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A selective supramolecular photochemical sensor for dopamine

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Dopamine (DA) is an important biomarker for diseases and biological disorders. Existing techniques for DA detection suffer from drawbacks including low sensitivity and selectivity as well as interfering signals from non-target molecules. A simple and selective photochemical sensor for the determination of DA in a supramolecular manner is presented. This approach utilises the complexation properties of a highly fluorescent water-soluble complex of perylene bis(diimide) dye with the macrocyclic host cucurbit[8]uril. The method can be used for the determination of DA in aqueous media, with detection limits below 2×10^{-5} M, even in the presence of known interferents including ascorbic acid and the catecholamines epinephrine and norepinepherine.

Keywords: cucurbit[8]uril; perylene bis(diimide); dopamine; sensor; supramolecular; host–guest

1. Introduction

Biogenic catecholamines such as dopamine (DA), norepinephrine (NE) and epinephrine (EPI) are important neurotransmitters in the central and peripheral nervous system (Figure 1(a)). DA is the most abundant of the three catecholamines. The detection of DA is of considerable clinical and pharmacological importance as it is associated with several neurological disorders and neurocrine tumours (*1–3*). In addition to DA imaging in live cells, DA can also be detected in various biological fluids, including serum, plasma, platelets, cerebral spinal fluid, saliva and urine (*1*). These samples often contain interfering compounds that cannot be easily resolved by commonly used electrochemical methods (*4*). Ascorbic acid (ASC), for example, is a major interferent in the electrochemical detection of DA (*5*, *6*). While interference can be reduced using various surface modification strategies on the electrodes, the resultant decrease in efficiency of electron transfer to the electrode surface compromises the sensitivity of the technique (*5*). New technologies are moving towards functionalised metal nanoparticles to improve sensitivity (*7*). Small organic molecules are also being designed for DA detection; however, they are often only selective for all catecholamines in complex mixtures, instead of single analytes of interest (*8*). Most of the existing methods (*9*) often rely on elaborate sampling-coupled techniques including liquid chromatography, mass spectrometry and capillary electrophoresis (*5*, *10–12*). The development of molecular probes for direct detection of DA remains challenging. An optical method, which is selective towards DA would, therefore, offer several advantages as a DA sensor over existing electrochemical systems such as fast detection with minimal interference.

A variety of synthetic receptors have been shown to bind DA with a wide range of equilibrium constants and selectivity depending on their design architectures (*13*). These systems could be implemented into non-intrusive, real-time sensing techniques for DA in biological media. Cucurbit[8]uril (CB[8]) is a macrocyclic host compound known for its ability to form 1:1:1 hetero ternary complexes in aqueous media with two different guest molecules (*14–16*). The inclusion of the guest molecules into CB[8] is driven by the release of the pre-encapsulated high-energy water from the cavity (*17*). The encapsulation of dye molecules as guests inside the CB[*n*] cavities exhibits well-known and often tuneable photophysical properties (*18–22*). Kaifer and co-workers have shown that CB[8] can form stable inclusion complexes with 2,7-dimethyldiazapyrenium with enhanced fluorescence when compared to the non-encapsulated dye (*23*). The fluorescence of the binary complex is quenched by the addition of a second guest including catechol (Cat) and DA, with more pronounced effect associated to Cat, thus indicating relatively low selectivity for DA. Our group has recently described that *N*,*N*-bis[diethylamine]-3,4,9,10-perylenetetra-carboxylic diimide (perylene diimide, PDI) can also be encapsulated in the cavity of CB[8] with a simultaneous and dramatic enhancement of the guest fluorescence as a result of breaking of the PDI π-stacks (*22*). This binary PDI·CB[8] host–guest system has been shown to be selective for different small molecules

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including azobenzene and dibenzofurane derivatives. Herein, we show the binding properties between PDI·CB[8] and DA and demonstrate the potential of utilising this supramolecular approach for the development of highly sensitive and selective sensors for DA (*24*).

