(ph)
	1037s					pyr ring breathing
1049w[a]		1052vw[a]	1051vw[a]		1051w[b]	δ(pyr)/δ(ph)
1070vw[a]		1060w[a]	1058vw[a]			δ(pyr)/δ(ph)
1100ms	1101s	1099m	1096m	1091m	1089m	$\delta(C_\beta-H)$, A_{1g} (II)
1145vw[a]					1122w[b]	δ(pyr)/δ(ph)
					1199w	δ(ph)
1186w[a]	1172mw[a]	1193m,br[a]	1188m,sh[a]	1188w,sh[a]		$\lbrace\delta$(pyr)/δ(ph) +
1206w[a]	1206m[a]		1192m[b]	1197m[b]		$\nu(N^+-CH_3)$, A_{1g} (III)
					1208w	$\delta(N^+(CH_3)_3)$
		1221ms	1222ms	1222s		δ(pyr) (IV)
			1238w[b]	1244m[c]	1249m[b]	$\lbrace\nu(C_m-pyr)$, $\nu(C_m-ph)$,
1260s[a]	1274s[a]	1256m[a]	1255s[a]			A_{1g} (V)
1276ms,sh		1278vw	1277vw	1277m		$\nu_s(C_\alpha-N)$, B_{1g}
1309s	1308s	1306w	1305w	1296w	1305w	$\nu_{as}(C_\alpha-C_\beta)$, B_{2g}
			1318w			$\nu_{as}(C_\alpha-C_\beta)$, B_{2g}
1378vs	1378s	1377s	1376ms	1375s	1376s	$\nu_s(C_\alpha-N)$, A_{1g} (VI)
1466vw		1425vw	1424vw			pyr ring stretch
1486vw	1479m	1475m	1473m	1476w	1473w	$\nu_s(C_\alpha-C_\beta)$, A_{1g} (VII)
1514w	1513m		1498w	1504vw		$\nu(C_\beta-C_\beta)$, B_{1g} or pyr ring stretch
1585s	1579s	1580vs	1578vs	1578s	1573s	$\nu(C_\beta-C_\beta)$, A_{1g} (VIII)
	1593s[a]		1601w[b]	1602w[b]	1609m[b]	pyr, ph ring stretch
1610w,sh	1612w,sh					pyr ring stretch
	1636m	1644s	1644s	1643s		pyr ring stretch (IX)

Abbreviations: s, strong; m, medium; w, weak; v, very; br, broad; sh, shoulder.
[a]Bands due to pyr (2-*N*-methylpyridyl)-, 3-*N*-methylpyridyl-, 4-*N*-methylpyridyl) groups.   [b]Bands due to ph (phenyl-, 4-*N,N,N*-trimethylaminophenyl) groups.   [c]$\nu(C_m-ph)$ + $\nu(C_m-pyr)$ coupled.
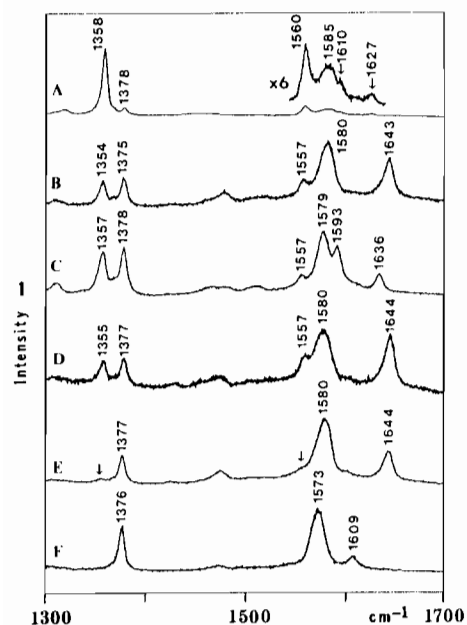

Fig. 6. RR spectra of four- and six-coordinate Ni(II) porphyrins (aqueous solutions, ~2 × 10^{-5} M): (A) Ni-1, (B) Ni-3, (C) Ni-2, (D) and (E) Ni-4, (F) Ni-7; λ_{exc} = 413.1 nm for A, B, C, E, F; 441.6 nm for D.

