Effect of host-guest complex formation on the fluorescence of 6-methoxy-1-methyl-quinolinium cation with 4-sulfonatocalix[4]arene: Utilization as a fluorescent probe for the study of difenzoquat binding

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Abstract

The complexation of the highly fluorescent 6-methoxy-1-methyl-quinolinium (C_1MQ) and the widely used herbicide, difenzoquat (DFQ), with 4-sulfonatocalix[4]arene (SCX4) macrocycle was studied by isothermal titration calorimetry, fluorescence and NMR spectroscopy in aqueous solutions at 298 K. Both guests produced 1:1 complexes with SCX4, but the binding affinity of C_1MQ was more than one order of magnitude larger than that of DFQ in neutral medium. The higher stability of C_1MQ–SCX4 complex originated from the significant enthalpy gain upon its formation. The encapsulation of C_1MQ in SCX4 in the ground state resulted in an efficient fluorescence quenching due to electron transfer from the host to the excited guest. The marked difference in the fluorescence quantum yields for free and bound C_1MQ was used to detect the competitive complexation of DFQ in SCX4.

Keywords: host-guest complex, competitive binding, fluorescence quenching, calorimetry, thermodynamics

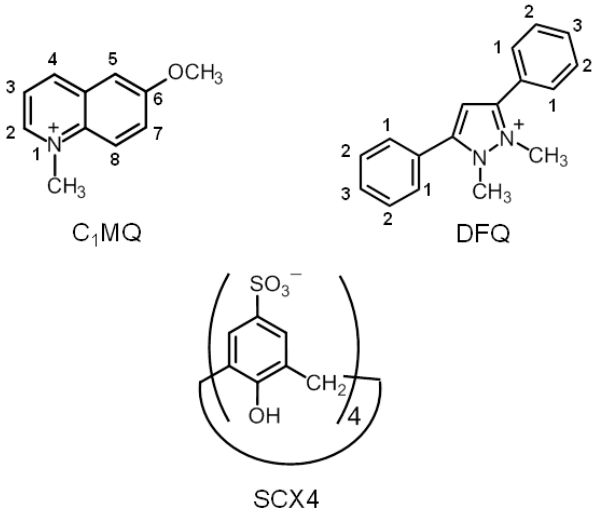
1. Introduction

4-Sulfonatocalixarenes (SCXn) are water-soluble biocompatible macrocyclic compounds whose π-electron rich cavity and negative charges promote complexation with a wide variety of organic guests and cations [1]. This type of cavitands have versatile applications in the formation of self-assembled nanoparticles [2], biology [3], pharmacology [4], and analytical chemistry [5]. They are also used to enhance solubility [6], and to modify the kinetics of photochromic reactions [7]. The encapsulation of fluorescent compounds in SCXn received particular attention [8] because of the possible utilizations in signaling and sensing. The marked emission intensity enhancement upon inclusion can be exploited to sensitively quantify alkaloids [9]. The fluorescence response upon the release of dyes from SCXn was applied to follow enzymatic reactions in real-time [10], and to quantify neurotransmitters with high selectivity [11].

Despite the intense activity in the field of SCXn complexes, only few studies dealt with the binding of pesticides. It was proposed that SCXn show considerable potential in the treatment of viologen poisoning due to the large stability and reduced toxicity of the produced complex [12]. The luminescence of CdTe quantum dots responded differently to pesticides in the absence and presence of 4-sulfonatocalix[4]arene (SCX4) [13]. The surface of silver nanoparticles was modified with SCXn to create colorimetric probe for optunal, also known as isocarbophos insecticide [14]. The effect of inclusion in 4-sulfonatocalix[6]arene (SCX6) on the fluorescence of benzimidazole fungicides was examined to enhance the sensitivity of their quantification [15].

In the present paper, we focus on the SCX4 complex formation of an intensely emitting fluorophor, 6-methoxy-1-methylquinolinium and on its potential use as a probe to reveal the binding properties of difenzoquat (DFQ), an important herbicide exhibiting also fungicidal activity. The chemical structure of the studied compounds is presented in Scheme

1. DFQ encapsulation has been studied only in cyclodextrins [16,17]. Negligible affinity was observed to α- and γ-cyclodextrins, whereas the pyrazole ring of DFQ was embedded in the cavity of β-cyclodextrin, but the binding constant was found to be small (around 70 M^{-1}).



