A new cucurbit[8]uril-based fluorescent receptor for indole derivatives†

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The dicationic guest 2,7-dimethyldiazaphenanthrenium forms a fluorescent inclusion complex with the cucurbit[8]uril host, which can be used to effectively bind and detect indole derivatives, such as serotonin and tryptophan.

Cucurbit[8]uril[1,2] (CB8) is capable of hosting two suitable aromatic guests and form stable ternary inclusion complexes. A few years ago, Kim *et al.*[3] reported the formation of stable complexes in which an aromatic electron rich molecule and an aromatic electron deficient molecule share the inner cavity volume of CB8, giving rise to an included charge transfer complex. The same group also reported that CB8 can effectively bind two viologen[4] or tetrathiafulvalene[5] cation radicals, providing additional stabilization to the dimers formed by these species. The unique ability of CB8 to stabilize by inclusion complexation π donor–acceptor charge transfer complexes or homodimers formed by viologen cation radicals has been used to advantage in the design of molecular machines,[6–9] as well as in the redox control of dendrimer self-assembly.[10] Urbach and co-workers have utilized the stable inclusion complex formed between methylviologen (MV^{2+}) and CB8 as a receptor to bind aromatic amino acids, such as tryptophan, tyrosine and phenylalanine, as well as tryptophan-containing peptides.[11] Similarly, our group has used the stable inclusion complex formed between the fluorescent dication, 2,7-dimethyldiazapyrenium (DAP^{2+}) and CB8 to bind and detect catechol and dopamine.[12] However, in the latter inclusion complex the large cross-section of DAP^{2+} results in a slight elliptical distortion of the D_{8h} host symmetry and limits the volume available for inclusion of an additional π donor guest within the cavity. Thus, indole and its derivatives are only weakly bound along DAP^{2+} inside the CB8 cavity. In order to increase the molecular rigidity of the central aromatic section of methylviologen, affording fluorescent properties without reaching the large cross-section of DAP^{2+}, we decided to prepare the 2,7-dimethyldiazaphenanthrenium (DPT^{2+}) dication as an obvious compromise (see Fig. 1 for structures). Here, we report on the guest properties of DPT^{2+} with the CB8 host and the application of the DPT^{2+}@CB8 complex as a fluorescent receptor for indole and their biologically relevant derivatives, tryptophan and serotonin.

2,7-Diazaphenanthrene was prepared as reported by Gill and Bracher.[13] Exhaustive methylation with iodomethane and treatment with the chloride form of ion-exchange resin beads (Amberlite7) yielded the pure dichloride salt ($DPT{\cdot}Cl_2$). In aqueous solution DPT^{2+} exhibits a strong UV absorption band

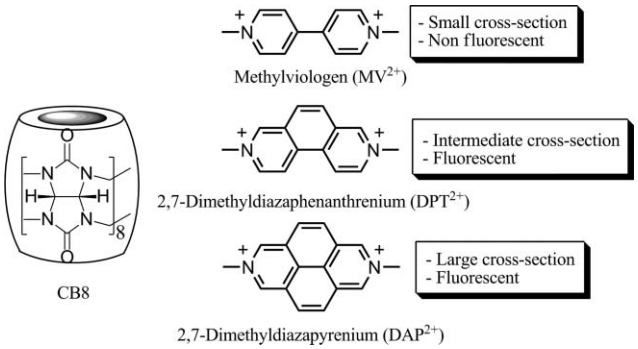

Fig. 1 Structures of the cucurbit[8]uril host and various electron acceptor guests.

(λ_{max} = 248 nm, $\varepsilon = 6.1 \times 10^4$ M^{-1} cm^{-1}) and two minor absorption bands at 370 and 390 nm. A strong fluorescent emission was also observed (λ_{max} = 405 nm), as anticipated from the rigid character of the aromatic region of this dication.