2. Results and discussion

Initial investigation of the ternary complex formation between PDI·CB[8] and a variety of catecholamines as second guests was carried out by nuclear magnetic resonance (NMR) spectroscopy. First, a series of ^{1}H NMR experiments were performed in D_2O to test for complex formation between PDI·CB[8] and selected guests DA, EPI and NE (see Supporting Information). The formation of ternary complexes can be observed as shifts and broadening of proton signals from both the first and second guest molecules in ^{1}H NMR. It is well known that the cavity region of CB[*n*] host molecules constitutes an NMR-shielding region, whereas the region just outside the carbonyl-lined portals is deshielding (*25–28*). The signals corresponding to the aromatic protons of both DA and EPI shift upfield and show broadening in the presence of PDI·CB[8] (Figure S1). Similar changes were observed for signals corresponding to the aromatic protons of PDI (in the ternary complex) compared to PDI·CB[8] (*22*). The shifting of the signals of both PDI and second guest is slightly larger in the case of the DA ternary complex, which may be attributed to a higher binding association. These preliminary results indicated that PDI·CB[8] exhibited some degree of selectivity towards DA. Ternary complex formation was further confirmed by a series of diffusion-ordered (DOSY) NMR experiments (Figure 2). The NMR signals of CB[8], PDI and DA share a single diffusion coefficient as they all diffuse as one single ternary complex.

In general, the binding of a second guest to the PDI·CB[8] binary complex quenches the fluorescence emission and also results in changes in the absorbance properties of the encapsulated PDI (*22*). Therefore, a UV/vis spectroscopic titration of DA into the PDI·CB[8] complex was carried out in order to determine the strength of the second guest binding event and the stoichiometric ratio of each component in the interaction (Figure 3) (*29*). The titration shows a significant decrease in the peak at $\lambda_{max} = 537$ nm, while a new peak arises at $\lambda_{max} = 572$ nm, indicating an interaction of DA with the PDI·CB[8] binary complex. A plot of the concentration of DA against normalised absorbance at 537 nm was fitted to the empirical logistic function (*30*) shown in the following:

$$y = \frac{1}{K_a + e^{(-a/(x-b))}}, \quad (1)$$

where y is the observed property proportional to complex formation, K_a is the association constant (M^{-1}), a is the point of inflection of the logistic curve (M), x is the concentration of DA (M) and b is a constant (M).

Interestingly, the shape of the binding curve imitates a typical 'sigmoidal dose–response' curve commonly found in biological systems, which is associated with ligand and macromolecular interactions that show some degree of cooperative binding. The binding isotherm of DA with PDI·CB[8] gave a relatively strong association constant of $10^6\,M^{-1}$, while the point of inflection at 3.2×10^{-5} M indicated a 1:1 DA:PDI·CB[8] stoichiometry (Figure 3(a)). A Job plot constructed from the change in absorbance at

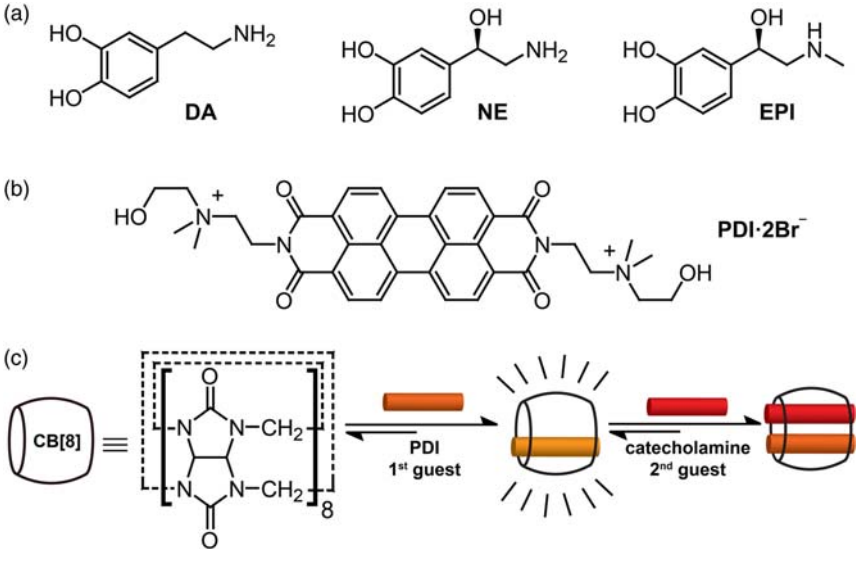

Figure 1. (Colour online) Structures of (a) DA, NE and EPI, (b) PDI and (c) schematic showing the formation of the PDI·CB[8]·catecholamine ternary complex.

