Cyclodextrin carriers of positively charged porphyrin sensitizers†

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The cationic sensitizer 5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl)porphyrin (TMPyP) forms supramolecular complexes with native, per-methylated, sulfonated and dimethyl-sulfonated cyclodextrins (CDs). Binding interactions were proved by NMR, mass spectra, capillary zone electrophoresis, UV-Vis and fluorescence spectroscopy. The 2D-NMR experiments on native CDs indicate that the interaction of TMPyP with the external CD surface is the dominant binding mode. The high binding affinity of TMPyP towards sulfonated CDs is due to electrostatic interactions. Binding is accompanied by an increase of the TMPyP basicity. Whereas βCD does not affect the lifetime of the TMPyP triplet states, binding with sulfonated CDs causes the protonation of the TMPyP triplet states even in neutral solution. The diprotonated anionic sensitizer 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin ($TPPSH_2^{2+}$) forms host–guest complexes with native βCD and γCD, similarly as in its non-protonated state. The positive charge of pyrrole nitrogen atoms does not significantly influence the mode of the interaction. In contrast to TMPyP, the lifetimes of the triplet states of bound $TPPSH_2^{2+}$ to native CDs increase.

Introduction

Natural cyclodextrins (CDs) are cyclic oligosaccharides made by six, seven or eight α-1,4-D-glucopyranose units arranged in a truncated cone and denoted as αCD, βCD or γCD, respectively (Fig. 1).[1] The interior of the cavity, lined with C–H groups and glycosidic oxygen bridges, is hydrophobic while the exterior is hydrophilic due to the presence of hydroxyl groups. Cyclodextrins are water-soluble and their solubility can be controlled by functionalization of hydroxyl groups. The cavity of native and modified CDs can accommodate various molecules to form host–guest inclusion complexes,[1–3] which have potential applications in catalysis,[4] mimicking enzyme–substrate interactions,[5] pharmacology,[6–8] chiral separations,[9] *etc.* The driving force for the formation of inclusion complexes is a combination of hydrophobic, van der Waals, and hydrogen-bonding interactions.[2] It has been shown that noncyclic oligosaccharides form external, non-inclusion complexes.[10,11] The most important pharmaceutical applications are oriented towards the improvement of the resistance of pharmaceuticals to thermal and oxidative degradation, limitation of side effects, enhancement of solubility and bioavailability of drug molecules. CDs in a solution or in the form of nanoparticles can be considered as inert nontoxic carriers of compounds of special pharmacological interest, such as photosensitizers in photodynamic therapy of cancer or atherosclerosis (PDT)[12,13] or photodynamic antimicrobial chemotherapy (PACT).[14]

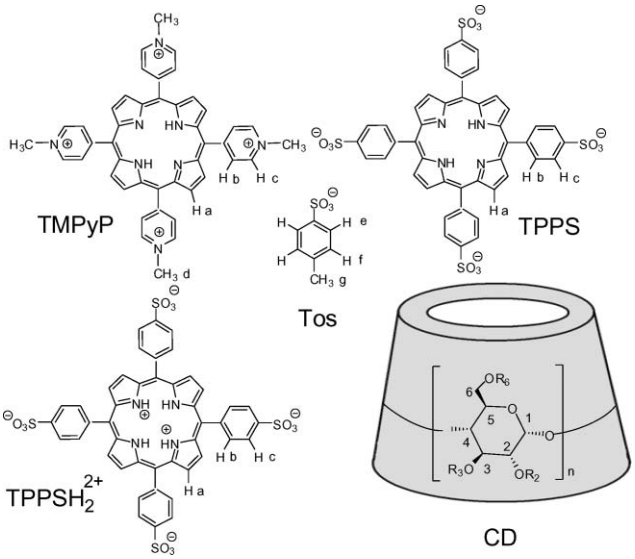

Fig. 1 Structure of porphyrins and cyclodextrins: R_2,R_3,R_6 = H (α.β or γ-CD); R_2,R_3 = H, R_6 = SO_3^-(SO_3βCD); R_2,R_3 = CH_3, R_6 = SO_3^-(diMeSO_3βCD); R_2,R_3,R_6 = CH_3 (triMeβCD); R_2,R_3 = H,R_6 = 2-hydroxypropyl (hpCD).

