Formation of Supramolecules in Solution. Interaction between Transition-metal Complexes and Water-soluble Porphyrins

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The rates of copper(II) insertion into water-soluble porphyrins in the presence of ethylenediamine have been studied near pH 7. Unusual kinetic profiles result which are interpreted as arising from the formation of supramolecular assemblies in solution. Confirmatory evidence for this model is provided by light scattering, conductance and temperature-variation experiments. The influences on assembly formation of reactant concentration, porphyrin charge and basicity, ionic strength, as well as buffer and electrolyte identity are reported.

The important biological roles porphyrins play in the living cell as well as their clinical and industrial potential have contributed to the sustained interest shown in the synthesis and reactivities of these pyrrolic compounds.[1–4] Among the processes most extensively studied is porphyrin metallation and a considerable number of studies over the past several years have been addressed to the elucidation of the mechanisms involved in these metal insertion reactions.[5–7] Most of the reports on reactions in aqueous solution are limited to low pH conditions to avoid hydrolysis of metal ions and precipitation of hydroxy species.[8–10] A rate law (1) (H_2L = porphyrin) has been

$$\text{Rate} = k[\text{M}^{2+}][\text{H}_2\text{L}] \qquad (1)$$

determined which is generally applicable to these systems at low pH. Pseudo-first-order conditions are usually maintained so that rate = $k_e[\text{H}_2\text{L}]$ with $k_e = k[\text{M}^{2+}]$.

Another aspect of porphyrin chemistry quite actively pursued in recent years is the interaction of these species with DNA and the reactivities of the resultant complexes.[11,12] It has been shown that cationic porphyrins can bind to DNA in a variety of ways: as territorial binders in which electrostatics are the primary driving force, through external groove binding or intercalation in which the purine/pyrimidine bases play an important role. It occurred to us that the kinetics of metallation reactions of DNA-bound free-base porphyrins should reflect the mode of binding and, indeed, this has been found to be the case for copper(II) insertion reactions. For these studies to be carried out with native DNA conformations low pH conditions have to be avoided and, thus, these reactions were investigated near pH 7 using imidazole or ammonia complexes of copper(II) as the metallation reagents.[13] Only a limited analysis of these results proved possible because of the large number of metal-containing species present in solution.

In an effort to simplify the analysis we selected ethylenediamine (en) as ligand[14,15] at a molar ratio to copper sufficient that well over 95% of the metal ion is in a single form, $[\text{Cu(en)}_2]^{2+}$. In preparation for studies in the presence of nucleic acids, it is first necessary to consider the metallation reaction at pH 7 in the absence of biopolymer. What was discovered in the course of these experiments is an anomalous and heretofore unreported kinetic effect which is the subject of the present paper.

Table 1 Metal concentration dependence of k_e for different buffer solutions *

	$10^3k_e/\text{s}^{-1}$		
$10^3[\text{Cu}^{2+}]/\text{mol dm}^{-3}$	Collidine	hepes	Tris
0.05	—	3.10	—
0.10	—	19.31	4.80
0.15	—	—	5.96
0.25	0.10	18.04	5.67
0.50	0.20	13.22	4.50
0.75	9.89	9.80	3.82
0.83	8.93	—	—
0.90	8.91	—	—
1.00	6.16	8.62	—
1.10	5.40	—	—
1.25	0.10	5.13	—
2.50	—	—	—

* I = 0.15 mol dm^{-3} (NaNO_3), pH 7.0, [buffer] = 20 mmol dm^{-3}, 25 °C.

