Allosteric Ligand Binding to Cofacial Metalloporphyrin Dimers: the Mechanism of Porphyrin Disaggregation

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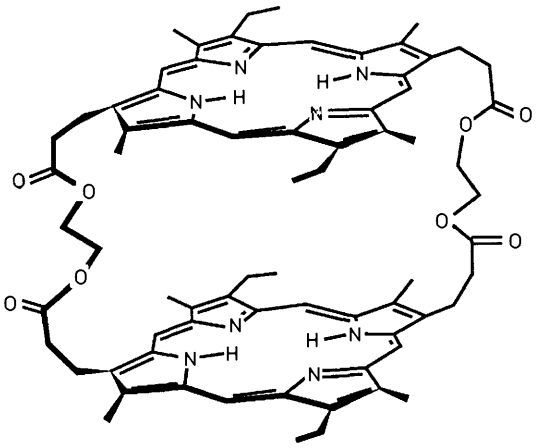
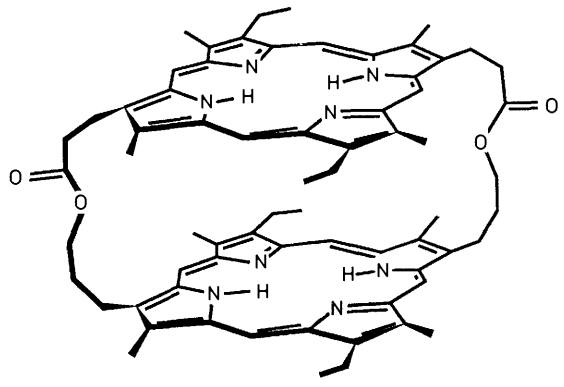
Intramolecular π–π interactions in two cofacial zinc porphyrin dimers have been probed as a model for porphyrin–porphyrin aggregation. Comparative studies of the binding of substituted pyridines using n.m.r., u.v./visible absorption and emission spectroscopy have allowed separation of the steric and electronic contributions to disaggregation and have shown that disruption of the π–π interaction in metalloporphyrins by ligands is mainly a steric effect. The electronic effect of ligand co-ordination is to moderate the interaction slightly by reducing the polarisation of the porphyrin by the metal. When ligand binding to a dimer causes disaggregation, the process of binding is allosteric, the second ligand binding being stronger than the first.

The creation of fully synthetic enzymes based on porphyrins requires that we understand the steric and electronic factors controlling their conformations and reactivity.[1,2] In this paper we address the question of porphyrin-porphyrin π–π interactions, which are a poorly understood aspect of this wider problem.[3] In an effort to develop a model for π-stacking phenomena, we designed two porphyrin cofacial dimers, (**1**) and (**2**), with flexible bridging chains. The synthesis and properties of the free base and zinc derivatives were recently reported,[3c] and it will be necessary to reproduce some spectroscopic data from that report to develop our arguments coherently in his paper. We have used these dimers to investigate how metallation and co-ordination of the central metal ion affects porphyrin π–π interactions.

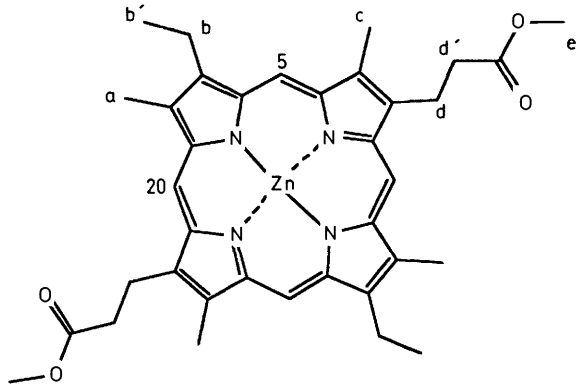


(**1**)



(**2**)

We have used three techniques to monitor the porphyrin–porphyrin interaction in the dimers: u.v./visible absorption, emission and n.m.r. spectroscopy. The first two techniques used *ca.* 10^{-6} M solutions, while the n.m.r. solutions were *ca.* 10^{-3} M. These solutions are all sufficiently dilute that intermolecular interactions are negligible; any evidence found for porphyrin aggregation is due to intramolecular interactions. In addition, measurements were made relative to, or may be compared with the control, meso-II-porphyrin dimethyl ester [denoted (**3**)] or its zinc derivative [denoted Zn(**3**)], so that any effects observed



Zn(**3**)

are solely due to porphyrin–porphyrin interactions. It should be further noted that in the following discussion, unless otherwise indicated, addition of pyridine refers to the addition of a large excess of pyridine so that the porphyrins are fully bound.