PDT is based on the delivery of a photosensitizer toward tumor tissues followed by irradiation leading to tissue destruction due to photoproduced reactive oxygen species. In this respect porphyrin-based sensitizers are efficient producers of singlet oxygen ($^1O_2$) after energy transfer from the triplet state of a sensitizer molecule to the oxygen ground state.[15] Singlet oxygen is a highly reactive oxidation agent, and it is generally accepted that $^1O_2$ (mostly $O_2(^1\Delta_g)$) is the main cytotoxic species in PDT.

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The properties of porphyrins and cyclodextrins can be modulated by functionalization, allowing the systematic study of mutual binding interactions. After binding, some dynamic and static properties of guest porphyrin molecules can dramatically change, namely physical, photophysical, and photochemical properties and the equilibrium between monomer and self-assembled aggregates.

The binding interactions between cyclodextrins and water-soluble anionic *meso*-tetraphenylporphyrins have been extensively investigated.[16–21] The main binding mode is the inclusion of porphyrin substituents, *e.g.*, sulfonatophenyls or carboxyphenyls, into the cyclodextrin cavity *via* the primary or secondary face. The direction, depth, and strength of penetration are sensitive to the size and functionalization of the CDs as well as to the peripheral substituents of porphyrins. The binding interactions were also utilized for the construction of large molecular self-assemblies.[22,23] We have found that inclusion complexes of anionic porphyrins remain good photosensitizers, and in addition, the shielding effects of CDs protect bound photosensitizers from aggregation and binding to other components in a solution.[15] In accordance with it, a porphyrin–CD supramolecular complex is an efficient sensitizer for photooxygenation reactions.[24]

The ^{1}H NMR studies of tetraarylporphyrins in the presence of CDs suggest that the microscopic environment of the positively polarized cavity of cyclodextrin promotes the inclusion of the anionic peripheral substituents. In contrast to anionic porphyrins, it was suggested that cationic porphyrin 5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl)porphyrin (TMPyP) bearing the positive charge on the peripheral *N*-methylpyridinium substituents does not interact with native CDs and that TMPyP can hardly enter the cavity of CDs.[16,20,25] In case of the positive charge being more distant from the porphyrin unit, the hydrophobic part of the substituent is threaded through the cavity with the positively charged end in a solution.[20] However, recent results on inclusion complexes of 5,10,15,20-tetrakis(4-pyridyl)porphyrin,[26] and reported spectral changes of TMPyP in the presence of CDs,[15,27] lead us to the hypothesis that the binding between TMPyP and CDs is possible.

Cationic TMPyP has attracted considerable attention as effective sensitizer.[28] Due to its binding affinity towards nucleic acids, TMPyP can selectively photocleave DNA[29,30] and inhibit telomerases.[31,32] The photoinactivation of extremely resistant bacteria[33] and antiviral activity[34] was also reported. In this report, we present the systematic study of binding interactions between CDs and TMPyP (Fig. 1). The results are compared with those of diprotonated anionic 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin ($TPPSH_2^{2+}$) where the positive charges are located on the pyrrole rings while negative charges of sulfonate groups are on the periphery. We aim to bring clear evidence on binding interactions between positively charged porphyrins and CDs and to determine how the presence of CDs affects the physicochemical and photophysical properties of TMPyP with respect to its possible applications in PACT.