Results and Discussion

The reaction of interest, an example of a porphyrin metallation, can be summarized as in equation (2). First-order kinetics was

$$[\text{Cu(en)}_2]^{2+} + \text{H}_2\text{L} \xrightarrow{k_e} [\text{CuL}] + 2\text{Hen}^+ \qquad (2)$$

observed for all conditions used as outlined in the Experimental section. We begin with experiments conducted under 'standard conditions' (*cf.* Experimental section) and consider the result of varying the copper(II) concentration from 0.25 to 1.25 mmol dm^{-3}. At the lower end of the range the reaction is so slow as to be inconvenient to measure with half-lives in hours (*cf.* column 1 of Table 1). A threshold value of 0.75 mmol dm^{-3} is reached at which k_e suddenly rises to a value of 9.9×10^{-3} s^{-1} ($t_{\frac{1}{2}}$ = 70 s). As the copper(II) concentration is further increased (maintaining en:Cu^{2+} = 3.0:1), k_e decreases but at a slower pace than its rapid rise (see Fig. 1).The copper(II) concentration at the maximum rate constant (k_e^{max}) is designated as $[\text{M}]_c$. Concentration profiles of the various minor copper(II)-containing species {*i.e.*, Cu^{2+}, $[\text{Cu(OH)}]^+$, $[\text{Cu(en)}]^{2+}$, $[\text{Cu(en)}_3]^{2+}$} have been calculated but none of them resembles the reactivity profile shown in Fig. 1.

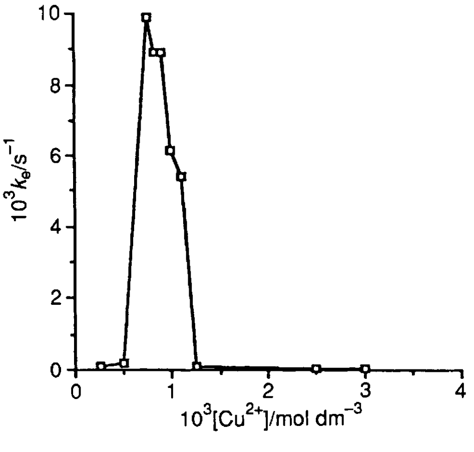


Fig. 1 Metal concentration dependence of k_e under 'standard conditions', as defined in the Experimental section; $[H_2tmpp] = 3.50 \times 10^{-5}$ mol dm^{-3}

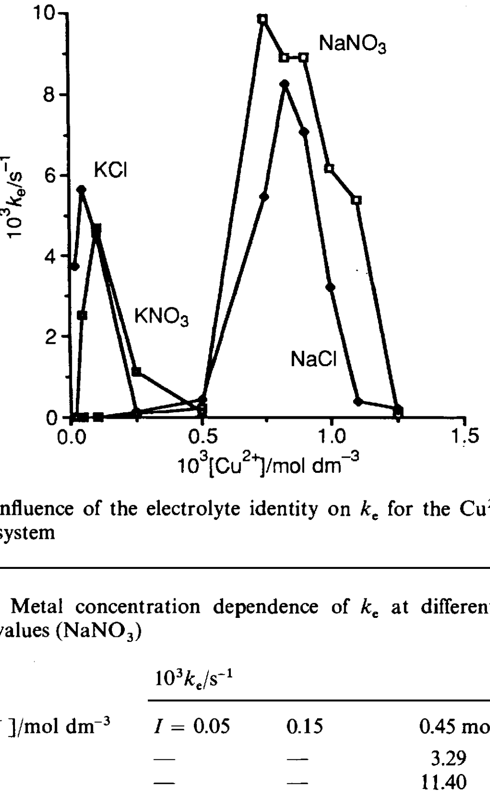


Fig. 2 Influence of the electrolyte identity on k_e for the Cu^{2+}–en–H_2tmpp system

Table 2 Metal concentration dependence of k_e at different ionic strength values ($NaNO_3$)

	$10^3k_e/s^{-1}$		
$10^3[Cu^{2+}]$/mol dm^{-3}	$I = 0.05$	0.15	0.45 mol dm^{-3}
0.05	—	—	3.29
0.10	—	—	11.40
0.25	12.10	0.10	11.20
0.50	13.40	0.20	7.96
0.75	8.45	9.89	6.57
0.83	—	8.93	—
0.90	—	8.91	—
1.00	4.85	6.16	—
1.10	—	5.40	—
1.25	4.45	0.10	—
2.50	—	—	—