The ^1H NMR spectra of DPT^{2+} in D_2O solution shows four signals corresponding to the four types of aromatic protons in the dication (the methyl protons overlap with the HDO signal). Addition of 0.5 equiv. CB8 causes broadening and shifting of all the aromatic proton signals to higher field. Addition of a slight excess CB8 leads to the observation of all four aromatic signals, broadened and upfield shifted (see ESI†). These NMR spectroscopic data reveal that, as anticipated, DPT^{2+} forms an inclusion complex inside CB8. The rate of chemical exchange between the free and bound guest is fast in the NMR time scale.

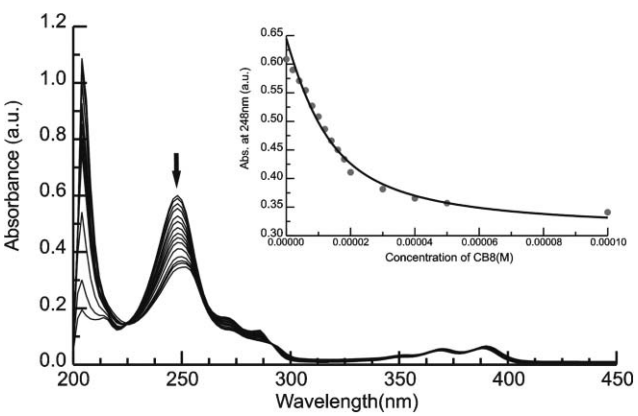

Fig. 2 Electronic absorption spectrum of 10 μM DPT^{2+} in the presence of increasing CB8 concentrations (0–0.1 mM CB8, increasing in the direction of the arrow). The inset shows the best fit of the experimental data to the 1 : 1 binding model.

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The binding interactions between DPT^{2+} and CB8 can also be monitored by electronic absorption spectroscopy. As it is the case with methylviologen,[14] the molar absorptivity coefficient of DPT^{2+} at 248 nm is depressed upon CB8 inclusion. Therefore, the decreasing absorbance as a function of the added CB8 concentration can be fitted to a 1 : 1 binding model[15] to determine the association equilibrium constant (K), which was found to be $(1.0 \pm 0.3) \times 10^5$ M^{-1} ($\Delta G^\circ = -28.7 \pm 0.6$ kJ mol^{-1}) in pH 7 0.1 M phosphate buffer (see Fig. 2). The fluorescent emission of DPT^{2+} was significantly enhanced by complexation inside CB8, which affords a second method to determine the corresponding K value. From fluorescence emission measurements we obtained a value of $(7.8 \pm 0.3) \times 10^4$ M^{-1} ($\Delta G^\circ = -27.9 \pm 0.1$ kJ mol^{-1}). Both K values are reasonably close to each other.

All these data clearly reveal that DPT^{2+} forms a stable inclusion complex inside the cavity of CB8. This point was further verified computationally using PM3 semi-empirical methods. Energy minimization of the complex, using the reported crystal structure of the host and an energy minimized structure for the guest as the starting point for the calculations, leads to the structure shown in Fig. 3. We conclude that the complex is strongly stabilized by ion–dipole interactions between each of the quaternized nitrogens on the guest and the carbonyl oxygens lining the host cavity portals. In addition to this, hydrophobic contacts that develop between the aromatic surface of the guest and the inner surface of the host cavity also contribute to the stability of this complex.

