Host–guest assembly of squaraine dye in cucurbit[8]uril: its implication in fluorescent probe for mercury ions†

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Received 1st February 2010, Accepted 23rd March 2010
First published as an Advance Article on the web 20th April 2010
DOI: 10.1039/c002219p

The binding interactions between SQ2 squaraine dye and cucurbit[8]uril (CB8) effectively removes the aggregation of SQ2 in aqueous solution by forming a 1 : 1 inclusion complex. The resulting SQ2·CB8 complex exhibits highly selective fluorescence quenching by Hg^{2+} ions, as a result of the synergetic binding between CB8, SQ2 and metal cation.

Mercury ion is one of the most toxic environment pollutants and can be accumulated in the organs of human or animal bodies through the food chain. Even low concentrations can give rise to a wide variety of disease and health problems.[1] In recent years, many studies have been directed to develop various probes specifically for Hg^{2+} ion detection.[2] The most attractive approach in this field is fluorescence-based sensors owing to their intrinsic sensitivity and selectivity.[3] However, while a few near-infrared (NIR) fluorescent Hg^{2+} probes are available[2c,d] it remains a greater challenge to design NIR Hg^{2+}-specific fluorescent probes.

As a class of well-studied organic dyes, squaraines exhibit intense absorption and fluorescence in the red to near-infrared region.[4] These dyes exhibit high tendency to form aggregates in solution, which significantly affects their optical properties. Depending on surrounding media, squaraine dyes can form J-aggregates that give red-shifted absorption bands (as compared to monomer absorption), or H-aggregates that give blue shifted absorption bands. Sensitivity of aggregate formation to the surrounding media has made squaraines suitable candidates for cation sensors.[5] The different aggregates show different emission properties with H-aggregates usually being poor emitters while J-aggregates show efficient luminescence.[6] Formation of a supramolecular guest–host assembly represents an effective strategy to control the molecular aggregation. Successful examples include encapsulating squaraine dyes to form rotaxanes,[7] formation of an inclusion complex between thioflavin and cucurbit[8]uril (CB8),[8] and using cationic[9] and nonionic[10] surfactants. The H- and J-aggregates of a cyanine dye can be controlled by using cucurbit[7]uril.[11] Utilization of surfactant has been shown to improve the fluorescence response of semisquaraine dye[9] and phenylene-ethynylene dendrimer[10] to achieve the selective detection of Hg^{2+} cation. To our best knowledge, no study has been reported to use CB8 to aid the Hg^{2+} chelation and selective detection.

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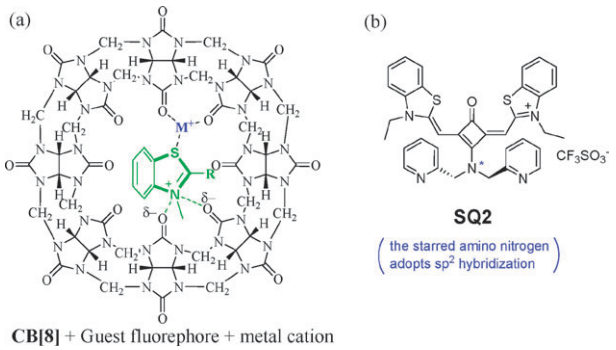
† Electronic supplementary information (ESI) available: Synthetic procedures, characterization of **SQ2**, and additional spectroscopic data. CCDC 764291. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c002219p



Scheme 1 Schematic representation of metal ion binding in the inclusion complex with a benzothiazolium derivative (a) and structure of **SQ2** (b).

Cucurbit[8]uril (CB8), a synthetic pumpkin-shaped cation receptor, is a member of the host family of cucurbit[*n*]urils, which show a strong affinity for cationic species such as protonated amines.[12,13] The binding between a cationic organic molecule and CB is thought to be driven by an interplay of hydrophobic interactions with the inner cavity as well as polar interaction with the carbonyl groups at the portals.[13] The inclusion complex with the cationic benzothiazolium as guest could be very interesting (Scheme 1), as the polar interaction between the positively charged nitrogen atom and carbonyl will place the sulfur atom near the portals of CB8. The unique geometry of CB8 portals, in conjunction with appropriate location of the sulfur atom, could enhance the selective interaction of metal ion with benzothiazolium moiety while restricting the access of other interfering chemicals. Herein we demonstrate such concept by using the squaraine dye (**SQ2**) which forms a guest–host complex with CB8. The resulting complex exhibits improved selectivity in recognizing Hg^{2+} cation.