Experimental

Native cyclodextrins (αCD, βCD and γCD), heptakis(2,3,6-tri-*O*-methyl)-βCD (triMeβCD), heptakis(6-*O*-sulfo)-βCD (SO_3βCD), heptakis (2,3-*O*-dimethyl-6-*O*-sulfo)-βCD (di$MeSO_3$βCD), tetratosylate salt (Tos) of 5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl)porphyrin (TMPyP/Tos) (all from Fluka) were used as received. Tetrachloride salt TMPyP/Cl was prepared on an ion exchange column from TMPyP/Tos (Dowex 1 × 2–200 Aldrich, 10 × 1 cm). The purity of TMPyP/Cl was checked by mass spectroscopy and NMR. 5,10,15,20-Tetrakis(4-sulfonatophenyl)porphyrin (TPPS) was synthesized and purified as described elsewhere.[35]

The UV-Vis absorption spectra were measured using a Unicam 340 or a Varian Cary IE spectrophotometer in 10 mm plastic or quartz cells. The fluorescence spectra were recorded using a Perkin Elmer LS 50B luminescence spectrometer. The emission spectra were corrected for the characteristics of the detection monochromator and photomultiplier.[36] The samples were excited at the $Q_y(1,0)$ absorption band at around 520 nm because this band is much less affected by the interaction of TMPyP with CDs than the Soret band. Unless otherwise stated, experiments were performed in deionized water at room temperature (22 °C).

The stoichiometry of porphyrin–CD complexes was determined by the Job method of continuous variations. The equimolar solutions of porphyrins and corresponding CDs were mixed to a standard volume, while leaving the total concentration of both components constant (8×10^{-6} M). The Job plots were constructed at the wavelength of the maxima of absorbance differences.

The binding constant K_b is defined by the expression

$$K_b = [\text{TMPyP–CD}_n]/((\text{CD}_T - n[\text{TMPyP–CD}_n])^n [\text{TMPyP}]), \quad (1)$$

where [TMPyP–CD_n] and [TMPyP] are the equilibrium molar concentrations of a complex and free porphyrin, respectively, CD_T is the total molar concentration of CD, and n expresses the stoichiometry. The binding isotherms were constructed from the absorption data of a set of 10 solutions containing TMPyP (5×10^{-6} M) and CDs in a range of concentrations from 6.2×10^{-7} M to 3.8×10^{-3} M. In the case of $TPPSH_2^{2+}$ (2×10^{-6} M in 0.01 M Britton-Robinson buffer, pH 3.0) the CD concentrations vary from 6.2×10^{-5} M to 3.8×10^{-3} M.

The measured absorbance changes ($\Delta A = A - A_0$) were cast into the binding isotherms. In the case of 1:1 complexes, the binding constants, K_b, of complexes can be obtained by a non-linear fitting procedure using the hyperbolic relationship

$$\Delta A = \Delta A_\infty K_b[\text{CD}]/(1 + K_b[\text{CD}]), \quad (2)$$

where

$$[\text{CD}] = (K_b \cdot \text{CD}_T - K_b \cdot \text{TMPyP}_T - 1)/2K_b + ((K_b\,\text{TMPyP}_T - K_b \cdot \text{CD}_T + 1)^2 + 4K_b \cdot \text{CD}_T)^{1/2}/2K_b,$$

$\Delta A_\infty = A_\infty - A_0$, and A_0, A and A_∞ are absorbances at selected wavelengths in the absence of CD, the presence of CD and of the complex, respectively. $TMPyP_T$ and CD_T are the total molar concentrations of TMPyP and CD, respectively, and [CD] stands for the equilibrium molar concentration of CD. All measurements were repeated 4 to 6 times.

Acido-basic properties of complexed porphyrins were studied in Britton-Robinson buffers or pure water acidified by drops of HCl. The concentrations of free, diprotonated and complexed (1000-fold molar excess of CDs) forms were calculated from the corresponding absorbances of the Soret bands. The apparent values of $pK_{3,4}$ were evaluated from the concentration *vs.* pH plots.