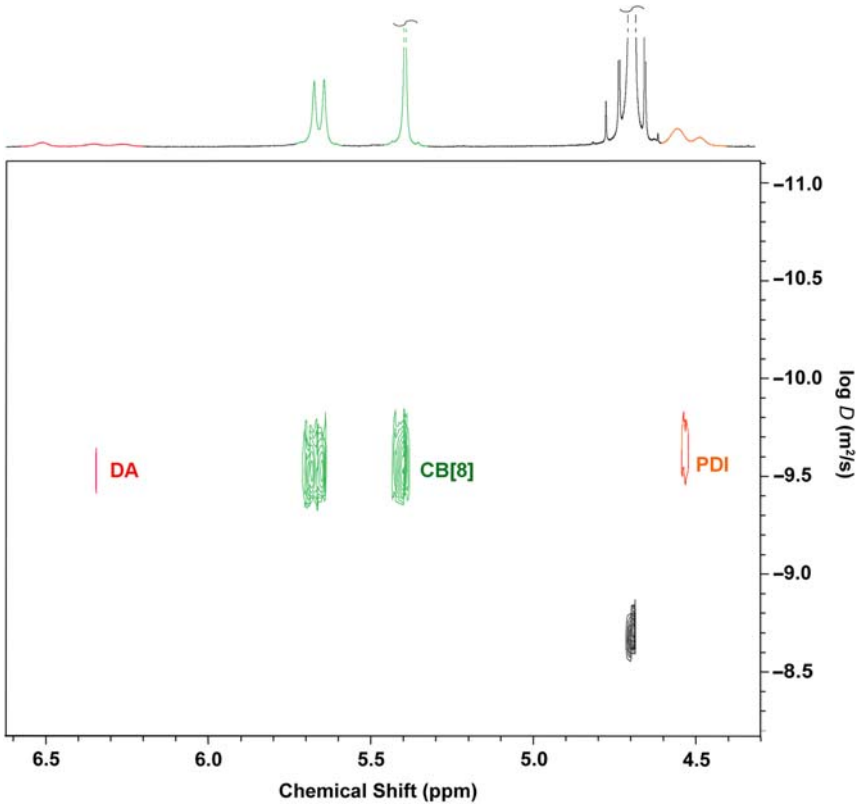

Figure 2. (Colour online) ^{1}H NMR DOSY plot of an equimolar mixture of CB[8] (green), PDI (orange) and DA (red) (0.5×10^{-3} M, 298 K).

572 nm also gave a stoichiometry of 1:1 as expected (Figure 3(b)). Results using isothermal titration calorimetry confirmed the high binding association of DA towards PDI·CB[8] (10^5 M^{-5}) (Figure S2). UV/vis binding studies performed with EPI and NE against the PDI·CB[8] binary complex showed substantially lower binding strengths of 10^3 and 10^1 M^{-1} for EPI and NE, respectively.

Kaifer and co-workers have previously shown that the fluorescence of 2,7-dimethyldiazapyrenium (DAP) encapsulated in CB[8] is also quenched in the presence of DA and Cat (see Section 4.2 for details of curve fitting) (*23*). A series of qualitative studies indicated that Cat binding is weaker in comparison to DA, likely on account of both the charged ammonium group of DA and the better fitting of Cat inside the DAP·CB. In our case, the structures of the three neurotransmitters are relatively similar (Figure 1(a)), thus a series of experiments were performed to investigate the higher affinity of the system towards DA. In order to determine whether the aromatic Cat or the aliphatic ethylamine unit plays a greater role in the selective binding of DA in the ternary complex, comparative UV/vis titrations were carried out with Cat and NE. NE was