Absorption spectroscopy can be used to determine the relative orientation and proximity of the two porphyrin moieties by the presence or absence of exciton coupling, and the co-ordination state of the zinc in the metalloporphyrin. When the zinc becomes five-co-ordinate a characteristic blue shift in the wavelength of the Soret band occurs; the magnitude of the shift depends on the basicity of the ligand and whether oxygen or nitrogen binds to the metal. For example, pyridine binding

causes a blue shift of 10—11 nm. Thus it is possible to examine simultaneously ligand binding and disaggregation by following changes in the absorption spectrum during titration of the metallated dimers with various ligands.

In these titrations it is not possible to measure independently the absorption of the mono-pyridine adducts. This can lead to large errors in the interpretation of such data when dealing with sequential binding at a single site.[4] However, in this work we have two separate sites whose properties, before any ligand binds, are identical. Thus, as a starting point for analysis, we assume that, to a good approximation, the extinction coefficients of the mono-pyridine adducts at any wavelength are given by equation (1). The absorption of the dimer is, in effect,

$$\varepsilon_{\text{monopyridine adduct}} = \tfrac{1}{2}(\varepsilon_{\text{free dimer}}) + \tfrac{1}{2}(\varepsilon_{\text{bispyridine adduct}}) \quad (1)$$

the sum of the absorptions of the two individual porphyrin units within the dimer. We show below that further justification for this assumption comes from curve-fitting routines: using the above values for the mono-pyridine adduct extinction coefficients generally yields a considerably better fit to the data than any other values.

The binding data can be used to determine whether or not there is any co-operativity in the binding of the ligands. If there is no co-operativity, then we can treat the problem as a simple single-site binding problem because the two binding sites behave independently and identically. Equation (2) then holds

$$\ln\{(A - A_0)/(A_f - A)\} = x\ln([\text{free ligand}]) + \ln(K) \quad (2)$$

where A is the absorption at a particular wavelength (λ), A_0 is the initial absorption at λ, A_f is the final absorption at λ, K is the binding constant, and x is a constant defining the number of ligands bound per site.

Thus a plot of $\ln\{(A-A_0)/(A_f-A)\}$ *vs.* ln([free ligand]) should yield a straight line of slope, x, of 1 for independent, identical binding at the two sites; this is a Hill plot.[5] Co-operative binding, where the second binding is aided by the first gives a slope >1, while negative co-operativity gives a slope of <1. In cases where binding at the two sites is not identical and independent, then equation (1) does not necessarily hold; the data were then analysed by a least-squares curve-fitting routine to ensure that accurate values for the binding constants were obtained. In simpler systems, these curve-fitting results merely confirmed those obtained by the above analysis.

We used two additional spectroscopic techniques to investigate ligand binding by these porphyrin dimers. Their emission (fluorescence) spectra were used to determine whether or not the porphyrin moieties are close in space, since proximity of the two porphyrins leads to fluorescence quenching.[3c] Finally, ^{1}H and ^{13}C n.m.r. studies of these systems provided independent information about the geometries of the porphyrin–porphyrin interactions. The use of n.m.r. in this way has been described in detail elsewhere.[3c,6]

Results

U.v./Visible Absorption Spectra.—Absorption spectra of co-facial porphyrin dimers show clear signs of exciton coupling of the two chromophores.[3c,7] (**1**) and (**2**), and their zinc derivatives are no exceptions, and their Soret bands are broadened, hypochromic, and hypsochromically shifted relative to (**3**) and Zn(**3**) (Table 1). This indicates that the two porphyrin planes are parallel and in close proximity, presumably as a result of strong π–π interaction between them.