In order to probe this highly unusual kinetic behaviour, light scattering and conductivity methods were applied to these systems. At high and low values of metal concentration (relative to $[M]_c$), where the reactions are quite slow, very little light is scattered. However, at $[Cu^{2+}] = [M]_c$, the scattered light signal is enhanced by about six-fold. These results imply the existence of some type of molecular assembly in solution at the critical value $[M]_c$, formed by the spontaneous association of a number of solute components, having microscopic organization and macroscopically detectable characteristics similar to vesicles or micelles.[16] In other words, the abnormal kinetic effect observed might be attributable to the presence of organized entities of a supramolecular nature, resulting from the association of the metal complex and porphyrin species in solution.[17]

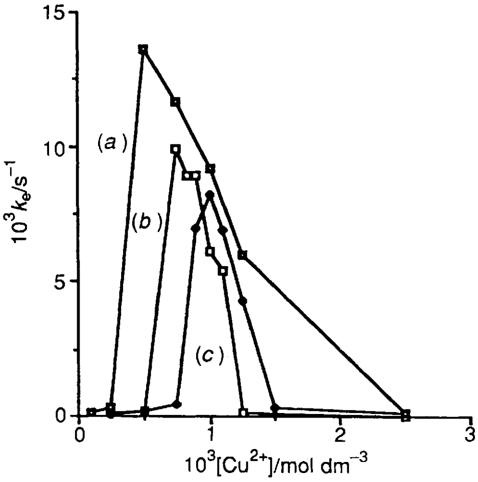


Fig. 3 Metal concentration dependence of k_e at different concentrations of H_2tmpp: (*a*) 4.10×10^{-5}, (*b*) 3.50×10^{-5} and (*c*) 1.70×10^{-5} mol dm^{-3}

Conductivity measurements confirm and extend the above hypothesis and consequently the existence of structural entities in solution. Systems showing slow kinetics have conductivity values of about $8.0 \times 10^{-3}\ \Omega^{-1}\ cm^{-1}$ while at $[Cu^{2+}] = [M]_c$ the conductivity is only $2.3 \times 10^{-3}\ \Omega^{-1}\ cm^{-1}$. This result demonstrates the presence of assemblies of low mobility in solution at the critical value of $[Cu^{2+}]$.

Furthermore, these conductivity measurements point out the complexity of these assemblies. Most of the solution conductance is due to the mobility of the electrolyte; the lowering of the conductance implies that the electrolyte is also involved in the assembly. As indicated in the Experimental section, these structures very efficiently scatter light of 480 and even 690 nm. Therefore, these are very large entities composed of vast numbers of molecules and ions. As might be predicted from these arguments the nature and concentration of the electrolyte play important roles in determining the critical parameters. Table 2 shows the influence of changing $NaNO_3$ concentration while Fig. 2 shows the effect on the kinetic profile of changing the electrolyte from $NaNO_3$ to NaCl, KNO_3 or KCl. Note that the cation seems to exert a greater influence than the anion. Although the composition of the buffering solution does not markedly influence the pattern (profile) of the kinetic phenomenon, it affects both k_e^{max} and $[M]_c$ (Table 1).

The concentration of porphyrin is also involved in determining the critical parameters for this kinetic process. The same anomalous pattern is observed at various concentrations of 5,10,15,20-tetrakis(*N*-methyl-4-pyridinio)porphyrin (H_2tmpp) (see Fig. 3) with k_e^{max} increasing and $[M]_c$ decreasing with increasing $[H_2tmpp]$. The influence of porphyrin concentration was explored further *via* light scattering measurements in which $[Cu^{2+}]$ was kept constant and $[H_2tmpp]$ was varied. Fig. 4(*a*) shows the effect of increasing $[H_2tmpp]$ at $[Cu^{2+}] = 0.5$ mmol dm^{-3}, a concentration at which the rate of metallation under standard conditions is appreciable. A maximum appears in the light scattering curve at a concentration of 38 μmol dm^{-3} H_2tmpp but then the light scattering decreases and eventually levels off at higher $[H_2tmpp]$. On the other hand, at $[Cu^{2+}] = 2.5$ mmol dm^{-3}, a concentration at which the reaction rate is virtually zero, the light scattering is negligible at all $[H_2tmpp]$ concentrations. The basicity and charge of the porphyrin also have a marked