Scheme 1. Chemical structure of the studied compounds

2. Experimental

4-Sulfonatocalix[4]arene (SCX4) (Acros Organics) and difenzoquat methyl sulfate (DFQ) (Fluka) were used as received. Double distilled water served as solvent. The molar weight of SCX4 was calculated taking into account the reported water content of the crystals [6]. 6-Methoxy-1-methylquinolinium iodide (C_1MQ) was synthesized as described previously [18]. The molar absorption coefficient of 5500 $M^{-1}cm^{-1}$ at 315 nm was used for the spectrophotometric determination of the C_1MQ concentration. The SCX4 stock solutions were neutralized by Na_3PO_4, and pH was adjusted to the value of 2 by HCl. Measurements were carried out in aqueous solution at 298 K. The UV-visible absorption spectra were taken on an Agilent Technologies Cary60 spectrophotometer. Jobin-Yvon Fluoromax-4 photoncounting spectrofluorometer was used to obtain corrected fluorescence spectra. The results of fluorescence titrations were analyzed with a homemade program written in MATLAB 7.9. ^1H NMR spectra were recorded in D_2O on a Bruker Avance 300 NMR

spectrometer equipped with a 5 mm inverse $^{1}H/^{13}C$-selective probe. The details of the 2D ^{1}H ROESY experiments are given in the Supporting Information. Isothermal titration calorimetry (ITC) experiments were performed on a MicroCal VP-ITC microcalorimeter as described [19]. The heat evolved after each injection was always corrected with the dilution heat. Quantum chemical calculations were performed with HyperChem 8.0 program (Hypercube Inc., Gainesville, FL).

3. Results and discussion

C_1MQ binding to SCX4 studied by fluorescence spectroscopy

The pKa values of the first and second dissociation steps of the phenolic OH groups in SCX4 were reported to be 3.28 and 11.5, respectively [20]. Consequently, SCX4 has 5 negative charges in neutral solution. C_1MQ has a high fluorescence quantum yield (Φ_F = 0.46) in water, and the double exponential fluorescence decay with 19.4 and 37.1 ns lifetimes has been assigned to the two torsional isomers differing in the orientation of the methoxy moiety

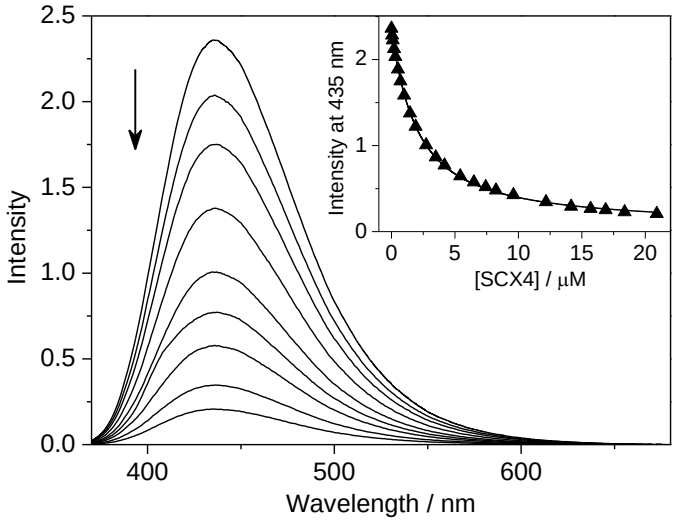


Fig. 1 Fluorescence titration of 0.63 μM C_1MQ with 0, 0.3, 0.7, 1.5, 2.7, 4.2, 6.5, 12, 21 μM SCX4. The 21 μM SCX4 stock solution was neutralized by 69 μM Na_3PO_4 and contained 0.63 μM C_1MQ. Excitation was performed at 350 nm. Inset: fluorescence intensity diminution with the SCX4 concentration increase at 435 nm.

