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Very Strong Binding and Mode of Complexation of Water-Soluble Porphyrins with a Permethylated β-Cyclodextrin

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Abstract: Heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin in a pH=7.0 aqueous solution binds *meso*-tetrakis(4-carboxyphenyl) porphyrin and its zinc complex to yield 2:1 complexes with exceptionally high binding constants. The mode of binding, involving the inclusion of two opposite aryl groups in the cyclodextrin cavity, is clearly defined by a detailed NMR analysis. Copyright © 1996 Elsevier Science Ltd

The central importance of porphyrins in heme proteins has stimulated much interest in their water-soluble derivatives[1]. These are mainly investigated and employed as catalysts of oxidation processes, as building blocks for relevant biomimetic systems and supramolecular devices[2], and as contrast agents for magnetic resonance imaging[3], to cite a few fields of interest.

One serious drawback in the use of water soluble porphyrins derives from their well documented tendency to form aggregates in aqueous solution[4] above a critical concentration and this may seriously compromise their functions. One possible way to circumvent the problem of self-assembly without impairing the properties of porphyrinic systems is to form inclusion complexes with proper receptors. Recently, cyclodextrins (CD) and their partially O-methylated derivatives have been shown to interfere with the self-aggregation of water soluble aryl porphyrins by forming inclusion complexes[5]. Among the various possible host-guest stoichiometries, that of a 2:1 CD-porphyrin complex, in which two opposite aryl moieties of the porphyrin are included into CD's cavity through its wide end, has been indicated as the most stable using a large excess of CD. The binding process is generally considered to involve two equilibria the first one leading to a 1:1 complex, which evolves to the 2:1 complex and the values of the stepwise binding constants, K_{11} and K_{12}, have been estimated, of the same order of magnitude, in the 10^3-10^4 M^{-1} range[5k] .

The need to operate under high dilution without a large excess of the complexing agent and still preserve the structural integrity of a supramolecular complex requires much higher binding constants. In the search for more effective CD derivatives, we thought that the heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin, TMCD, was a good candidate owing to the extended and more hydrophobic cavity provided by the full methylation of the parent CD. This was indeed the case and we here report the results of a study of the binding of TMCD with the water soluble *meso*-tetrakis(4-carboxyphenyl)porphyrin, **1a**, and its zinc complex, **1b**, in 0.1 M aqueous

phosphate buffer at pH=7.0.

The spectrophotometric analysis of very dilute solutions of **1a** following a characteristic band at 412 nm, showed that the Beer's law was obeyed up to a concentration of *ca.* 5 x 10^{-6} M; the marked deviations observed for more concentrated solutions were taken as diagnostic of the onset of self-aggregation of the porphyrin derivatives. Addition of a 3-fold excess of TMCD to a 1 x 10^{-6} M solution of **1a** caused a red shift, from 412 to 417 nm, and a 14% increase of the Soret band. Although small, the changes observed allowed us to define the titration diagram following the absorbance changes at 412 nm reported in Fig. 1.

Similar behavior was also observed in the case of **1b**. The titration curve of Fig.1 shows an increase of the absorption intensity up to a 2:1 TMCD:**1a** ratio followed by a plateau region. The sharp transition indicates that the microscopic binding constants are very large, too high to be evaluated from these spectroscopic data. A reliable estimate of such constants was possible by means of fluorescence experiments carried out for very diluted solutions.

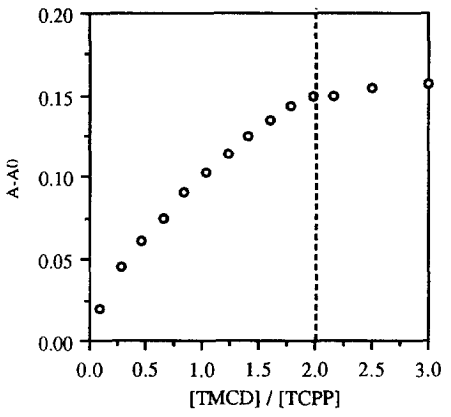

Figure 1: UV-Visible titration, at 412 nm, of **1a** (2.05 x10^{-6} M) with TMCD (1.0 x10^{-5} M), in 0.1 M phosphate buffer (pH=7.0) at 25°C.

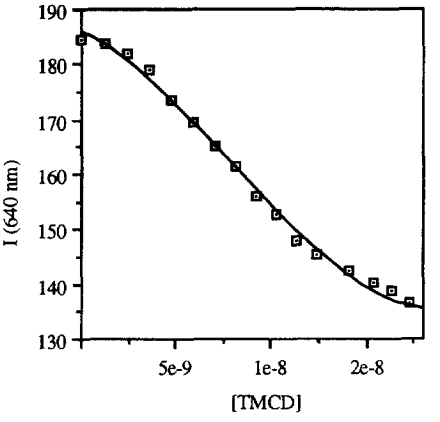

Figure 2: Fluorescence titration, at 640 nm, (λ_{ex} = 414 nm) of **1a** (2.5x10^{-9} M) with TMCD (2.0x10^{-7} M), in 0.1 M phosphate buffer (pH=7.0) at 25°C. The continuos line represents the best fitting results.

$$\text{TCPP} + \text{TMCD} \overset{K_{11}}{\rightleftharpoons} \text{TCPP(TMCD)} \quad (1)$$

$$\text{TCPP(TMCD)} + \text{TMCD} \overset{K_{12}}{\rightleftharpoons} \text{TCPP(TMCD)}_2 \quad (2)$$

$$\text{TCPP} + 2\,\text{TMCD} \overset{K_{13}}{\rightleftharpoons} \text{TCPP(TMCD)}_2 \quad (3)$$