TABLE 5. RR frequencies (cm^{-1}) and band assignments of six-coordinate Ni porphyrins[a]

Ni-1	Ni-2	Ni-4	Assignment
1006	1012	1005	$\nu_s(C_\alpha-C_m)$, A_{1g} (I)
1094	1098	1096	$\delta(C_\beta-H)$, A_{1g} (II)
1185/1212			δ(pyr) + $\nu(N^+-CH_3)$, A_{1g} (III)
1317	1310		$\nu_{as}(C_\alpha-C_\beta)$, B_{2g}
1358	1357	1355	$\nu_s(C_\alpha-N)$, A_{1g} (VI)
1458	1465		$\nu_s(C_\alpha-C_\beta)$, A_{1g} (VII)
1560	1557	1557	$\nu(C_\beta-C_\beta)$, A_{1g} (VIII)
1595			pyr ring stretch
1627[b]			pyr ring stretch (IX)

[a]Only those frequencies are listed for which a difference between the four- and six-coordinate form was found.
[b]Not observed for four-coordinate form.

the Ni complexes are listed in Tables 4 and 5 together with empirical band assignments which are based on those given previously for related porphyrins [37–42], for benzene, pyridine, the pyridinium ion and their derivatives [43–48]. The assignments for the Co(III) porphyrins are analogous to those for the Ni complexes. The spectra of the

Cu porphyrins are not shown since they are very similar to those of the Co and Ni complexes. See also 'Supplementary Material'.

Since extensive vibrational coupling occurs throughout the whole porphyrin ring system [37–41], all the assignments denote only those coordinates which make main contributions to a given normal mode. The following discussion will be limited to the frequency range between 950 and 1700 cm^{-1} since the major structure-sensitive bands are found in that region. As reported previously [42], metal complexes of 3 exhibit nine major RR bands (Bands I to IX) in this frequency range. In order to identify those bands of the metal complexes of the porphyrins 1, 2, 4, 5, 6 and 7 which correspond to the nine key bands of 3 derivatives, we use the assignments and notations of Blom et al. [42] in this paper (Fig. 5, Tables 4, 5).

A survey of Figs. 5 and 6 and Tables 4 and 5 shows that the RR bands of the water-soluble metalloporphyrins can, as a first approximation, be divided into two groups: vibrations of the porphyrin core (tetrapyrrole system) which are common to all the porphyrins, and internal vibrations of the phenyl(ph)/N-methylpyridyl(pyr) meso substituents.

Porphyrin core vibrations

All the water-soluble metalloporphyrins exhibit RR bands of medium to very high intensity near 1000 (Band I), 1100 (Band II) and 1370 (Band VI) cm^{-1} which show very little dependence on the meso substituent and, thus, are mainly vibrations of the tetrapyrrole system. Traces D, E, F and G of Fig. 5 show that Band II ($\delta(C_\beta-H)$) is continuously shifted towards lower frequency as the 4-N-methylpyridyl groups are replaced by the phenyl (and 4-N,N,N-trimethylaminophenyl) groups. Among other reasons, this may reflect an effect of the orientation of the aromatic meso substituents. From X-ray crystallography work it is known that the phenyl rings in tetraphenylporphyrin (TPP) derivatives are almost perpendicular to the tetrapyrrole plane, with angles near 80°, whereas in 3 this angle is only 66–72° [49–51]. Thus, the steric hindrance experienced by the β hydrogens of the porphyrin core should decrease in going from 3 to 4 to 5/6 to TPP. As the steric hindrance decreases, the frequency decreases.

The narrowest range of variation is observed for Band VI which is assigned to the $C_\alpha-N$ stretching mode; its position depends only on the central ion and its coordination number. For all Cu(II) porphyrins investigated by us it is found at 1365–1368 cm^{-1}; for four-coordinate Ni(II), at 1375–1378 cm^{-1}; for six-coordinate Ni(II), at 1354–1358 cm^{-1}; and for Co(III), at 1372–1375 cm^{-1}.

Other porphyrin core vibrations are found near 1250 (Band V), 1475 (Band VII; very weak in Co

porphyrins (Fig. 5), but stronger in the Cu and Ni complexes) and near 1575 (Band VIII) cm^{-1}. However, they are not always clearly distinguished from the ph/pyr in-plane C–H bending modes (~1150–1300 cm^{-1}) and the ph/pyr ring stretches (~1420–1650 cm^{-1}), since a certain degree of coupling is expected between them [41]. These regions will be discussed in more detail below.

Resonance Raman spectra of the positional isomers 1, 2, 3

Traces A, B and C of Fig. 5 show the RR spectra of the Co(III) complexes of 1, 2 and 3 taken by Soret excitation. The $\delta(pyr)$ vibrations at 1180–1300 cm^{-1} and the $\nu(pyr)$ vibrations at ~1600–1650 cm^{-1} (Band IX) are sensitive to the substitution pattern and responsible for the main differences in the spectra. There seems to be a tendency that those vibrations which are mainly pyr in character become weaker as the N-methylpyridyl group is moved closer to the porphyrin core. Thus, the pyr vibrations of the metal complexes of 3 near 1190, 1220 and 1645 cm^{-1} are in their intensity comparable to the porphyrin core vibrations, whereas the corresponding bands in 1 derivatives are very weak, so that the spectrum shows almost exclusively porphyrin vibrations. 2 complexes are intermediate between 1 and 3. It is interesting to note that the derivatives of 2 exhibit a very intensive ring-breathing mode at ~1037 cm^{-1} which is characteristic of meta substitution [43–45].

