Binding of Porphyrins in Cyclodextrin Dimers

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Received July 20, 1995
Revised Manuscript Received November 13, 1995

As part of our research program aimed at the development of new supramolecular catalytic systems, we are studying the use of cyclodextrin dimers (CD-dimers) to encapsulate porphyrins.[1] In this communication we describe the formation of complexes between water-soluble porphyrins and CD-dimers containing flexible spacers. These complexes show a syn- and anti-binding geometry. We also describe an unusual 2:2 (porphyrin:CD-dimer) complex in which two porphyrin molecules are encapsulated by four CD units. The latter units are in dimeric pairs, with the CDs connected via bipyridine spacers. The formation of this complex is facilitated by the addition of a coordinating metal ion.

CD-dimers **1a**−**c** (Figure 1) were synthesized as previously reported by us.[2] The aggregation of porphyrins **2a** and **2b** in aqueous solution was investigated by fluorescence spectroscopy.[3] Graphs of the fluorescence intensity versus the concentration of the porphyrins were linear up to 5×10^{-7} M. At higher concentrations deviations from linearity were observed due to self-quenching processes as a result of aggregation of the porphyrins. In the following studies we used concentrations of **2a** and **2b** below this critical concentration.

The binding of the porphyrins in the cavities of the CD-dimers resulted in a decrease of the fluorescence intensities, yielding titration curves that corresponded to the formation of 1:1 (porphyrin:CD-dimer) complexes (Figure 2a). The binding constants (K_bs) of these complexes are summarized in Table 1. For comparison we also determined the binding constants of the complexes between unsubstituted β-CD monomer and the two porphyrins. For porphyrin **2b** we measured a value of 1700 M^{-1}, which is much smaller than the value of $K_b = 7.0 \times 10^4$ M^{-1} reported by Zhao and Luong.[4]

The very high binding constants found for our CD-dimers, as compared to those of β-CD monomer, indicate a strong chelate effect.[5] The affinity of **1b** for the two porphyrins is lower than that of **1a**, which is the result of the self-encapsulation of the octamethylene spacer in one of the cavities

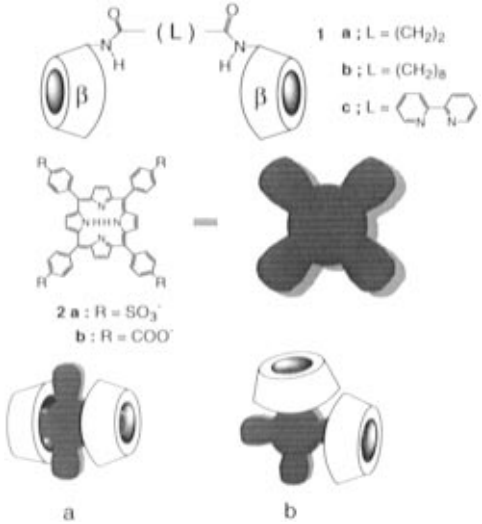

Figure 1. Schematic representation of the structures of **1a**−**c** and **2a**,**b** and the geometries of the porphyrin−CD-dimer complexes: (a) anti complex; (b) syn complex.

Table 1. Binding Constants of Complexes between Porphyrins and CD-Dimers[a]

host	binding constant (M^{-1})	
β-CD	guest **2a**: 1400	guest **2b**: 1700
1a	0.8×10^6	1.9×10^6
1b	0.4×10^6	0.9×10^6
1c	$>50 \times 10^6$ [b]	

[a] Binding constants were obtained by varying the concentration of the host molecules at a fixed porphyrin concentration (2.0×10^{-7} M) and directly monitoring the fluorescence intensity changes. Assays were performed in duplo at 25.0 ± 0.1 °C, phosphate buffer 0.1 M, pH 7.0, $\lambda_{ex} = 415$ nm, $\lambda_{em} = 635$ nm. Binding constants were obtained by curve fitting assuming a 1:1 complex. Errors in K_bs are less than 10%. [b] Estimated value.