SQ2 was synthesized by using a modified literature procedure.[14] The crystal structure of **SQ2** reveals that both sulfur atoms are pointing to the same side. The squaraine skeleton is nearly planar (Fig. 1), while two benzothiazole rings are twisted away slightly by $\sim 10^{\circ}$ from the coplanarity.

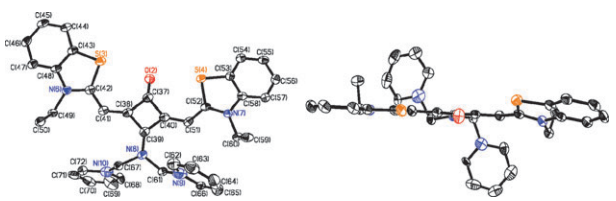


Fig. 1 ORTEP view of the molecular structure of **SQ2** from the front (left) and side (right). All hydrogen atoms are omitted for clarity.

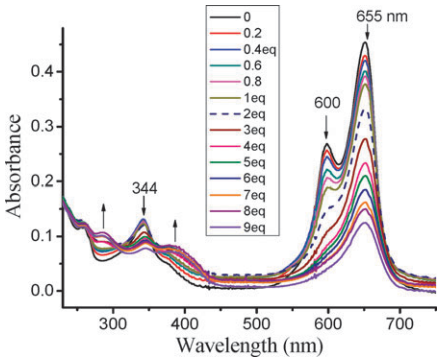


Fig. 2 Absorption spectra of **SQ2** (5 μM) with different equivalent of CB8 in water. The molar extinction coefficients (ε) of the **SQ2**·CB8 complex at 655 nm appears to be lower than that of non-complexed form.

The amino nitrogen (N8) adopts the sp^2 hybridization, indicating a strong interaction present between the nitrogen atom and the π-conjugated backbone of **SQ2**. The crystal structure also shows that the two pyridinyl rings in the di(2-pyridinylmethyl)amine (DPA) group are located on the opposite sides of the squaraine main plane.

In solution, **SQ2** exhibits absorption peaks at 344 and 655 nm at low concentration (1 μM), which can be attributed to the DPA segment and **SQ2** backbone, respectively. At 5 μM concentration, a new absorption peak appears at ~600 nm (Fig. 2), which is assigned to H-aggregates on the basis of the observed spectral blue-shift (~55 nm) in comparison with the absorption of the monomer. The assignment is consistent with the crystal packing of **SQ2**, as the X-ray crystal structure reveals significant parallel overlap of two conjugated backbones.

The binding interaction between **SQ2** and CB6 and CB8 were investigated in aqueous solution. Addition of CB6 (by as much as 8 equiv.) shows nearly no visible influence on the aggregation absorption band of **SQ2**. In sharp contrast, the presence of about 3 equiv. of CB8 causes the complete disappearance of the H-aggregate absorption band at ~600 nm (Fig. 2), suggesting the formation of the guest–host complex **SQ2**·CB8. Mass spectrometry detected **SQ2**·CB8 (m/z = 1942.5974) without the $CF_3SO_3^-$ counterion (Fig. S1, ESI†). The complexation is observable in the ^1H NMR spectrum (Fig. S2, ESI†). The corresponding equilibrium association constant (K) is determined to be 2.4×10^5 M^{-1} on the basis of 1 : 1 stoichiometry of the complexes (revealed from Job plot, Fig. S3, ESI†).