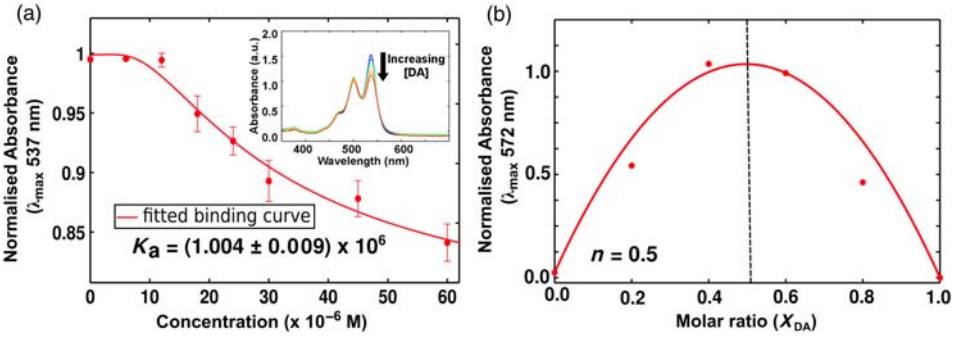

Figure 3. (Colour online) (a) Binding isotherm of DA titration into PDI·CB[8] monitored by UV/vis spectroscopy (titration spectra shown inset), see Section 4.2 for details of curve fitting; (b) Job plot showing 1:1 DA:PDI·CB[8] binding stoichiometry (maximum complex formation at molar ratio, $n = 0.5$).

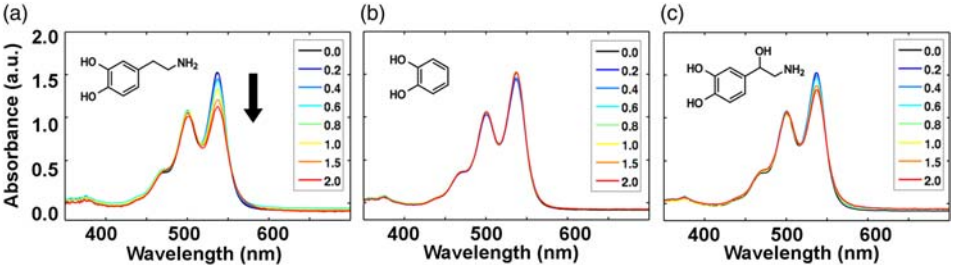

Figure 4. (Colour online) Titration of (a) DA, (b) Cat and (c) NE into 1:1 PDI·CB[8] (3×10^{-5} M) solution.

chosen as one of the controls as it contains a parent Cat unit and has a similar structure to DA, except that NE contains an additional hydroxyl group at the β-C position of its ethylamine group. The results of the UV/vis spectroscopic titration of DA, Cat and NE into PDI·CB[8] are shown in Figure 4. The addition of DA clearly shows a significant decrease in the absorbance band of PDI at 537 nm, whereas Cat and NE show negligible changes comparatively.

PDI·CB[8], therefore, appears to be highly selective towards the ethylamine moiety of the DA as Cat alone does not show binding with PDI·CB[8]. The difference in the binding behaviour of DA compared to NE also indicates that the alkyl components of these molecules play a major role in binding. The presence of the hydroxyl group on the alkyl arm of the NE appears to hinder binding. This could be a result of steric hindrance and/or unfavourable interactions of the hydroxyl group with the electronegative carbonyl portals. Although it is clear from these experiments that binding to DA is favoured by the PDI·CB[8] complex, it is important to understand whether the PDI·CB[8] is still selective for DA in the presence of interferents commonly found in biological systems. Two different types of interferents can be associated with the detection of DA in biological systems. The first is the presence of other catecholamine metabolites and the second is the high concentration of ASC in biological fluids. In fact, interference from ASC is a major hindrance in most of the existing detection techniques for DA (*5*). Therefore, the binding affinity of PDI·CB[8] towards DA was studied in mixtures containing DA, EPI and NE, both in the presence and absence of ASC in order to determine the selectively for DA in 'real' systems (Figure 5).