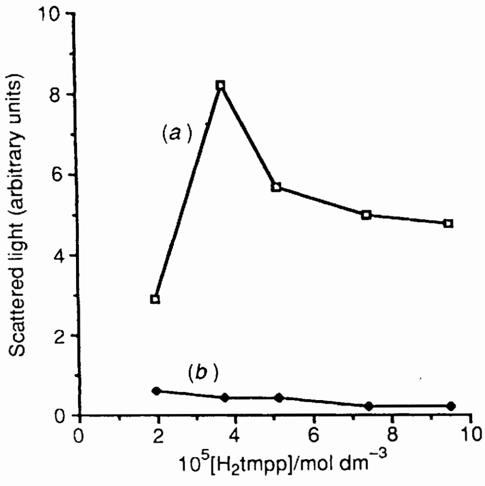


Fig. 4 Variation of light scattering with porphyrin concentration at (*a*) 0.5 and (*b*) 2.5 mmol dm^{-3} Cu^{2+}

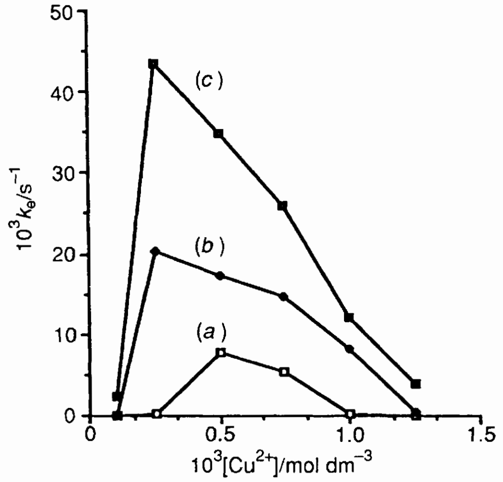
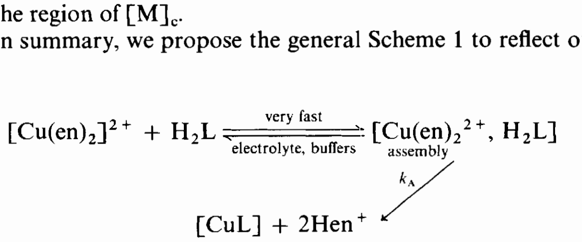


Fig. 5 Metal concentration dependence of k_e for several water-soluble porphyrins: (*a*) H_2tmpp, (*b*) H_2ttap and (*c*) H_2tspp

effect on the metallation kinetics (Fig. 5) although the anomalous profile remains for all cases tested thus far. Indeed, it has been shown that for more standard metallation reaction pathways the nature of the peripheral substituents on *meso*-porphyrins influences the rate of metal ion incorporation.[7] Our results, where a comparison can be made, parallel these other studies in that the critical rate constant k_e^{max} increases with increased basicity and negative charge. For example, for 5,10,15,20-tetrakis(4-sulphonatophenyl)porphyrin (H_2tspp) a tetraanionic porphyrin, stopped-flow methods were required in the region of $[M]_c$.

In summary, we propose the general Scheme 1 to reflect our



$$[Cu(en)_2]^{2+} + H_2L \underset{\text{electrolyte, buffers}}{\overset{\text{very fast}}{\rightleftharpoons}} \underset{\text{assembly}}{[Cu(en)_2{}^{2+}, H_2L]} \xrightarrow{k_A} [CuL] + 2Hen^+$$