relative to the heterocyclic ring. [21] The stepwise addition of SCX4 results in an efficient quenching, but very small change appears in the absorption spectrum. Fig. 1 presents the alteration of the fluorescence spectra and the intensity at 435 nm (inset) as a function of SCX4 concentration. The vanishing of the emission is attributed to ground-state complex formation because no dynamic quenching via a bimolecular process with the short-lived [21] singlet-excited C_1MQ is possible at the low SCX4 concentrations used in these experiments. The unchanged position of the band maximum and the negligible emission intensity in the presence of a large excess of SCX4 (1 mM) indicate that the inclusion complex is practically nonfluorescent. The light absorption of C_1MQ in SCX4 macrocycle induces rapid electron transfer from the phenolate unit of the host to the excited guest, which is followed by electron back transfer resulting in efficient energy dissipation. The driving force of the excited state electron transfer (ΔG) can be estimated by the following relationship [22]:

$$\Delta G = (E_{ox} - E_{red}) - E(S_1) + C \qquad (1)$$

$E(S_1)$ = 3.2 eV denotes the energy of the singlet-excited C_1MQ [21], E_{ox} and E_{red} stands for the oxidation and reduction potentials of the reactants in the ground state. The Coulomb term (C) is negligible in highly polar solvents. Cyclic voltammetric measurements provided E_{red} = –1.06 V and E_{ox} = 0.84 V vs. Ag/AgCl electrode for the reduction [23] of C_1MQ and the oxidation [24] of SCX4 at pH 7.17, respectively. On the bases of these quantities and eqn. 1, ΔG = –1.3 eV is obtained implying that the photoinduced electron transfer within the inclusion complex is thermodynamically highly favorable.

Because the neutral pH of the SCX4 stock solution was reached by addition of Na_3PO_4, the competitive binding of Na^+ cations to SCX4 has to be taken into account [25] when the binding constant (K) is calculated assuming 1:1 association between C_1MQ and SCX4. The equilibrium constant of Na^+–SCX4 complex formation (183 M^{-1}) was taken from the literature [25]. The fitting of the results of the fluorescence titration led to K = (6.2 ±

0.3)×10^5 M^{-1} for C_1MQ binding to SCX4. It is worth noting that if we had neglected the competitive binding of Na^+, K = (5.9 ± 0.3) × 10^5 M^{-1} would have been derived. The difference between this apparent binding constant and the correctly calculated real binding constant is close to the limits of experimental errors indicating that Na^+ binding plays a negligible role under our experimental conditions.

Thermodynamics of C_1MQ–SCX4 complex formation

To gain insight into the driving force of association, isothermal titration calorimetry (ITC) measurements were performed. Fig. 2 presents the enthalpogram obtained for the titration of 19 μM SCX4 with 360 μM C_1MQ solution. Exothermic binding occurs and an inflexion

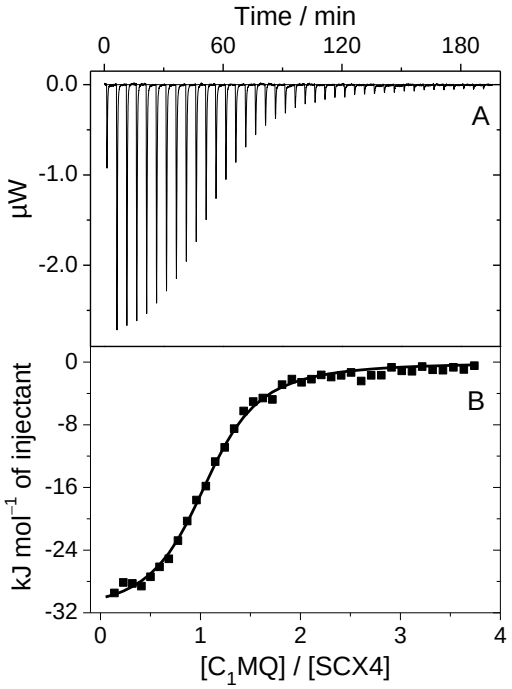


Fig. 2 (A) Results of isothermal calorimetric titration of a mixture of 19 μM SCX4 and 68 μM Na_3PO_4 with 360 μM C_1MQ solution at pH 7 after subtraction of the dilution heat of C_1MQ. (B) Squares represent the integrated heat released per injection, whereas the line is the best fit with the one binding site model.

point appears at 1:1 mixing ratio confirming that only a single C_1MQ is complexed with a SCX4 macrocycle. Nonlinear least-squares fit provided $K = (6.6 \pm 0.5) \times 10^5\ M^{-1}$ for the binding constant and $\Delta H = -(32.2 \pm 0.8)$ kJ mol^{-1} for the enthalpy change. The standard free enthalpy (ΔG) and the entropy (ΔS) changes upon confinement were calculated on the basis of the following relationship:

$$\Delta G = -RT \ln K = \Delta H - T\Delta S \qquad (2)$$

where T stands for the temperature and R denotes the gas constant. The binding constant and the thermodynamic parameters are summarized in Table 1. K values obtained by ITC and fluorescence titration agree well. The result of the latter method is used in further