In the absence of TMCD, the fluorescence intensity increases linearly with the concentration of **1a**. Addition of TMCD to a 2.5 x 10^{-9} M solution of the porphyrin derivatives causes a decrease of the fluorescence intensity at 640 nm (λ_{ex} = 414 nm) as shown by the titration curve of Fig. 2. These titration data, in principle, can be fitted with a two step binding model (equations 1 and 2) or with an overall equilibrium model (equation 3).The shape of the fluorescence titration curve in Fig. 2 does not show any clear evidence of two distinct steps.
That means either that the overall equilibrium described by equation 3 is operative, or that the two binding constants K_{11} and K_{12} are very close or even that K_{12} is bigger than K_{11}. The fitting procedure[6] gives

unambiguous answers only using the model of equation 3. The K_{13} constants (which corresponds to the product of K_{11} and K_{12}) for **1a** and **1b** are 1.4×10^{16} M^{-2} and 1.9×10^{16} M^{-2} respectively. Such binding constants are the highest ever reported in the literature for analogous inclusion compounds.

The termolecular complex of TMCD with **1b** was studied in detail by NMR spectroscopy in D_2O. Only two sets of signals were observed in the ^1H NMR spectrum. This clearly indicates that two facts: only an *anti* complex is formed in which the TMCD units bind two opposite aromatic residues of **1b**. A *syn* complex with two TMCD units complexing two adjacent residues, would give rise to four sets of signals. Moreover, a rapid exchange regime between the ligands is ruled out as no saturation transfers were observed between the *endo* and *exo* signals of **1b** protons during the NOE experiments described in Table. A ligand exchange process involving both an *anti* $\rightarrow$ *syn* $\rightarrow$ *anti* pathway or a complete freeing of both TMCD units is quite unlikely in view of the very high binding constants evaluated for the trimeric complex.

The 2:1 complex has a total weight of 3651 daltons, which puts this system in the negative NOE region. In order to control spin diffusion, truncate driven NOE (TOE)[7] experiments were performed, with eight irradiation times from 0.1 to 0.8 secs. The data, fitted into equation (4)[8], give the cross relaxation rates σ_{IS} reported in Table.

$$f_{I\{S\}}(t) = (\sigma_{IS}/\rho_{IS})(1-\exp(-\rho_{IS}\, t)) \qquad (4)$$

The TMCD signals have been assigned with a COSY experiment, except those of the methyl groups, which could be attributed by means of further TOE experiments.

		TCPPZn: observed					
TMCD: saturated		o_e	m_e	p_e	o_x	m_x	p_x
	δ	8.26	8.04	8.76	7.22	8.01	8.90
1	4.81						
2	2.73						
Me^2	2.86				-0.08	-0.09	-0.05
3	2.97	-0.16		-0.11			-0.16
Me^3	2.28	-0.10		-0.11	-0.56	-0.31	-0.49
4	3.35						
5	3.62	-0.28	-0.33	-0.31			-0.05
6	3.73	-0.09	-0.20	-0.08			
6'	3.59	-0.13	-0.24	-0.12			
Me^6	3.30						

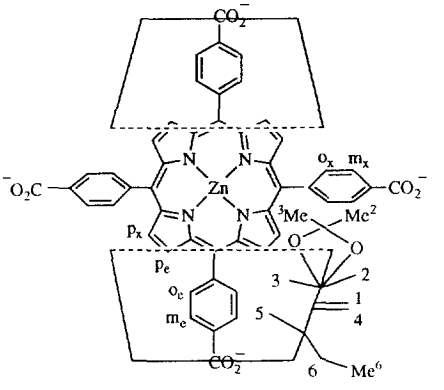

Table: Cross relaxation rates, σ_{IS}, of ortho, meta and pyrrolic **1b** protons. Protons inside and outside the TMCD cavity are called *endo* (indexed with e) and *exo* (indexed with x) respectively. The chemical shifts (δ) are also given. Solutions of **1b** (1.0×10^{-2} M) and TMCD (2.0×10^{-2} M) in 0.1 M phosphate buffer (pD=7.0) were employed.

The cross relaxation data in Table 1 allow the easy attribution of the *endo* and *exo* signals of **1b**. They also allow us to draw some conclusions regarding the conformational preference of complexed TMCD. Relevant cross relaxations of *endo* and *exo* **1b** protons were observed upon irradiation of TMCD H3 and Me^3 protons,

while much smaller, if any, cross relaxations were observed from irradiation of H2 and Me^2 protons. In accordance with the conformational preference observed in free TMCD, H3 and Me^3 are both pointing toward the internal side of TMCD (more correctly, Me^3 is pointing upwards) while H2 and Me^2 are pointing toward the external side. By the same arguments, it may be concluded that the H6 protons are directed inward, while the Me^6 groups point outward. It may finally be noticed that relevant cross relaxations from irradiation of Me^3 are observed for all *exo* and *endo* signals of **1b**, with the exception of aromatic *endo* meta protons. This indicates that although TMCD is strictly bound to the aromatic ring of **1b**, nevertheless different degrees of penetration within a large oscillation band are equally probable.

Studies are in progress to better define the factors responsible for the strong interactions observed and to test the functions of the complexed porphyrin *vis-a-vis* the uncomplexed ones.

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