To explain the resonance enhancement of pyr vibrations by Soret excitation, it is necessary to assume some π overlap between the pyridyl group and the porphyrin core. This overlap should be more effective if it is possible for the meso substituent to rotate into the plane. In 1 and its complexes, such a rotation is effectively prevented by the N-methyl group located at the ortho position. The rotation barriers in the meta (2) and para (3) isomers should be much lower and allow an increasing amount of $\pi-\pi$ overlap.

Phenyl versus N-methylpyridyl groups

Traces D, E, F and G of Fig. 5 show that the band patterns in the regions ~1150–1300 and ~1550–1650 cm^{-1} change not only when the methylated nitrogen atom is moved away from the porphyrin core, but also when the N-methylpyridyl groups are replaced by the phenyl groups. We will focus on the former range since the ph/pyr ring stretches in the latter range are very weak in the RR spectrum, with the exception of the 1645 cm^{-1} band, IX.

Metal derivatives of 3 show a characteristic pattern of three bands near 1190 (Band III), 1220 (Band IV) and 1255 (Band V) cm^{-1} [42]. When the phenyl groups are introduced, Band IV, which is assigned

to $\delta(pyr)$ (= pyr in-plane CH bending), remains nearly unchanged. Band III, $\delta(pyr) + \nu(N^+–CH_3)$ [42, 47], is broadened and eventually split into two components, one probably corresponding to $\delta(pyr)$ and the other to $\delta(ph)$. Band V, near 1255 cm^{-1}, is the counterpart of the strong band at 1230–1240 cm^{-1} in metal tetraphenylporphyrins [37–41] and near 1250 cm^{-1} in metal complexes of 7. In the TPP system, it has been assigned to either $\nu(C_m$–phenyl) [37, 40] or an internal phenyl mode [38]; a recent normal coordinate treatment has shown that this band is mainly $\nu(C_m$–phenyl) in character [41], confirming the original assignment [37]. By analogy, the band of 3 complexes at ~1255 cm^{-1} is assigned to $\nu(C_m$–pyr) [42]. The metal complexes of 4 and 5 exhibit both $\nu(C_m$–ph) near 1240 cm^{-1} (as shoulder in 4, as resolved band in 5 derivatives) and $\nu(C_m$–pyr) near 1255 cm^{-1} (unshifted with respect to 3). The '*trans*' porphyrin 6 exhibits two bands at 1247 and 1279 cm^{-1} which may correspond to $\nu(C_m$–phenyl) and $\nu(C_m$–pyr), respectively. These frequencies are markedly different from those of the '*cis*' isomer because the nature of vibrational coupling with porphyrin core modes is different between the isomers.

The RR spectra of the metal complexes of 7 (Fig. 5, trace G) are, as expected, quite similar to those of tetraphenylporphyrins [37–41]. A weak band at 1208 cm^{-1} is probably not due to a C–H in-plane bending mode of the aromatic ring but rather to an internal deformation of the $N^+(CH_3)_3$ group since a similar band at 1200–1220 cm^{-1} is observed for the $C(CH_3)_3$ group [45]. The bands to which mainly the phenyl groups contribute (for example the ring stretch near 1609 cm^{-1}) are much weaker than the pyridyl vibrations in 3 complexes; thus, the π systems of the phenyl groups and the core are only weakly coupled in this porphyrin, and the angle between these planes is probably close to 90° due to the bulky *para* substituent.

Four- versus six-coordination in Ni porphyrins

As mentioned above, an equilibrium of the type $NiP + 2H_2O \rightleftharpoons NiP(H_2O)_2$ is a characteristic feature of some water-soluble Ni(II) porphyrins. We have shown previously [42] that the vibrations of four-coordinate Ni-3 ($\lambda_{max} \sim 419$ nm) are selectively enhanced by 406.7 nm excitation whereas the vibrations of the corresponding diaquo species ($\lambda_{max} \sim 441$ nm) are selectively enhanced by 441.6 or 457.9 nm excitation. The most notable changes are exhibited by Bands VI ($\nu(C_\alpha$–N), ~1370 cm^{-1}), VII ($\nu_s(C_\alpha$–C$_\beta$), ~1480 cm^{-1}) and VIII ($\nu(C_\alpha$–N), ~1580 cm^{-1}) which are all shifted downward by ~20 cm^{-1} upon going from four- to six-coordination.