Table 1 Binding constant and thermodynamic quantities of C_1MQ–SCX4 complex formation at pH 7

$K / 10^5\ M^{-1}$		ΔG / kJ mol^{-1}	ΔH / kJ mol^{-1}	$T\Delta S$ / kJ mol^{-1}	ΔS / J mol^{-1} K^{-1}
Fluorescence	ITC				
6.2 ± 0.3	6.6 ± 0.5	–33.0 ± 0.3	–32.2 ± 0.8	0.8 ± 1.1	3 ± 4

calculations because of its smaller error limit. The substantial heat release indicates that the complexation is enthalpy-driven. The electrostatic interactions between the oppositely charged host and guest significantly contribute to the stability of the complex. The small entropy change upon complexation may arise from the compensation of the reduced degrees of freedom of the components within the complex by the release of water molecules from the solvate shell of the reactants. The K value of association with SCX4 is a factor 3 smaller than the corresponding quantity for the inclusion in cucurbit[7]uril [21]. This difference stems from the more negative binding enthalpy in the latter macrocycle. The interaction among the water molecules in the extremely nonpolarizable and apolar interior [26] of the rigid CB7

macrocycle is energetically less favorable than in the flexible widely opened cavity of SCX4. Consequently, the expulsion of the high-energy water upon encapsulation of C_1MQ contributes more significantly to the exothermicity of the complex formation when CB7 serves as a host. Recent studies demonstrated that the release of high-energy water from cucurbiturils plays a decisive role in the binding affinity [27-29].

Structure of C_1MQ–SCX4 complex

^{1}H NMR experiments confirmed the complexation of C_1MQ with SCX4. (Fig. 3) As the molar ratio of the host was increased, the most substantial chemical shift variation was observed for H2, H3, H4, and NCH_3, whereas the shielding was much less pronounced for