These interactions can be abolished by the addition of trifluoroacetic acid to the free base dimers. The protonated porphyrin rings repel each other and move to their maximum

Table 1. Electronic absorption and emission properties of porphyrins

Compound	U.v./visible absorption spectra[a] Soret band $\lambda_{max.}$/nm ($\varepsilon \times 10^{-3}$)	FWHM[c] (nm)	Emission spectra[b] λ_{em}(nm)	Φ_f(rel)[d]
Free Base				
(**1**)	387 (182)	57	626	0.47
(**2**)	385 (192)	58	624	0.54
(**3**)	397 (164)	39	621	1.0
Cations				
H_4(**1**)$^{4+}$	401 (601)	14	598	0.79
H_4(**2**)$^{4+}$	397 (610)	19	596	0.71
H_2(**3**)$^{2+}$	401 (406)	12	596	1.0
Zinc derivatives				
Zn_2(**1**)	388 (272)	28	586	0.15
Zn_2(**2**)	388 (166)	31	581	0.20
Zn(**3**)	402 (314)	13	572	1.0
Zinc derivatives + pyridines				
Zn_2(**1**) + Py	409 (461)	15	583	0.96
Zn_2(**2**) + Py	408 (200)	28	585	0.37
Zn(**3**) + Py	413 (318)	12	582	1.0
Zn_2(**1**) + 4-EtPy	411 (385)	19	580	0.56
Zn_2(**1**) + 4-Bu^{t}Py	411 (326)	21	580	0.68

[a] In dichloromethane solution. [b] Recorded in dichloromethane solution at concentrations of *ca.* 10^{-6} M with irradiation at the Soret maximum. [c] Full width at half height of the maximum absorption of the Soret band. [d] Fluorescence emission per porphyrin moiety, relative to (**3**) or the corresponding derivative as appropriate.

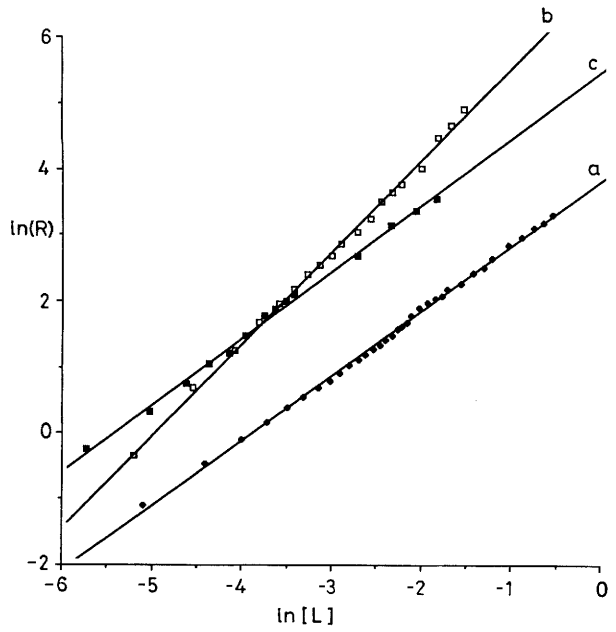


Figure 1. Hill plots for the binding of pyridines to Zn_2(**1**) and Zn_2(**2**): (a) Zn_2(**2**) + pyridine, (b) Zn_2(**1**) + pyridine, (c) Zn_2(**1**) + 4-t-butylpyridine. Data are taken from the Soret absorption of the porphyrin, where R = $(A-A_0)/(A_f-A)$ and [L] is the concentration of added ligand

possible separation, so the exciton coupling should be reduced to the minimum allowed by the length of the connecting chain. Indeed exciton coupling is effectively abolished for (**1**), but the short bridging chains of (**2**) hold the porphyrins closer together so that weak exciton coupling is observed.

Addition of pyridine to Zn_2(**1**) abolishes much of the exciton coupling and produces an absorption spectrum that is virtually normal, showing that we have successfully disaggregated the porphyrins. However, addition of pyridine to Zn_2(**2**) reduces the exciton coupling seen in the Soret band much less dramatically; we have not disaggregated the two porphyrins.

There is essentially no structural difference between the two dimers apart from the length of the bridging chains and the size of the potential cavity between the two porphyrin faces. We reasoned that the difference in behaviour of the two systems must be due to the ability of the ligand to enter the cavity between the two porphyrins and disrupt the π–π stacking. CPK models suggested that pyridine was indeed too big to fit inside Zn_2(**2**) but should fit inside Zn_2(**1**). We decided, therefore, to investigate the effects of binding more bulky pyridines.