of **1b** as we showed previously.[6] Before binding of a guest can take place, this spacer must first be removed, which leads to a lower binding constant. At the bottom of Figure 1 are presented two possible structures for the porphyrin:CD-dimer complex: (a) an anti and (b) a syn arrangement of the cyclodextrins.[7] In the ^{1}H NMR spectrum of the **1a**−**2a** complex recorded in CD_3OD/D_2O (25:75, v/v) at −10 °C, the porphyrin ring protons appear as four broad signals ($\delta = 9.17$, 8.92, 8.82, and 8.61 ppm). In the case of complex **1b**−**2a** the same four signals ($\delta = 9.17$, 8.90, 8.83, and 8.62 ppm) are present as well as two extra peaks at $\delta = 8.95$ and 8.51 ppm (spectra not shown).[8] For the anti complex only two signals are to be expected, and for the syn complex, four signals. The observed patterns therefore indicate that the **1a**−**2a** complex has a syn structure, and the **1b**−**2a** complex**,** a mixed syn/anti structure. From the intensity of the signals the ratio of the syn/anti conformers could be calculated to be 2:1. The geometry of the anti complex could also be deduced from the δ values of the methylene spacer protons which showed an upfield shift (broad signals at δ 1 to −2 ppm) due to the shielding effect of the porphyrin core. More details will be presented in a full paper.

The titration curve for CD-dimer **1c** and porphyrin **2a** is shown in Figure 2b. This curve clearly is different from those recorded for dimers **1a** and **1b** and could not be fitted assuming simply 1:1 (dimer:porphyrin) complex formation. The sigmoidal shape of the curve can be explained if first a 1:1 complex

(1) For other examples of porphyrins encapsulated in CDs, see: (a) Manka, J. S.; Lawrence, D. S. *Tetrahedron Lett.* **1989**, *30*, 7341. (b) Manka, J. S.; Lawrence, D. S. *J. Am. Chem. Soc.* **1990**, *112*, 2440. (c) Mosseri, S.; Mialocq, J. C.; Perly, B.; Hambright, P. *J. Phys. Chem.* **1991**, *95*, 2196. (d) Mosseri, S.; Mialocq, J. C.; Perly, B.; Hambright, P. *J. Phys. Chem.* **1991**, *95*, 4659. (e) Dick, D. L.; Venkata, T.; Rao, S.; Sukumaran, D.; Lawrence, D. S. *J. Am. Chem. Soc.* **1992**, *114*, 2664. (f) Jiang, T.; Lawrence, D. S. *J. Am. Chem. Soc.* **1995**, *117*, 1857.

(2) Venema, F.; Baselier, C. M.; van Dienst, E.; Ruël, B. H. M.; Feiters, M. C.; Engbersen, J. F. J.; Reinhoudt, D. N.; Nolte, R. J. M. *Tetrahedron Lett.* **1994**, *35*, 1773. It should be noted that the opening of the epoxide at C-3 leads to a cyclodextrin in which one of the glucose rings has undergone a flip to give an altrose ring; see: Breslow, R.; Czarnik, A. W. *J. Am. Chem. Soc.* **1983**, *105*, 1390.

(3) Fluorescence spectroscopy is more sensitive than UV/vis spectroscopy to study the aggregation of porphyrins; see also: (a) Margalit, R.; Shaklai, N.; Cohen, S. *Biochem. J.* **1983**, *209*, 547. (b) Ravikant, M.; Reddy, D.; Chandrashekar, K. *J. Chem. Soc., Dalton Trans.* **1991**, 2103 and references cited therein.

(4) Zhao, S.; Luong, J. H. T. *J. Chem. Soc., Chem. Commun.* **1994**, 2307. These authors report an *increase* in fluorescence intensity on complex formation, which is in contrast with the *decrease* in intensity that we observed. Since the concentration of **2b** (5×10^{-6} M) used by the authors is in the aggregation range of the porphyrin, it is likely that they have observed the breakdown of porphyrin aggregates. The reported binding constants are probably incorrect. Also the working mechanism for the reported complex as a chemosensor for pentachlorophenol is probably incorrect since this guest (size 9−10 Å) does not fit into a CD cavity (6−6.5 Å); see: Zhao, S.; Luong, J. H. T. *J. Chem. Soc., Chem. Commun.* **1995**, 663.

(5) Breslow, R. *Pure Appl. Chem.* **1994**, *66*, 1573.

(6) Venema, F.; Baselier, C. M.; Feiters, M. C.; Nolte, J. M. *Tetrahedron Lett.* **1994**, *35*, 8661.