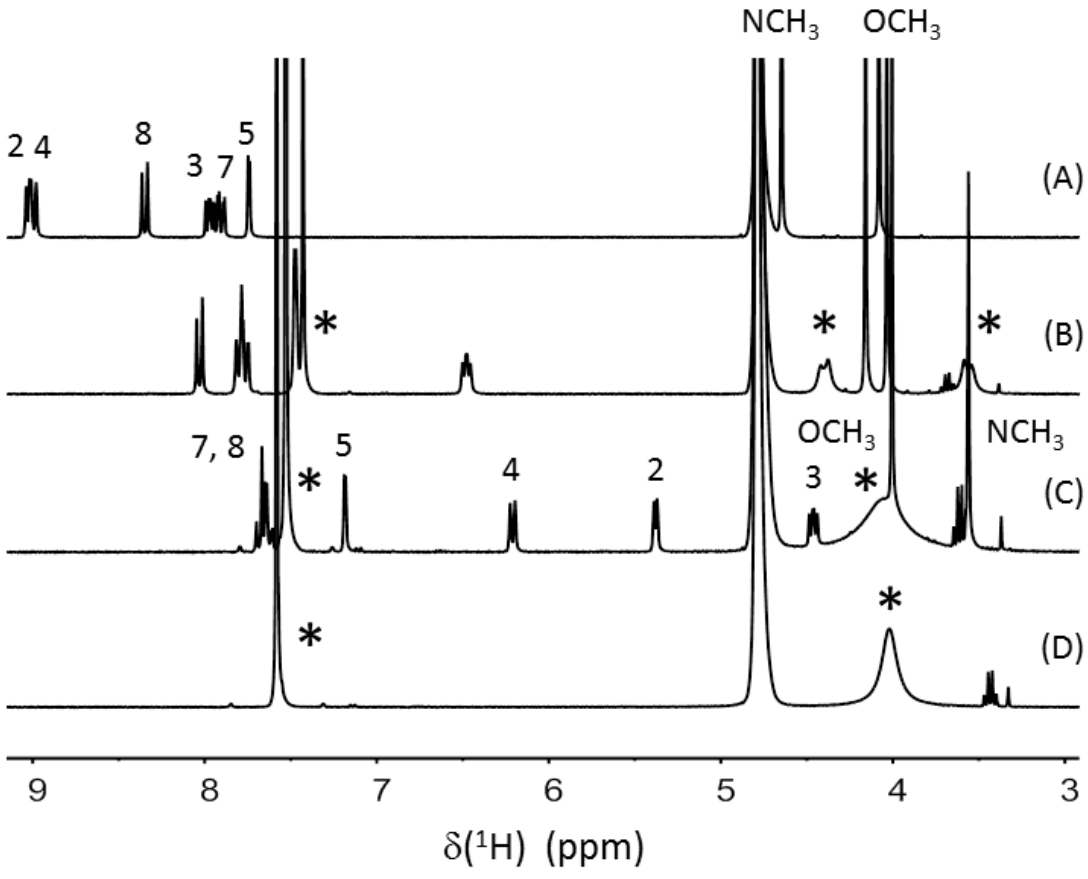


Fig. 3 ^{1}H NMR spectra for C_1MQ (A), [C1MQ]:[SCX4] ratio 2:1 (B) and 1:2 (C), and for SCX4 (D) in D_2O (total concentration of 10 mM). The asterisks denote the peaks related to the SCX4 protons.

Table 2 Displacement of proton resonances upon the complexation of C_1MQ with SCX4

	H2	H3	H4	H5	H7	H8	N–CH_3	O–CH_3
Δδ (ppm)	–3.695	–3.565	–2.848	–0.553	–0.287	–0.669	–1.074	–0.085

H5, H7, H8, and OCH_3 signals (Table 2). Additional 2D ^{1}H ROESY experiments were performed on a solution of SCX4 and C_1MQ with a [C_1MQ]:[SCX4] ratio of 1:2 (see Supporting Information, Figure S1). This composition was selected to ensure that high fraction (about 95 %) of C_1MQ is complexed. The 2D ROESY spectrum displays cross peaks between the aromatic protons of SCX4 and the protons of C_1MQ implying host-guest interaction. While C_1MQ undergoes a fast exchange between the free and complexed states over the corresponding NMR timescale, a transition from a moderately fast to a slow exchange regime is clearly detected for the axial and equatorial CH_2 protons of SCX4 (see Fig. 3). This feature indicates that the ring inversion displayed by SCX4 is slowed down upon C_1MQ binding. A similar dynamical behavior has been observed upon the coordination of Ca(II) and La(III) ions to SCX4 [30].

To visualize the structure of C_1MQ–SCX4 complex, molecular mechanic calculations

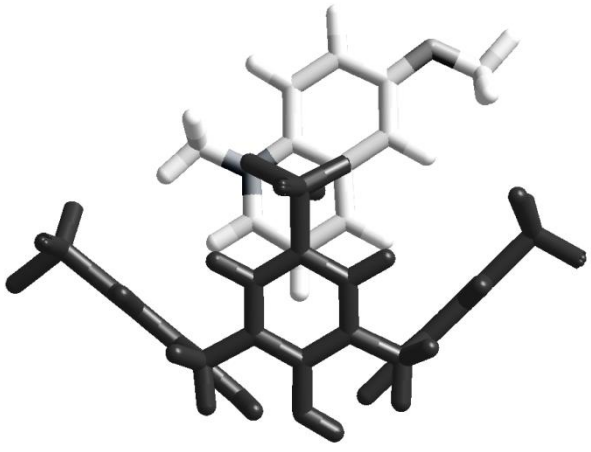


Fig. 4 Energy-minimized structure of C_1MQ–SCX4 complex in the ground state calculated by MM+ molecular mechanics method with HyperChem 8.0 program.

with MM+ method was performed. The energy-minimized structure in Fig. 4 suggests that the heterocyclic ring is located within the macrocycle, while the methoxy group is oriented outwards. Such a result may rationalize the chemical shift variation of the aromatic protons of C_1MQ observed by NMR. Hydrophobic and charge–π interactions, as well as electrostatic interactions between the oppositely charged components stabilize the inclusion complex.