Models indicated that 4-substituted pyridines would not fit inside Zn_2(**1**). The results for binding 4-t-butyl- and 4-ethylpyridine to Zn(**1**) show that these ligands are indeed less effective in disrupting the porphyrin aggregation: the Soret bands still show considerable exciton coupling (Table 1). There is little electronic effect involved in using this range of pyridines [pK_b(Py) = 8.64 and pK_b(4-EtPy) = pK_b(4-Bu^{t}Py) = 8.00]; indeed the substituted pyridines should bind more strongly to the zinc, so that the observed effects must be purely steric.*

Plots of the titrations of Zn_2(**2**) and Zn_2(**1**) with pyridine, and of Zn_2(**1**) with 4-t-butylpyridine are shown in Figure 1, and the results are summarised in Table 2. For the Zn_2(**2**)·pyridine and Zn_2(**1**)·hindered-pyridine systems there is a simple binding process at each site, the slope being one. Curve-fitting analysis reveals that, as expected for simple two-site systems with non co-operative binding, $K_1 = 4K_2$. However, the Zn_2(**1**)·pyridine system behaves very differently. The slope of the Hill plot is 1.39 because there is co-operativity in the binding process: the fact that the first site is co-ordinated to pyridine increases binding to the second site by a factor of *ca.* 7. This is, in fact, an artificial allosteric system with a surprisingly large Hill coefficient.[5]

* Water is present in most samples, but has a low affinity for zinc and it should not compete significantly with pyridine. Titrations were repeated three times with variable amounts of water but the results were identical.

Table 2[a] Binding properties of porphyrins

Compound	Ligand	K_1[b] (M^{-1})	K_2[c] (M^{-1})	Hill coefficient
Zn_2(**1**)	Pyridine	105	202	1.39
Zn_2(**1**)	4-t-Butylpyridine	426	115	1.01
Zn_2(**2**)	Pyridine	105	23	0.99

[a] The errors are *ca.* 5%. [b] K_1 = [mono-Py adduct]/([Py][free dimer]). [c] K_2 = [bis-Py adduct]/([Py][mono-py adduct]).

The very large change in the absorption spectrum of Zn_2(**1**) resulting from disaggregation on pyridine-binding raises the question of whether the change results from binding the first or the second ligand molecule. We can estimate the effect of binding without disaggregation by comparison with the other compounds in this study, and then attempt curve-fitting with the disaggregation effects added either at the first or second binding stage. The best fit is obtained if the absorption properties of the mono-pyridine adduct are midway between those of the pyridine-free dimer and bis-adduct [*i.e.* equation (1) is, coincidentally, applicable for this molecule]. It seems, then, that both steps in pyridine binding contribute to the intramolecular disaggregation of Zn_2(**1**). However, this result cannot tell us exactly how much the two porphyrin moieties are separated in the mono-adduct, or what proportion of the molecules are separated rather than aggregated.

Emission Spectra.—A characteristic feature of the emission spectra of porphyrin dimers is that the fluorescence is strongly shifted and significantly quenched by the chromophore–chromophore interactions. These interactions are disrupted in the free base compounds (**1**) and (**2**) by addition of trifluoroacetic acid (Table 1), which forces the two porphyrin entities to their maximum separation.[3c] The substantial increase in fluorescence in both dimers on protonation again shows that we have largely effected disaggregation.

The zinc dimers also show fluorescence quenching; in fact they show a substantial increase in quenching compared with the free base compounds, indicating that, as expected, the π–π interaction has been enhanced by metallation.[1,3] However, a remarkable difference in behaviour between Zn_2(**1**) and Zn_2(**2**) is seen in their emission properties on addition of pyridine. Addition of pyridine to Zn(**3**) causes a 50% decrease in the intensity of fluorescence; this is a standard feature of metalloporphyrin fluorescence, and is in marked contrast to the behaviour of Zn_2(**1**) (Table 1 and Figure 2). Fluorescence quenching is completely abolished on co-ordination of Zn_2(**1**), again indicating that complete disaggregation has been achieved. As expected from the absorption results above, Zn_2(**2**) shows a small reduction in the degree of fluorescence quenching; despite co-ordination by pyridine, the chromophores show only a slight weakening in their interaction. Similarly the addition of 4-t-butyl- or 4-ethyl-pyridine to Zn_2(**1**) does not completely abolish the fluorescence quenching.