(7) The formation of an anti arrangement between **2a** and β-CD was recently proposed by Ribó et al. based on ROESY spectra; see: Ribó, J. M.; Farrera, J.-A.; Valero, M. L.; Virgili, A. *Tetrahedron* **1995**, *51*, 3705.

(8) ^{1}H NMR spectra (400 MHz) were recorded in a mixture of D_2O and CD_3OD (75:25, v/v), [**2a**] = 1.5×10^{-3} M, [**1a**] = [**1b**] = 4.5×10^{-3} M.

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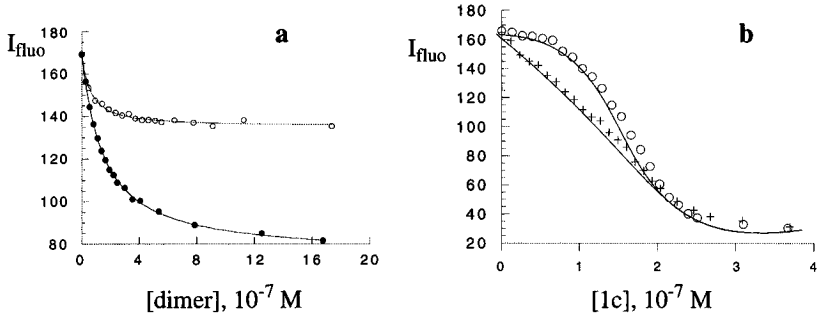

Figure 2. Titration curves: (a) complexation of **2a** (2.0×10^{-7} M) with **1a** (○) and **1b** (●) (observed points and calculated curves); (b) complexation of **2a** (2.0×10^{-7} M) with **1c** in the absence (○) and presence (+) of Zn(II) ions (1.0×10^{-7} M) (observed points and observed curves).

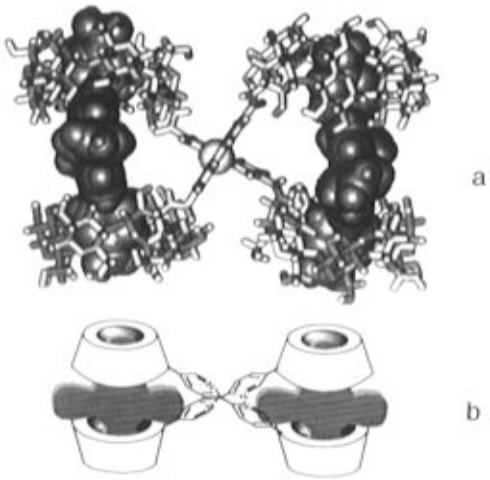

Figure 3. (a) Computer-generated structure and (b) schematic representation of the 2:2 complex between **2a** and **1c**.

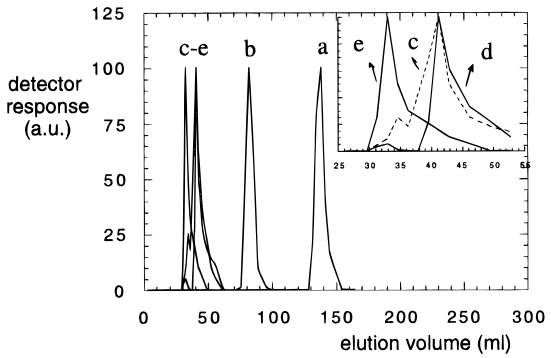

Figure 4. Gel chromatograms of (a) β-CD; (b) **1c**; (c) complex **1a**−**2a**; (d) complex **1b**−**2a**; (e) complex **1c**−**2a**. Inset: expanded tracks of the complexes.

is formed, then a 2:1 (CD-dimer:porphyrin) complex is formed, and finally the two remaining empty CD units encapsulate a second porphyrin to form a 2:2 complex (Figure 3).