Association of DFQ with SCX4

The absorption bands of DFQ and SCX4 strongly overlap. Therefore, complex formation between these compounds cannot be examined either by spectrophotometric or by fluorescence titrations. ITC measurements showed exothermic interaction when neutralized SCX4 was introduced into DFQ solution. (Figure 5) The results were consistent with a 1:1

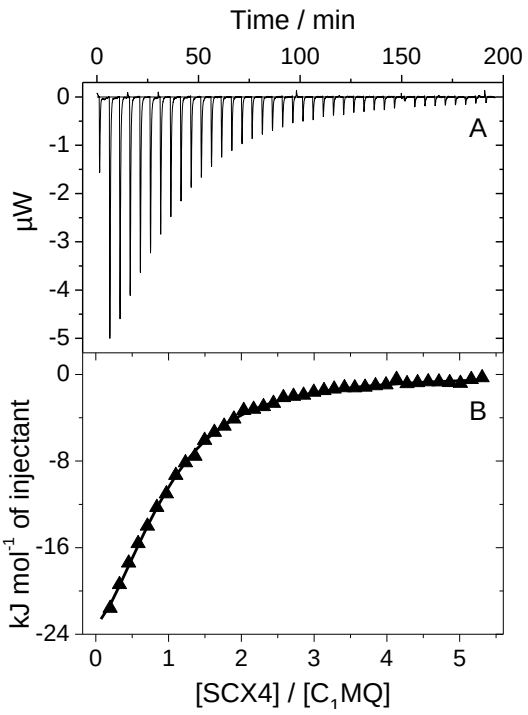


Fig. 5 (A) Isothermal titration calorimetry data obtained by adding the mixture of 1.03 mM SCX4 and 3.13 mM Na_3PO_4 into a 0.04 mM DFQ solution. (B) The integrated heat released per injection after subtraction of the dilution heat. The line displays the results of the fit with a one binding site model.

Table 3 Binding constant and thermodynamic quantities for DFQ–SCX4 complexation at pH 7

$K / 10^4 M^{-1}$		ΔG / kJ mol^{-1}	ΔH / kJ mol^{-1}	$T\Delta S$ / kJ mol^{-1}	ΔS / J mol^{-1} K^{-1}
Fluorescence[a]	ITC				
4.9 ± 0.4[a]	5.0 ± 0.3	–26.8 ± 0.3	–29.2 ± 0.7	–2.4 ± 1.0	–8 ± 4

[a] using C_1MQ fluorescence probe (vide infra)

association. Table 3 lists the binding constant and thermodynamic parameters derived from the analysis of the experimental data. The binding affinity of DFQ is more than one order of magnitude lower than that of C_1MQ. This difference arises from the smaller enthalpy gain and also from an unfavorable entropy contribution to the free energy in the case of the former compound. Nevertheless, the complexation is enthalpy-driven for both guests.

Structure of DFQ–SCX4 complex

Complexation of DFQ with SCX4 is also confirmed by 1D ^{1}H NMR experiments, reported in Figure 6, as well as 2D ^{1}H ROESY measurements (see Supporting Information, Figure S2). As can be seen on Figure 6, when the molar ratio of the host was increased, the resonances of the aromatic protons displayed a substantial upfield shift, whereas the signals due to the proton of the heterocyclic ring and its methyl substituents were displaced in much smaller extents. The spectra indicate fast exchange between the free and complexed DFQ. The chemical shift variations suggest that one of the aromatic rings is included, on average, in the macrocycle and the heterocyclic ring with the other phenyl group is oriented outwards. The most probable structure of the complex, obtained by MM+ molecular mechanic calculations is presented in Fig. 7. A phenyl group of DFQ is located in the vicinity of the negatively charged sulfonato moieties of SCX4 suggesting that charge–π interactions significantly contribute to the stabilization of the complex. RM1 semiempirical calculations