Table 3. ^{1}H N.m.r. chemical shifts for Zn_2(**1**) + various pyridines

Proton	Zn(**3**) + Pyridine	Zn_2(**1**)	+Py	+4-Bu^{t}Py	+4-EtPy
5-H	10.11	9.18	9.57	9.50	9.48
20-H	10.09	8.35	9.30	8.93	8.92
H_e	—	4.51	3.84	3.93	3.92
H_b	4.10	4.03	3.90	4.04	4.04
H_d	4.37	2.84	3.55	2.63	2.68
H_a	3.62	3.14	3.35	3.40	3.40
H_c	3.57	2.77	3.02	2.49	2.53
$H_{d'}$	3.28	2.74	2.69	2.27	2.31
$H_{b'}$	1.87	1.78	1.72	1.90	1.89

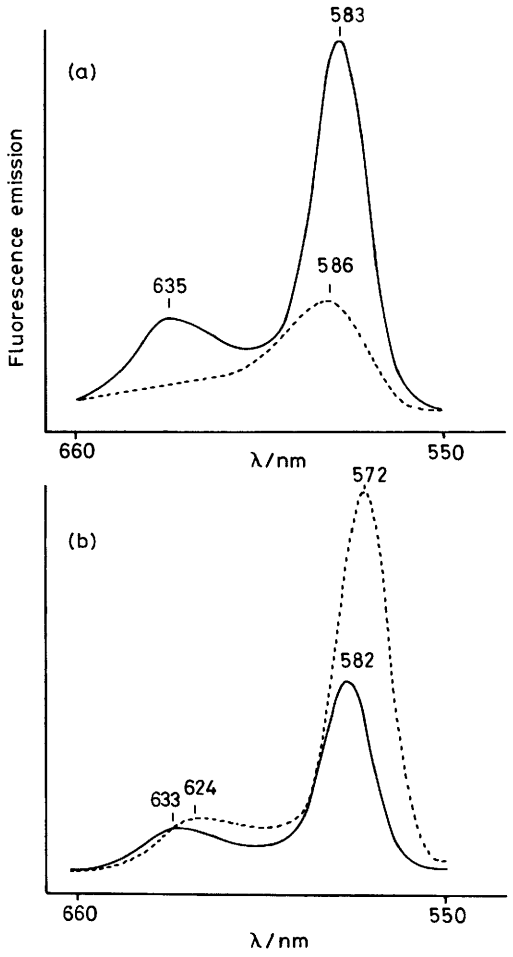


Figure 2. Fluorescence emission spectra: (a) ----- Zn_2(**1**) and ——— Zn_2(**1**) + pyridine; (b) ----- Zn(**3**) and ——— Zn(**3**) + pyridine

N.m.r. Spectra.—In the n.m.r. spectra of Zn_2(**1**) and Zn_2(**2**) we observe large ring current-induced shifts in the dimer signals indicating close, cofacial proximity (Table 3). Addition of pyridine to Zn_2(**1**) dramatically reduces these upfield shifts. The ring-current shifts for the Zn_2(**1**) pyridine complex were fitted to a best geometry using a porphyrin ring-current model.[3c,6] The parameterisation of the model and the limitations of the approach have been described in detail previously.[6] The calculations gives a good fit (Table 4) to the geometry described by a displacement $x = 3.5$ Å, $y = 2.0$ Å, $z = 6.0$ Å of the centre of one porphyrin relative to the centre of the other. The r.m.s. error is 0.06 p.p.m. and the agreement factor, AF, is 0.12. However, in the calculation we cannot distinguish a single geometry with

Table 4. Observed and calculated best-fit chemical shifts for Zn_2(**1**)·pyridine adduct[a]

Proton	Observed	Calculated
5-H	0.40	0.49
20-H	0.70	0.77
H_b	0.20	0.13
H_d	0.82	0.85
H_a	0.27	0.30
H_c	0.55	0.56
$H_{d'}$	0.59	0.47
$H_{b'}$	0.15	0.18

[a] P.p.m. upfield shift relative to Zn(**3**)·pyridine adduct.