As expected, the stability of the DPT^{2+}@CB8 complex is similar to those of the previously investigated MV^{2+}@CB8[14,16] and DAP^{2+}@CB8.[12] However, the DPT^{2+}@CB8 is strongly fluorescent and has more free volume available within its cavity than the also fluorescent DAP^{2+}@CB8 complex. In order to verify the latter point we investigated binding of indole, as well as two indole derivatives (tryptophan and serotonin), along the acceptor guest DPT^{2+} in the cavity of the CB8 host. Indole was found to be an excellent guest for the formation of these ternary complexes. ^{1}H NMR spectroscopy of an equimolar mixture of DPT^{2+} and indole in aqueous solution shows the aromatic proton signals of both components at chemical shifts similar to those observed individually for each of them. This reflects the lack of association – in the absence of CB8 – between these compounds at the mM concentrations used in these experiments. However, the addition of CB8 leads to pronounced upfield shifts for all the aromatic proton resonances of the π acceptor (DPT^{2+}) and the π donor (indole), clearly suggesting that CB8 enhances markedly the association of these two aromatic compounds inside its cavity (Fig. 4). We note that the CB8-induced upfield shift on the signal for protons 9 and 10 on DPT^{2+} is more pronounced in the presence of 1 equiv. indole (*ca.* 0.7 *vs.* 1.0 ppm), which reflects the combined effect of DPT^{2+} inclusion inside CB8 and π stacking interactions with indole. The final proton chemical shifts observed in the equimolar mixture of DPT^{2+}, indole and CB8 were the same regardless of the order of addition of the three components. Similar data were obtained with tryptophan and serotonin, revealing that the indole moiety in these two compounds is responsible for the binding interactions with the DPT^{2+}@CB8 inclusion complex.

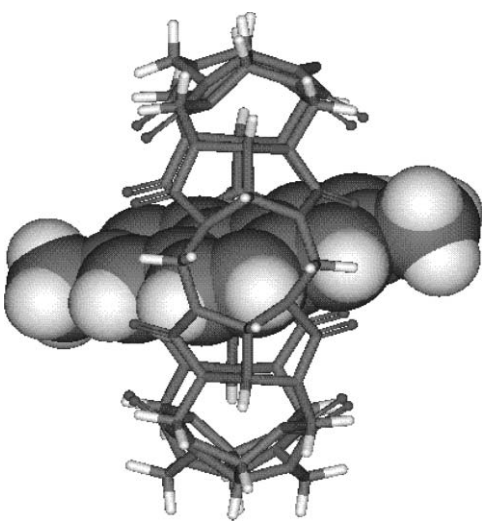

Fig. 3 Energy-minimized structure of the DPT^{2+}@CB8 complex (PM3).

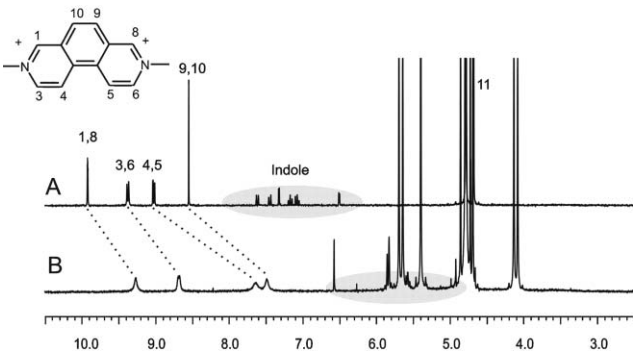

Fig. 4 ^{1}H NMR spectrum (0.1 M NaCl–D_2O, 300 MHz) of 2.5 mM DPT^{2+} and 2.5 mM indole (A) in the absence and (B) in the presence of 1 equiv. CB8.

We also investigated the formation of these ternary complexes by electronic absorption spectroscopy. The inclusion of indole, serotonin or tryptophan in the DPT^{2+}@CB8 complex is expected to give rise to charge transfer bands resulting from the strong interaction between the π acceptor guest (DPT^{2+}) and the π donor guest (indole derivative) inside the CB8 cavity. These bands were all clearly detected in our experiments (see ESI†) but we did not use them for any quantitative purposes due to their low intensity compared to the remaining absorption bands exhibited by the guests in our ternary complexes. As a much better alternative we monitored the formation of the ternary inclusion complexes using fluorescence emission measurements. In this regard, the fluorescence emission of DPT^{2+}@CB8 is anticipated to be quenched by association with a π donor such as indole. In order to verify this prediction, it is necessary to select carefully the excitation and emission wavelengths for the experiments, owing to the well-known fluorescent nature of indole and its derivatives. After inspection of the excitation spectra, we selected an excitation wavelength of 360 nm, because indole does not absorb at this wavelength, while DPT^{2+} and its CB8 inclusion complex do. Therefore, the fluorescence emission detected under these conditions originates from the excited state of the DPT^{2+} guest. Using these experimental conditions, we monitor the fluorescence of DPT^{2+}@CB8 as a function of the concentration of indole (I) added to the solution. The data show a pronounced but gradual quenching of the emission, which levels off at *ca.* 14% of the initial