When both NE and EPI are present together with the PDI·CB[8] in a control mixture without DA present, the combined effect of the two catecholamines (NE and EPI) also produces a significant change in absorbance. Currently, we are unable to definitively assign any complex arising from this marked change. However, one possibility is the formation of a 1:1:1 heteroternary complex of EPI·CB[8]·NE. In contrast to the DA-binding isotherm, the titration data of EPI, NE and MIX1 (EPI + NE) fit the following binding equation, which follows a rational function (*31*):

$$y = \frac{bK_a x}{1 + K_a x}, \tag{2}$$

where K_a is the association constant (M^{-1}), x is the concentration of EPI and NE (M) and b is a constant (M).

From Figure 5(a), it is clear that the binding isotherm for the mixture containing DA, EPI and NE with PDI·CB[8] follows a similar binding isotherm as for PDI·CB[8]·DA complex alone. The presence of EPI and NE in the mixture, together with DA, does not affect the generalised logistic curve shape of the DA-binding isotherm. This confirms that the presence of one equivalent or even higher amounts of NE and EPI in mixtures of these compounds has negligible effects on the selectivity of the PDI·CB[8] complex for DA (Figure 6). ASC does not

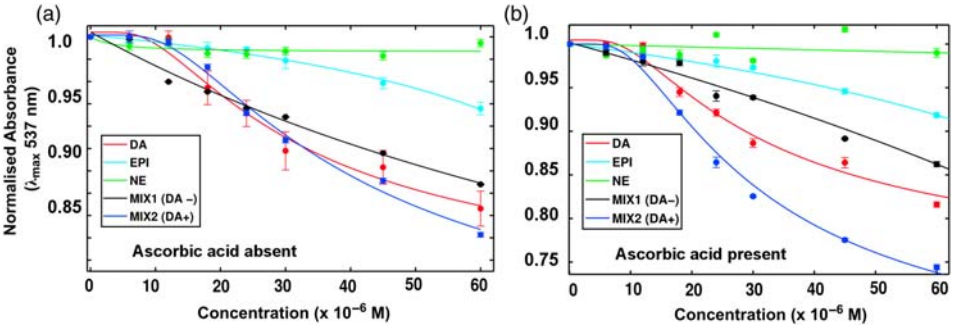

Figure 5. (Colour online) Binding isotherms for DA, EPI, NE, MIX1 (1:1 EPI + NE, without DA) and MIX2 (1:1 EPI + NE 3×10^{-5} M, with DA) in the (a) absence of ASC and (b) presence of ASC.

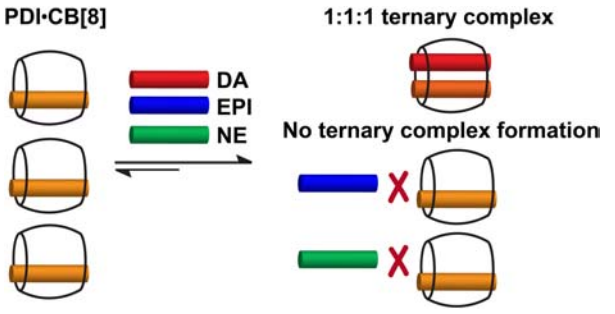

Figure 6. (Colour online) Schematic showing the selectivity of PDI·CB[8] towards DA in the presence of common interferents EPI and NE.

show binding to PDI·CB[8] (Figure S3). When ASC is added to the system, the binding isotherms maintain their original curve shapes (Figure 5(b)) and the association constants remain unaffected respectively (Table 1).

This shows that DA binding to PDI·CB[8] is the dominant event even in mixtures containing EPI, NE and ASC. The distinctive nature of the DA-binding curve allows determination of the presence of DA in the mixtures with certainty despite the small changes in absorbance of PDI·CB[8] from the presence of other two other catecholamines. Therefore, the presence of DA can be selectively determined in a mixture of catecholamines without prior sample preparation, thus giving a simple and efficient detector for DA in complex matrices at low concentrations. These are clearly important advantages of this sensor over those previously reported (5).