$z = 6$ Å, a family of conformations with $4 < z < 8$ Å, or an equilibrium between two well defined geometries with $z = 4$ Å and $z = 8$ Å. A similar calculation for Zn_2(**1**) in the absence of ligand gives a displacement of $x = 3.0$ Å, $y = 2.0$ Å, $z = 4.0$ Å.[3c] Hence from these calculations we can conclude that the effect of binding pyridine is to increase the vertical separation of the rings while maintaining a similar displacement in the xy plane.

Such a detailed study of pyridine binding to Zn_2(**2**) was not possible since this compound has lower symmetry and so the spectrum is more complicated. Nevertheless, the addition of pyridine to Zn_2(**2**) changes the spectrum only slightly, indicating only minor changes in the average geometry.

The titrations of Zn_2(**1**) with pyridine, 4-ethyl- and 4-t-butylpyridine were followed by ^{1}H n.m.r. spectrometry (Table 3). We do not see large upfield shifts of the pyridine signals in the ^{1}H n.m.r. spectrum, even when it is binding inside the cavity, because the pyridine signals are in fast exchange, the binding constant is small, and the solutions used were *ca.* 10^{-3}M. There is always a vast excess of free compared with bound pyridine, so the resultant upfield shifts are too small to be be of any diagnostic value.

The final porphyrin chemical shifts of the fully bound species in the n.m.r. titration (Table 3) clearly indicate that the hindered pyridine adducts have a radically different geometry from the pyridine adduct and that the two porphyrins are still in close proximity. This is because the substituted pyridines are too large to fit into the cavity and so cannot disrupt the porphyrin–porphyrin interaction. The geometry of the interaction is, however, altered when a hindered pyridine is bound. From the chemical shifts in Table 3 it is possible to describe this new geometry fairly clearly. Relative to Zn_2(**1**), in the hindered pyridine adducts H_d, $H_{d'}$, H_c, and H_e are shifted upfield (*i.e.* this part of one porphyrin moves over the face of the other porphyrin and experiences a greater ring current), while the other protons are shifted downfield. Most noticeably, $H_{b'}$ in these adducts experiences a small downfield shift. The porphyrins must thus be further offset approximately along the direction of the bridging chains. Addition of an excess of

pyridine to the 4-t-butylpyridine adduct again changes the conformation and yields the fully disaggregated dimer as expected.

Discussion

These results all indicate that the binding of pyridine to Zn_2(**2**) slightly reduces the π–π interaction between the two porphyrin entities. Similarly, binding of 4-alkylpyridines to Zn_2(**1**) disrupts the π–π interaction to a small extent. In contrast, binding pyridine to Zn_2(**1**) effects complete disaggregation. The conclusion we draw from this is that disaggregation of metalloporphyrins by ligand co-ordination is mainly a steric effect. The rate of ligand exchange is so fast that, despite zinc being exclusively 5-co-ordinate in porphyrins, there are effectively pyridine ligands on both porphyrin faces. Thus pyridine co-ordination blocks both porphyrin faces and prevents any π–π interaction. The n.m.r. data on the Zn_2(**1**)·pyridine complex suggest a range of geometries, either due to rotation of one porphyrin relative to the other or to simple opening and closing of the cavity, both of which would facilitate binding of the ligand in the cavity. The allosteric behaviour of the system can be explained on this model. When the first pyridine molecule binds, the π–π interaction is reduced slightly [as in the case of Zn_2(**2**)·pyridine complex] and this permits penetration of pyridine into the cavity, resulting in an equilibrium between pyridine bound on the inside and on the outside of the dimer. Penetration of pyridine into the cavity forces the porphyrins apart and significantly disrupts the π–π interaction. In this open form the second zinc site is exposed and so binds pyridine more strongly.