Scheme 1

results, where $[Cu(en)_2]^{2+}$ reacts with H_2L so slowly, as ordinary independent solute species, that this pathway is negligible. However, under appropriate conditions (which depend in part on pH, buffer concentration and identity, as well as electrolyte concentration and identity) a large supramolecular assembly forms in which the rate of metal insertion is enhanced by several orders of magnitude. In this assembly the metal complex plays a unique role; it may be profitable to think of $[Cu(en)_2]^{2+}$ as forming nucleation sites for assembly formation. If too few sites are present the assembly process is unfavourable. At a threshold value an assembly forms spontaneously in a highly co-operative process in which the solute species no longer behave as independent entities giving rise to extensive light scattering, lowered conductance and a metallation rate acceleration of about two orders of magnitude. As the number of nucleation sites increases the assemblies grow smaller in size and become less reactive with respect to metallation. Eventually, the rate constant returns to nearly zero as the assemblies approach molecular dimensions. Note, in contrast to the pattern for $[Cu(en)_2]^{2+}$, that as the concentration of porphyrin increases the assemblies are somewhat affected but after a certain point is reached the porphyrin no longer influences the size of the assemblies (using a light scattering criterion).

The influence of temperature on the kinetic profile has also been explored and is consistent with this model. Experiments were conducted at four temperatures between 283 and 328 K. Fig. 6 presents the metal concentration dependence of the rate constant at each temperature; the critical values are $10^3\ k_e^{max}$ = 10.5 at 283, 9.9 at 298, 10.8 at 313 and 10.5 s^{-1} at 328 K. These k_e^{max} values are a composite of a rate constant for metal insertion (k_A in Scheme 1) and an 'equilibrium constant' for assembly formation. The lack of temperature dependence in k_e^{max} results from opposite (and approximately equal) heat effects for the two steps. The position of the onset of assembly formation, $[M]_c$, as a function of temperature (Fig. 7) is consistent with this assignment; as the temperature increases, so does the concentration of $[Cu(en)_2]^{2+}$ needed to initiate the organization.

Many questions remain including the molecular organization of the assemblies. We are probing these structures chemically by considering among other issues whether ligands other than ethylenediamine lead to similar kinetic profiles and if macrocycles other than porphyrins can be used as metal ion receptors. Reports of these investigations are forthcoming.

Experimental

5,10,15,20-Tetrakis(*N*-methyl-4-pyridinio)porphyrin (H_2-tmpp) and 5,10,15,20-tetrakis(*N*,*N*,*N*-trimethyl-4-anilinio)porphyrin (H_2ttap), both in the chloride form, as well as 5,10,15,20-tetrakis(4-sulphonatophenyl)porphyrin (H_2tspp) in the sodium form were obtained from MidCentury Chemical company and used without further purification. Ethylenediamine, collidine(2,4,6-trimethylpyridine) and *N*-(2-hydroxyethyl)-piperazine-*N'*-ethane-2-sulphonic acid (hepes) were obtained from Sigma, while all other reagents including 2-amino-2-hydroxyethylpropane-1,3-diol (Tris) and electrolytes used in this study, $NaNO_3$, NaCl, KNO_3 and KCl, were Fisher Scientific products.

Kinetic studies of slow reactions as well as various spectroscopic measurements were conducted on either a Nicolet 9420 or a Varian 2200 ultraviolet–visible recording spectrophotometer, each fitted with a thermostatted cell compartment. Faster reactions were studied with a Nortec stopped-flow system. The metallation reactions were investigated at a fixed wavelength, either at the free base maximum near 520 nm or the metalloporphyrin absorption peak near 550 nm. No evidence was found for rate constant dependence on the wavelength of observation. The existence of an isosbestic point for each of the systems studied, between 520 and 550 nm (*e.g.* at 535 nm for H_2tmpp), implies that the porphyrin reactant and metalloporphyrin product are the only chromophores present in non-negligible concentration other than copper(II)–ethylenediamine complexes which absorb further into the red portion of the visible spectrum.