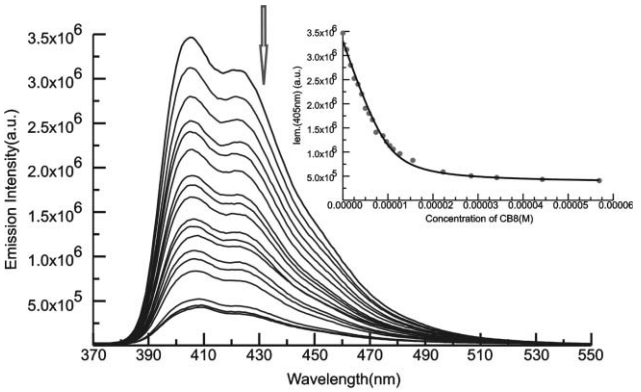

Fig. 5 Fluorescence intensity from a solution containing 10 μM DPT^{2+} and 10 μM CB8 as a function of the added indole concentration (0–57 μM, increasing in the direction of the arrow). The inset shows the fit of the data to the binding model described in the text.

fluorescent intensity (Fig. 5). In agreement with our NMR and UV-Vis spectroscopic data we interpret these results as a reflection of the formation of the $I{\cdot}DPT^{2+}@CB8$ ternary complex in which the fluorescence of the DPT^{2+} guest is strongly quenched by the close proximity of the indole guest. The overall binding process can be described by the following equilibria:

$$DPT^{2+} + CB8 \rightleftarrows DPT^{2+}@CB8 \quad (1)$$

$$DPT^{2+}@CB8 + I \rightleftarrows I{\cdot}DPT^{2+}@CB8 \quad (2)$$

The association constant for equilibrium (1) was obtained previously (*vide supra*). Since the corresponding K value is relatively large (*ca.* 10^5 M^{-1}) and the equilibrium is further shifted to the right because the binary complex is removed by complexation with indole, we assumed that the whole binding process can be simplified and treated as a single equilibrium between indole and $DPT^{2+}@CB8$, whose initial concentration was approximated from the concentrations of DPT^{2+} and CB8 present in the system. Using this approximation, we could fit the fluorescence intensities measured as a function of the concentration of indole added to the solution (Fig. 5) and estimate the association constants for equilibrium (2). For indole we obtained a K value of $(1.1 \pm 0.2) \times 10^6$ M^{-1}. Similarly, the values obtained for tryptophan and serotonin were $(4.2 \pm 0.1) \times 10^5$ and $(1.4 \pm 0.1) \times 10^5$ M^{-1}, respectively. It is interesting to compare the K value obtained here for the association of tryptophan and $DPT^{2+}@CB8$ with the value obtained by Urbach and co-workers[11] for the association of tryptophan and $MV^{2+}@CB8$ (4.3×10^4 M^{-1}). Replacement of MV^{2+} by DPT^{2+} as the electron acceptor guest in the CB8 complex leads to an increase of ca. one order of magnitude in the measured binding affinity for tryptophan. This is probably due to the largest aromatic area of the latter dication and confirms that DPT^{2+} is at least as effective as MV^{2+} in driving the formation of ternary complexes with CB8 and indole derivatives, while affording analytically useful fluorescent properties.

In summary, we have shown that the electron acceptor guest 2,7-dimethylphenanthrenium gives rise to a very stable inclusion complex with CB8, while leaving enough room inside the host cavity for the formation of stable ternary complexes with indole and its derivatives. The formation of these complexes can be easily detected by the quenching of the fluorescence emission of the $DPT^{2+}@CB8$ complex. These binding phenomena might be useful for the development of new fluorescence methods to detect and quantitate either serotonin or tryptophan.

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