3. Conclusion

To summarise, this paper reports a simple and sensitive supramolecular method for the detection of DA in a mixture of catecholamines, which has a limit of detection of less than 2×10^{-5} M based on the PDI·CB[8] complex. Typical measurements including sample preparation and titration can be made within a time frame of 5–10 min.

Table 1. Association constants of PDI·CB[8] towards catecholamines and their mixtures in the presence and absence of ASC.

Second guest	K_a (M^{-1}) without ASC	K_a (M^{-1}) with ASC
DA	$(1.0 \pm 0.009) \times 10^6$	$(1.0 \pm 0.013) \times 10^6$
EPI	$(1.0 \pm 0.007) \times 10^3$	$(1.3 \pm 0.008) \times 10^3$
NE[a]	–	–
Mixture 1 (EPI + NE)	$(1.4 \pm 0.003) \times 10^3$	$(1.5 \pm 0.005) \times 10^3$
Mixture 2 (EPI + NE + DA)	$(0.9 \pm 0.006) \times 10^6$	$(1.0 \pm 0.014) \times 10^6$

Note: ± Error values denote standard deviation.
[a]No data available (poor fit as a result of no binding, see Supporting Information).

This system requires no pre-sampling or functionalisation steps for the direct detection of DA. In addition, it has been shown that the presence of relatively high concentrations of ASC, EPI and NE in the analyte medium does not significantly affect the detection of DA in the system, which is a major advantage over several electrochemical methods. This new detector could open new prospects for fast and sensitive detection of DA in extra bodily fluids for medical diagnostics.

4. Experimental

4.1 Materials

DA hydrochloride, ± EPI hydrochloride and norepinepherine hydrochloride, ASC and Cat were purchased from Sigma-Aldrich (Dorset, UK) and used without further purification. Millipore 18 MΩ cm H_2O was used in all experiments unless stated otherwise. CB[8] was synthesised according to the method published by Day et al. (32). Standard solutions were freshly prepared prior to analysis.

4.2 Methods

4.2.1 NMR spectroscopy

^{1}H NMR and DOSY spectra were recorded on a Bruker Avance 500 BB-ATM (500 MHz) spectrometer (Bruker, Coventry, UK). DOSY experiments were carried out using a modified version of the Bruker sequence ledbpgp2s. Spectra were recorded in heavy water (D_2O) at 298 K. The concentration of CB[8] was fixed at 1×10^{-3} M for all the samples. The experiments were processed with standard Bruker 1D and 2D DOSY software. The diffusion coefficients were determined by fitting the intensity decays to the following equation:

$$I = I_0 \exp\left[-D\gamma^2 g^2 \delta^2 \left(\frac{\Delta\delta}{3}\right)\right], \quad (3)$$

where I and I_0 represent the signal intensities in the presence and absence of gradient pulses, respectively; D is the diffusion coefficient; γ is the ^{1}H gyromagnetic ratio; δ is the duration of the gradient pulse; Δ is the total diffusion time and g is the applied gradient strength.

4.2.2 UV/vis spectroscopy

UV/vis titration spectra were recorded on FLASHscan 550 (Jena, Germany) Analytik Jena (excitation wavelength = 510 nm). UV/vis measurements for the construction of the Job Plot was carried out on a Varian Cary 4000 UV/vis spectrophotometer (Varian, Agilent Technologies UK Ltd., Stockport, UK). Different equivalents of second guest compounds were titrated into PDI·CB[8] and the final volume was adjusted with H_2O. The concentration of PDI·CB[8] was

fixed at 3×10^{-5} M for all the measurements. Matlab curve fitting toolbox (MathWorks, Cambridge, UK) was used to fit the binding Equations (as mentioned in the main text) to the titration data. Absorbance data were normalised by division with the absorbance at the corresponding λ_{min} at 800 nm to correct for any minor baseline shifts prior to curve fitting. The reported association constants are an average of three measurements.

4.2.3 Isothermal titration calorimetry

Measurements were carried out on NanoITC (TA Instruments, Waters Ltd., Elstree, UK) where 1×10^{-3} M solution of DA was titrated into a 10 times lower concentration of PDI·CB[8]. The data were fitted to an independent binding model inbuilt within the instrument software.

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