In the RR spectra of the positional isomer, Ni-2, obtained by 413 nm excitation, strong bands are observed at 1378 and 1579/1593 cm^{-1} and other bands at 1357 and 1557 cm^{-1} (Fig. 6, trace C). The intensity of the latter two bands increases at the expense of the former when laser lines of longer wavelength are used to excite the spectra, and they disappear nearly completely upon addition of acetone to the solution. Thus, the behavior of Ni-2 is almost identical to that of Ni-3 [8, 42] (the only difference being a small difference in the relative amounts of four- and six-coordinate forms, see above), confirming the assignment of the 411 nm absorption band to the square-planar and of the 432 nm band (Fig. 2, trace C) to the diaquo form. The splitting of Band VIII of four-coordinate Ni-2 near 1580 cm^{-1} is probably due to coupling with a pyridyl ring stretch. Several other bands seem to be sensitive to the coordination number as well, but most of them are too broad and their shifts too small to permit resolution.

As discussed earlier, two bands are also found in the Soret region of Ni-1 (Fig. 2, trace A). The higher intensity of the 427 nm absorption relative to the 407 nm band suggests that the equilibrium mixture contains more six- than four-coordinate form, in contrast to Ni-2 and Ni-3. This is confirmed by the RR spectra (Fig. 6, trace A). In the $\nu(C_\alpha$–N) region, the 1358 cm^{-1} band of the diaquo form is much stronger than the 1378 cm^{-1} band of the square-planar complex obtained by 406.7 nm excitation, and it becomes even stronger than the latter when 413.1 or 457.9 nm lines are used. The band pattern around 1600 cm^{-1} is more complicated for Ni-1 than for its isomers. It consists of at least five bands (Tables 4, 5), three of which (1560, 1595 and 1627 cm^{-1}) are promoted under conditions favoring enhancement of the six-coordinate species (i.e. excitation with 457.9 nm) whereas the other two (1585 and 1610 cm^{-1}) become more intense at 406.7 nm excitation and upon adding acetone. The strongest of these bands, 1560 and 1585 cm^{-1}, respectively, are presumably due to $\nu(C_\beta$–C$_\beta$) of the pyrroles while the weaker ones represent different pyridyl ring stretches. The 1595 cm^{-1} band is too weak at 413.1 nm excitation to be seen in Fig. 6. By using 457.9 nm excitation for the diaquo complex and by using 406.7 nm excitation for a solution of Ni-1 in acetone:water = 9:1 (which contains almost exclusively the four-coordinate form) we were able to observe and assign most of the other bands of both forms. A comparison of Tables 4 and 5 shows that the major bands near 1010, 1100, 1210 and 1310 cm^{-1} are structure-sensitive. In particular, we note that Band II near 1100 cm^{-1} which is known as an intercalation marker in 3 derivatives [8] is upshifted by 6 cm^{-1} when the six-coordinate form loses its water ligands. This shift is of the same magnitude as that observed for this band when Cu- or Ni-3 is intercalated at the

G–C site. Analogous, but smaller shifts by $+3$ cm^{-1} upon going from four- to six-coordination are observed for Ni-2 (Tables 4, 5) and Ni-3 [8].

As stated earlier, Ni-4 exhibits only one Soret band at a frequency near the absorption maximum of four-coordinate Ni-3 (see Fig. 2, trace D). Consequently, the RR spectra obtained by 406.7 or 413.1 nm excitation show strong bands at 1377 and 1580 cm^{-1}, characteristic of four-coordination (Fig. 6, trace E). However, a weak band is still observed at 1355 cm^{-1}. At 441.6 or 457.9 nm excitation, this band gains intensity, a new band arises at 1557 cm^{-1} (Fig. 6, trace D), and the bands near 1000 and 1100 cm^{-1} are downshifted (Tables 4, 5). Clearly, a small amount of diaquo complex is present in aqueous solution. For Ni-5, Ni-6 (not shown) and Ni-7 (Fig. 6, trace F), no bands attributable to six-coordination can be detected, even by using 441.6 or 457.9 nm excitation. As discussed above, the porphyrins 5, 6 and 7 are very basic so that their Ni complexes are exclusively four-coordinate in water.

Supplementary Material

Complete listings of the RR frequencies and band assignments of all the Cu(II), Ni(II) and Co(III) porphyrins are available from the authors on request.

Acknowledgements

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