A molecular modeling study (AM1) further shows that the **SQ2** can fit into CB[8] (Fig. 3). The two pyridinyl groups, which point to the opposite sides of the conjugated plane (as seen from the crystal structure), appears to still allow the guest molecule **SQ2** to thread through the cavity of CB8. The result also explains why CB6 of smaller cavity is ineffective to remove aggregation of **SQ2**, since an adequate cavity size from the cucurbit[*n*]uril is essential to fit the guest molecule. The bulky DPA group also prevents the inclusion of a second **SQ2** in the CB8 cavity for dimer formation, which can occur when the guest is less hindered.[8]

The photophysical behaviors of **SQ2** in the absence and presence of CB8 were examined upon addition of various

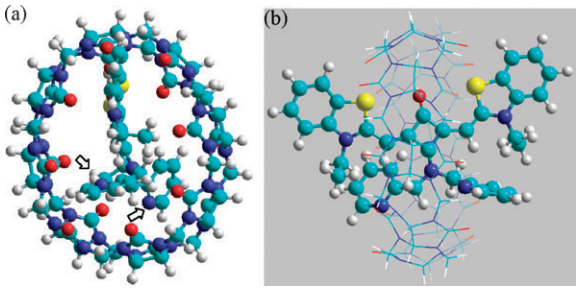


Fig. 3 Optimized geometry of the **SQ2**·CB8 complex in side (a) and front view (b), where the C, N, S and O atoms are in cyan, blue, yellow and red, respectively. The pyridinyl groups in view (a) are indicated by double arrows, and the CB[8] in view (b) is shown in the stick model.

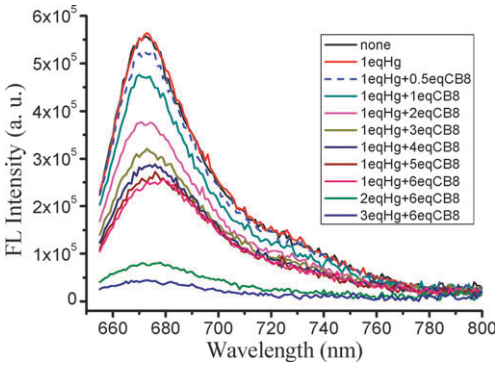


Fig. 4 Fluorescence spectra of **SQ2** (5 uM) in water in the presence of different equivalents of CB8 and Hg^{2+}.

metal ions in aqueous solution. The fluorescence of **SQ2** at ~673 nm is nearly unaffected upon addition of 1 equiv. of Hg^{2+} cation (Fig. 4). Introduction of CB8 to the solution containing **SQ2** and Hg^{2+}, however, leads to gradual fluorescence quenching, which is accompanied with disappearance of the H-aggregate absorption band at 600 nm (Fig. S4, ESI†). The results reveal that the CB8 has a profound effect on the **SQ2**–Hg^{2+} interaction.

The response of the host–guest supramolecular assembly **SQ2**·CB8 to Hg^{2+} was further evaluated by addition of three equiv. of CB8 to **SQ2** aqueous solution, which ensures all **SQ2** molecules are incorporated into the CB host. The fluorescence signal of **SQ2**·CB8 is completely quenched after addition of 3 equiv. of Hg^{2+} ion (Fig. 4). In the absence of CB8, however, 50 equiv. of Hg^{2+} are required to completely quench the fluorescence of **SQ2**. Dramatic difference in the optical response of **SQ2** to Hg^{2+} cation (Fig. 5) strongly indicates the involvement of the guest molecule CB8. The absorption and fluorescence titration data indicate a 1 : 2 binding model between **SQ2** and Hg^{2+} cation (Fig. S5, ESI†). The effective Hg^{2+}-binding constants are determined to be $(4.3 \pm 0.6) \times 10^{10}$ M^{-2} and $(7.3 \pm 0.1) \times 10^7$ M^{-2} for **SQ2**·CB8 complex and **SQ2**, respectively (by using fluorescence titration data). In other words, formation of the CB8 complex increases the Hg^{2+}-binding efficiency by nearly three orders of magnitude. The 1 : 2 binding ratio suggests the possibility that two Hg^{2+} cations are bonding to each end of CB8 to quench the fluorescence of **SQ2**·CB8.

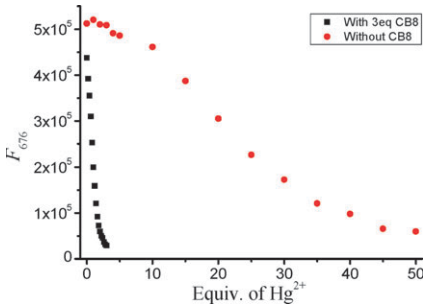


Fig. 5 Fluorescence response of **SQ2** (5 μM) in water to Hg^{2+} ion in the presence (black squares) and absence (red circles) of 3 equiv. of CB8.