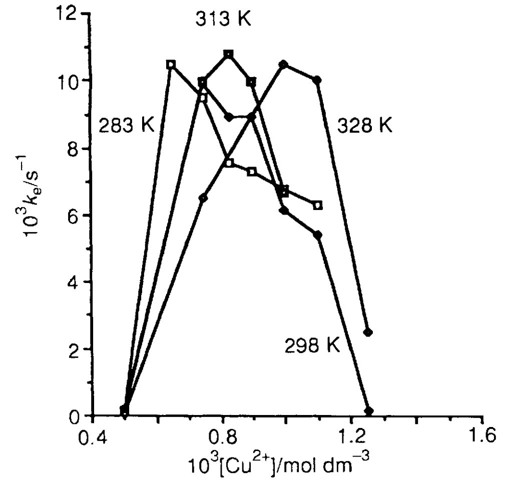


Fig. 6 Metal concentration dependence of k_e at various temperatures

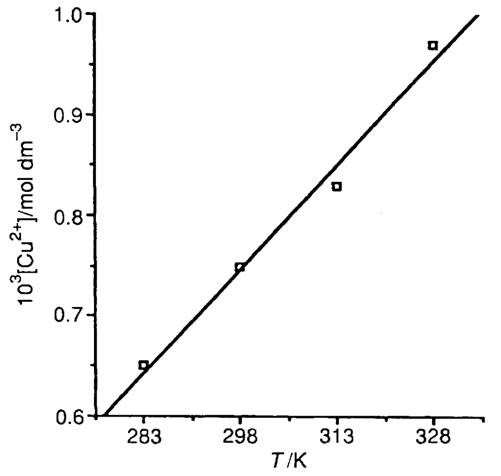


Fig. 7 Variation of the critical copper(II) concentration with temperature

To summarize the design of these studies, we find it convenient to define arbitrarily our 'standard experimental conditions': pH maintained at 7.0 with 20 mmol dm^{-3} collidine, ionic strength (I) 0.15 mol dm^{-3} ($NaNO_3$), 25 °C and H_2tmpp concentration 35 µmol dm^{-3}. For the kinetic studies the copper concentration was allowed to vary between 50 µmol dm^{-3} and 2.5 mmol dm^{-3} while the molar ratio of ethylenediamine: Cu^{II} was maintained at 3.0:1 (the reagents were added in the sequence Cu^{2+}, en, $NaNO_3$, buffer). Under these conditions, the predominant copper-containing form ($\geqslant$98.4%) is $[Cu(en)_2]^{2+}$. Throughout the entire copper(II) concentration range, from $[Cu^{2+}]_0 \approx [H_2tmpp]_0$ through $[Cu^{2+}]_0 > 10[H_2tmpp]_0$, first-order kinetics were obtained over a minimum of 3–4 half-lives.

The influence on the kinetics of the metallation reaction of temperature, ionic strength, nature of the electrolyte and buffer as well as porphyrin identity and concentration was studied. All the experiments were conducted in the pH interval 6.8–7.2 over which the first-order rate constant, k_e, proved not to change significantly, all other conditions remaining fixed. The temperature variation was over a 45 °C range from 283 to 328 K, the ionic strength was studied from I = 0.05 to 0.45 mol dm^{-3} with $NaNO_3$ and the effect of changing electrolyte to NaCl, KNO_3 and KCl was also considered. The role played by the porphyrin concentration was examined for $10 \leqslant H_2\text{tmpp} \leqslant 41$ µmol dm^{-3} although some light scattering experiments (see above) were conducted at higher porphyrin concentrations, up to 95 µmol dm^{-3}. Moreover, the effect of porphyrin basicity and charge was considered using another tetracationic porphyrin (H_2ttap) which is more basic than H_2tmpp (pK_a = 4.7 *vs.* 2.0) as well as a tetraanionic porphyrin H_2tspp (pK_a = 5.0). Finally, the buffering system was also changed with some experiments conducted with hepes or Tris.

Light scattering experiments were carried out with a Perkin-Elmer 512 double-beamed spectrofluorimeter. Solutions containing Cu^{II}–en and H_2tmpp were prepared as in a standard experiment at several copper(II) concentrations. The excitation wavelength was 480 or 690 nm, chosen because very little absorption and virtually no porphyrin fluorescence occurs at these values. Conductivity measurements were carried out, either with a Crison 525 or Cole Palmer J-1491-05 conductimeter fitted with a glass cell, using the same solutions as were used for the light scattering studies. Results of light scattering experiments are expressed in arbitrary units; conductivities are expressed in Ω^{-1} cm^{-1}.

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