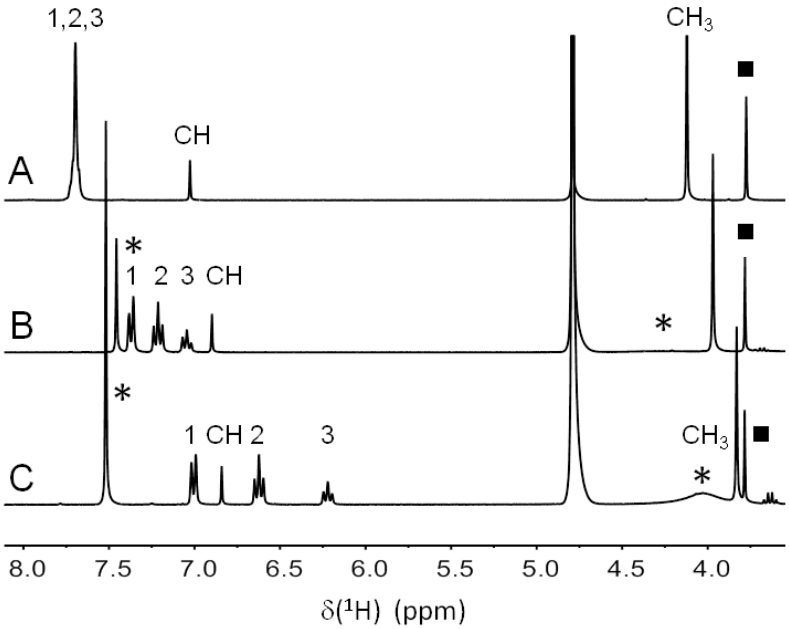


Fig. 6 ^{1}H NMR spectra of DFQ (A), [DFQ]:[SCX4] ratio 2:1 (B), and 1:2 (C) in D_2O (total concentration of 10 mM). The asterisks denote the peaks related to the SCX4 protons and the filled squares mark the resonance due to the protons of the methyl sulfate counterions.

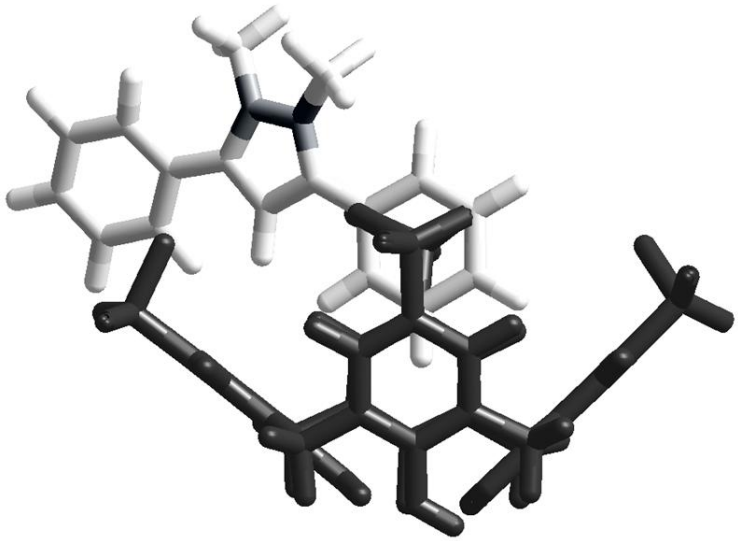


Fig. 7 Structure of DFQ–SCX4 complex calculated by MM+ molecular mechanics method with HyperChem 8.0 program.

showed the delocalization of the positive charge all over DFQ. The largest positive charge density appeared on the carbon atoms linked to the heterocyclic nitrogens and the hydrogen of the pyrazolium ring leading to electrostatic interaction with the SO_3^- substituents of the cavitand.

Utilization of C_1MQ as a fluorescent probe

The several orders of magnitude lower fluorescence intensity of C_1MQ–SCX4 complex compared to that of free C_1MQ can be used to develop highly sensitive methods for the determination of the SCX4 binding affinity of nonfluorescent or only UV-excitable guests. As a representative example, we have chosen to examine the effect of DFQ, a widely applied herbicide, on the fluorescence in the solution of C_1MQ–SCX4 complex. DFQ absorbs[31] below 300 nm, which renders its sensitive and selective detection difficult. The equilibrium constant of DFQ association with SCX4 can be measured by fluorescence titration employing the competitive binding of C_1MQ, which serves as an off-on fluorescence probe. Fig. 8 shows the rise of the fluorescence intensity and the spectral change in a mixture of 67 μM C_1MQ

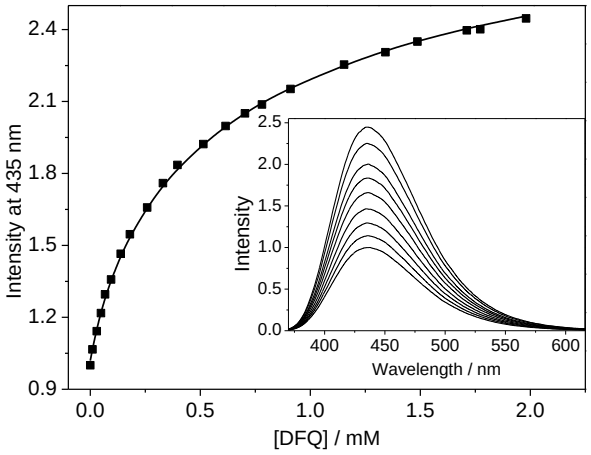


Fig. 8 Fluorescence intensity enhancement at 435 nm as a function of DFQ concentration in 67 μM C_1MQ, 49 μM SCX4, and 170 μM Na_3PO_4 solution. Inset: variation of the fluorescence spectra. Excitation took place at 350 nm.