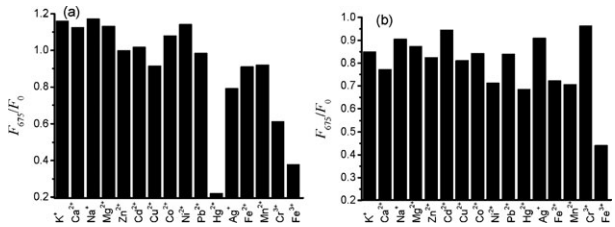


Fig. 6 Fluorescence quenching data for **SQ2** (5 μM) in water with (a) or without (b) 3 equiv. of CB8 upon addition of various metal ions (2 equiv.).

To validate the selectivity of **SQ2**·CB8 in practice, various cations are added to the solution of **SQ2** under the same conditions (in the presence of 3 equiv. of CB8). Addition of 2 equiv. of alkali, alkaline earth metal or transition metal ions (Mn^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Fe^{2+}, Ag^{+}, Pb^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}) does not induce any apparent fluorescence quenching (Fig. 6). The ratio of F/F_0 at 675 nm (here, F_0 indicates the fluorescence intensity of free **SQ2** and F_{675} indicates the fluorescence intensity upon addition of 2 molar equiv. of respective metal ions) is examined in the presence of various other metal ions. For Hg^{2+}, the F_{675}/F_0 value is almost 0.2, while the values for the other metal ions are approximately 1 (except for Fe^{3+}, the value is 0.4). Therefore, the host–guest supramolecular assembly is a highly selective chemosensor for Hg^{2+}.

In the absence of CB8, the F_{675}/F_0 value for Zn^{2+} cation is about 0.83, indicating moderate interaction. The observation is consistent with the ^{1}H NMR result, which detects no binding characteristics when the **SQ2** solution is titrated with Zn^{2+} in DMSO-d_6 (Fig. S6, ESI†). In the presence of CB8, however, the F_{675}/F_0 value for Zn^{2+} cation is 1, indicating no fluorescence response, despite the fact that DPA is a well known chelator for Zn^{2+} cation.[15] It appears that the middle segment of **SQ2** is shielded by the host CB8, making the DPA group less accessible. The trend observed from Zn^{2+} is also true for most of the other metal ions (except Hg^{2+}, Cr^{3+}, Fe^{3+}), exhibiting a larger F_{675}/F_0 value in the presence of CB8 as the chromophore is shielded from interaction with other cations to some extent.

The selective fluorescence quenching of **SQ2**·CB8 by Hg^{2+} is likely to occur *via* binding to the sulfur atom, which remains accessible in the complex. High affinity of sulfur to Hg^{2+} ion is also expected to play an important role in the observed selectivity.[16] Addition of EDTA to an aqueous solution of **SQ2**·CB8–Hg^{2+} recovers the emission of **SQ2**·CB8 (Fig. S7, ESI†), showing that the binding of Hg^{2+} to **SQ2**·CB8 is a reversible process.

In conclusion, we have demonstrated that H-aggregation of the NIR-emitting dye **SQ2** in aqueous solution can be effectively controlled by forming the inclusion complex **SQ2**·CB8. The array of the carbonyl groups at the CB8 portals provides strong ion–dipole interaction to stabilize the complex with the positively charged guest molecule **SQ2** (in 1 : 1 ratio). Utilization of the oxygen array from CB8, in connection with the fluorophore **SQ2** in the complex, leads to a highly selective and reversible Hg^{2+} sensor. On forming the inclusion complex with CB8, the binding strength between **SQ2** and Hg^{2+} can be enhanced by up to three orders of magnitude. The example illustrates a new approach to design and optimize the performance of fluorescent sensors by using a supramolecular strategy.

This work was supported by The University of Akron and Coleman endowment. We also wish to thank The National Science Foundation (CHE-9977144) for funds used to purchase the NMR instrument used in this work.

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