In order to confirm the presence of a 2:2 complex we carried out gel chromatography experiments.[9] The elution curves for the **1c**−**2a** complex and for reference compounds are shown in Figure 4. The elution volume of β-CD is much larger than that of compound **1c**, as is expected from their relative molecular weights. The three complexes **1a**−**2a**, **1b**−**2a,** and **1c**−**2a** show smaller elution volumes than **1c**, again in line with what is expected. From the expanded plots (Figure 4, inset) it can be seen that the **1c**−**2a** complex is eluted faster than the complexes **1a**−**2a** and **1b**−**2a**. This suggests that the former complex has a higher molecular weight, supporting the idea of the formation of a 2:2 complex. Figure 4 reveals that CD-dimers **1a** and **1b** also form small amounts of 2:2 complexes. Since the bipyridine spacer of CD-dimer **1c** is capable of binding metal ions, we also performed the titration of **2a** in the presence of 0.5 equiv of Zn(II) ions.[10] Figure 2b shows that under these conditions the formation of the 2:2 complex is facilitated, probably because the metal ion coordinates to the bipyridine unit in a tetrahedral fashion (Figure 3). The binding constants for both the **1c**−**2a** complex and the **1c**−**2a**−Zn^{2+} complex are very high: the estimated values are $K_b \gg 5 \times 10^7$ M^{-1}. UV measurements on **2a** confirmed that upon addition of 10 equiv of Zn(II) ions no metal ions were incorporated within the porphyrin macrocycle; hence changes in fluorescence for complex **1c**−**2a**−Zn^{2+} are the result of enhanced binding and not of a reduction in fluorescence intensity due to metal incorporation in the porphyrin.

Preliminary ^{1}H NMR measurements were carried out in order to verify the proposed 2:2 cross dimer structure.[11] High-field ^{1}H NMR spectra of the complex **1c**−**2a** revealed, in particular for the porphyrin and bipyridine resonances, significant line broadening indicative of an exchanging system. This line broadening prohibited spectral assignment. Upon the addition of 0.5 equiv of Zn(II) ions to this complex, however, line sharpening occurred, suggesting the presence of a more rigid complex. ^{1}H NMR COSY and NOESY experiments (200 MHz) were subsequently carried out only on the **1c**−**2a**−Zn^{2+} complex, enabling an assignment for the porphyrin and bipyridine resonances of this complex to be made. ^{1}H NMR spectra indicated a 1:1 ratio between porphyrin and cyclodextrin dimer and hence either a 1:1 or 2:2 complex. A highly unsymmetrical porphyrin environment was highlighted by the presence of four individual pyrrole resonances (δ = 9.21, 8.98, 8.82, and 8.34 ppm) and three different porphyrin phenyl AB quartets (δ = 7.83, 8.21; 7.98, 8.18; 8.16, 8.42 ppm in a ratio 1:1:2, respectively). The four inequivalent pyrrole resonances may be interpreted as resulting from a 1:1 complex in a syn arrangement similar to that seen for the complex **1a**−**2a**. Molecular modeling, however, predicts that in the case of the rigid bipyridine spacer a syn 1:1 complex is impossible. The unsymmetrical nature of the porphyrin phenyl ABs is also not in agreement with a 1:1 complex. The NMR spectra, however, can be interpreted fully in terms of a 2:2 complex with two sets of two identical porphyrin phenyls bound within the cyclodextrins (δ = 8.16, 8.42 ppm) in an anti manner (Figure 1a). In the case of the other set of phenyl groups, one is pointing out of the complex and one is pointing inside toward the zinc(II) ion at the center of the complex. Complexes of the latter type may be of interest in the study of the metal-mediated transfer of electrons between porphyrins. Studies along this line are currently in progress.

Acknowledgment. This research was financially supported by the Dutch Foundation for Technology (STW). We thank Mr. A. P. H. J. Schenning for stimulating discussions.

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(9) Fractogel, TSK HW-40 (F), bed volume 200 mL, eluted with water, 13.2 mL h^{-1}.

(10) To study the influence of Zn(II) ions, titrations of **2a** with **1c** were performed in 0.01 M Tris buffer, pH 7.0. The sigmoidal shape of the titration curve, which is observed in the absence of Zn(II) ions, was independent of the kind of buffer used (phosphate or Tris).

(11) Time of flight mass spectrometer measurements of most of the complexes including the 2:2 complex **1c**−**2a** were unfortunately unable to distinguish the presence of these high molecular weight complexes. This was due to significant contamination by Na^+ ions from the water-soluble porphyrin **2a**. (In the case of a dimer type complex there are eight Na^+ counterions present.)