The binding constants between pyridine and these dimers are significantly smaller than the value of 2 890 M^{-1} for pyridine binding to the simple monomer, Zn (**3**). This effect is the result of competition either with an internal ligand [6,8] or with some other factor that reduces the Lewis acidity of the metal ion. In the case of these dimers, the π–π interaction itself appears to be dramatically reducing the affinity of the zinc for external ligands. The higher affinity of Zn_2(**1**) for the first molecule of 4-t-butylpyridine than for pyridine itself presumably reflects the greater basicity of the substituted ligand; the ratio of the two K_1 values is precisely that expected for a pK_b difference of 0.6 units.

Our evidence demonstrates that metallation enhances the porphyrin–porphyrin interaction and the consequential aggregation. This enhancement has been explained in terms of polarisation of the porphyrin π-system by the metal ion.[3b] It seems that the greater the metal–porphyrin interaction, the greater the π–π stacking forces. The electronic effect of co-ordination of the metal ion is to reduce the metal–porphyrin interaction. The polarisation of the porphyrin by the metal is, therefore, reduced and so, in cases such as the Zn_2(**2**)·pyridine complex, the π–π interaction with the other face is weakened slightly. In support of this, the fluorescence results suggest that binding of a more basic ligand, a 4-alkylpyridine, reduces the π–π interaction in Zn_2(**1**) to a larger extent than pyridine binding to Zn_2(**2**). Clearly, however, co-ordinative saturation of the metal is not by itself capable of preventing aggregation.

We recently reported the syntheses of some new macrocyclic porphyrin dimers which provide additional evidence in support of these ideas.[1] The bridging groups were long enough to cap the porphyrin and produce a monomeric species. In the course of the dimer syntheses the corresponding capped species were indeed produced and the yield of the capped compound was found to be strongly dependent on the strength of the π–π interaction between the cap and the porphyrin: use of the zinc porphyrin considerably increased the yield of the capped compound even in the presence of a large excess of *N*,*N*-dimethyl-4-aminopyridine.* In other words, despite the co-ordination of the zinc by the pyridine derivative, the π-stacking was enhanced relative to the metal-free system.

Further corroboratory evidence is provided by the co-ordination chemistry of Zn_2(**1**) and Zn_2(**2**) with bifunctional ligands: a single molecule of an appropriate size will bind in the cavity between the two porphyrins in each of these dimers, inducing all the disaggregation phenomena exhibited by the Zn_2(**1**)·pyridine system. These results will be discussed in a future report.

Conclusions

We have shown that disaggregation of metalloporphyrins by basic ligands has a small electronic component, but that it is dominated by steric factors. It seems, therefore, that it will not be possible to exert subtle control on porphyrin–porphyrin interactions and conformation through the electronic effects of ligand co-ordination. Rather, this control will have to be built into the covalent structure of the macrocycle.

Experimental

The porphyrins were synthesized as described previously.[3c]

U.v./visible electronic absorption spectra were recorded on a Pye-Unicam PU 8800 spectrometer in 1 cm × 1 cm cuvettes. All measurements were made on dichloromethane solutions, *ca.* 10^{-6} M in porphyrin. Emission spectra were recorded on dichloromethane solutions in 1 cm × 1 cm cuvettes with a Perkin-Elmer 3000 spectrometer. The apparatus was initially rigorously cleaned and dried and the dichloromethane was distilled from calcium hydride. Accurately determined pyridine solutions of *ca.* 0.5 M were made up in 1 ml volumetric flasks and added to the porphyrin sample in 5 μl aliquots *via* a 10 μl Hamilton 800 Series syringe. Towards the end of the titrations, the neat pyridines were added in 1 μl aliquots. The analysis of the results allowed for the changes in volume which occurred during the titration. Titration data were analysed by graphical and curve-fitting programs on a Macintosh SE microcomputer. The curve-fitting programs are based on Simplex routines written by Dr. A. Crawford.

^{1}H N.m.r. spectra were recorded on Bruker AM-400 and WM-250 spectrometers. Data were accumulated over 16K data points with a spectral width of 12 p.p.m. Spectra were obtained in deuteriochloroform or deuteriodichloromethane solutions.

Acknowledgements

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* These capping groups are small enough that they will even π-stack with a magnesium porphyrin which is co-ordinated to two axial ligands, (C. A. H. and J. K. M. S.; unpublished results).

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