and 49 μM SCX4 upon the successive addition of DFQ at pH 7. The experimental results were evaluated taking into account the competitive binding of DFQ, C_1MQ, and Na^+ to SCX4. The association constants for C_1MQ–SCX4 and Na^+–SCX4 complexes were taken from Table 1 ((6.2 ± 0.3)×10^5 M^{-1}) and the literature (183 M^{-1}) [25], respectively. The nonlinear least-squares fit of the measured data led to (4.9 ± 0.4) ×10^4 M^{-1} for the equilibrium constant of DFQ–SCX4 formation. This quantity agrees well with the result of ITC measurements (vide supra).

We examined whether C_1MQ can also be used as a probe in acidic solution when none of the phenolic OH moieties of SCX4 dissociates. Fluorescence titrations at pH 2

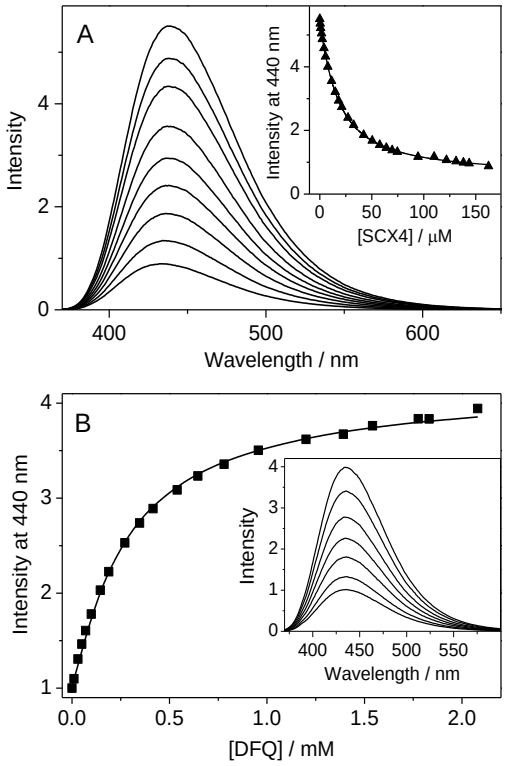


Figure 9. (A) Effect of SCX4 concentration enhancement on the fluorescence of 8 μM C_1MQ in 5 mM H_2SO_4. Inset: fluorescence intensity diminution with SCX4 concentration at 440 nm. (B) Fluorescence intensity increase at 440 nm as a function of DFQ concentration in 14 μM C_1MQ and 62 μM SCX4 solution in 5 mM H_2SO_4. Inset: variation of the fluorescence spectra. (Excitation at 350 nm.)

(Figure 9A) provided (7.4 ± 0.4) $\times 10^4$ M^{-1} for the equilibrium constant of C_1MQ binding to SCX4. This value is about 8-fold smaller than the one derived at pH 7. The significant pH effect probably arises primarily from the smaller negative charge of the host in strongly acidic solution. The stepwise addition of DFQ to a C_1MQ–SCX4 solution at pH 2 (Figure 9B) induced a fluorescence intensity rise like the one presented in Figure 8. The fit of the experimental data resulted in (2.2 ± 0.3)$\times 10^4$ M^{-1} for the equilibrium constant of DFQ–SCX4 complexation at pH 2.

4. Conclusions

In contrast with the slight fluorescence enhancement upon inclusion in cucurbit[7]uril, the complex formation of C_1MQ with SCX4 cavitand leads to fluorescence quenching due to the efficient electron transfer from the host to the singlet-excited guest. We have demonstrated that C_1MQ is a valuable off-on fluorescent probe for the study of the binding affinity of nonfluorescent or only at short wavelengths absorbing guests to SCX4 host. It can be used in a wide pH range because does not contain acid or base susceptible moiety. The high fluorescence quantum yield of the free C_1MQ and the negligible emission of its SCX4-bound form can be exploited to develop sensitive analytical methods for the quantification of DFQ. This popular herbicide was found to produce a stable complex with SCX4 via an enthalpy-controlled process.

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