The NMR spectra were recorded on a Varian–Inova 400 MHz spectrometer using a 5 mm gradient inverse probe and/or 5 mm broadband probe. The frequency of ^{1}H nuclei was 399.951 MHz. All samples were measured in D_2O using 2-methylpropan-2-ol as an internal reference (^{1}H NMR δ 1.25 ppm) at 298 K if not stated otherwise. Samples containing diprotonated $TPPSH_2^{2+}$ were measured in 0.01 M deuterated Britton-Robinson buffer, pD 2.2. Two-dimensional ROESY experiments were recorded using a standard pulse sequence with a mixing time set to a short value (100 ms) to ensure that the cross-peaks originate only from NOE. In some cases the 2D-NOESY spectra were measured with a 300 ms mixing time. The complete assignment of signals and determination of spatial contacts were acquired at the porphyrin:CD molar ratios of 1:2 and 2:1. The typical concentrations of porphyrins were 8×10^{-3} M and of CDs were 4×10^{-3} or 1.6×10^{-2} M.

Mass spectra were measured on an Esquire 3000 instrument (Bruker) with an electrospray and iontrap analyzator.

Electrophoretic experiments were performed on an HP^{3D} CE capillary electrophoresis system from Agilent Technologies (Waldbronn, Germany) equipped with a diode array detector. Additional conditions: a fused silica capillary (inner diameter 75 μm, total length 79.5 cm, effective length 71.0 cm), 40 mM acetate buffer of pH 4.7, separation voltage +30 kV, electrical current 36 μA, and 300 mbarsec sampling.

Laser flash photolysis experiments were performed with a Lambda Physik FL 3002 dye laser (wavelength of 420 and 425 nm, pulse width 28 ns). Transient absorption spectra were measured within 300–800 nm on a laser kinetic spectrometer (Applied Photophysics, U.K.). The time profiles were recorded using a 250 W Xe lamp equipped with a pulse unit and a R928 photomultiplier (Hamamatsu). The triplet lifetimes were measured at the maximum of the triplet band in oxygen-free solutions. The bimolecular rate constants of the triplet states quenching by oxygen were calculated from rate constants measured in oxygen-free, air-saturated, and oxygen-saturated aqueous solutions.

Results and discussion

TMPyP–CD interaction

UV/Vis, fluorescence and transient absorption spectra. In aqueous solution, the interaction of TMPyP with CDs was monitored by absorption and fluorescence spectroscopy. The presence of CDs led to a small red-shift of the TMPyP Soret band (Table 1). This is a typical sign of noncovalent interactions between the porphyrin moiety and CDs as a result of the reduced exposure to solvent molecules.[15] The observed shifts of isosbestic points during titrations of TMPyP by CDs can indicate the coexistence of complexes of several stoichiometries and/or of different binding modes.

More significantly, interaction resulted in dramatic changes of fluorescence emission spectra. The broad fluorescence emission of TMPyP with unresolved Q(0,0) and Q(0,1) bands (Fig. 2a) is caused by the mixing of the first excited state with a nearby charge transfer state because of rotational freedom and electron-accepting properties of the porphyrin methylpyridinium groups.[37,38] In the presence of all studied CDs, a clear separation of the emission bands at 659 nm and 717 nm was accompanied by

Table 1 Complexes of TMPyP with CDs: ε_{max} is the absorption molar coefficient at the Soret band maximum, λ_{max}, S is the stoichiometry of TMPyP/Tos-CD complexes, p$K_{3,4}$ stands for the apparent protonation constant

CD	λ_{max} (nm)	ε_{max} (M^{-1} cm^{-1})	p$K_{3,4}$	S
	422	2.2×10^5	1.1 ± 0.1	—
αCD	423	2.2×10^5	0.7 ± 0.1	—[b]
βCD	423	2.2×10^5	0.7 ± 0.1	1:1
γCD	423	2.3×10^5	0.7 ± 0.1	—[b]
SO_3βCD	427	2.2×10^5	2.5 ± 0.1	2:1
triMeβCD	422	2.3×10^5	—[b]	—[b]
diMeSO_3βCD	425	2.2×10^5	2.4 ± 0.1	2:1
hpβCD[a]	423	2.1×10^5	—	1:1
hpγCD[a]	423	2.4×10^5	—	1:1

[a] Published data.[27] [b] Small absorbance changes. For other experimental conditions, see Experimental.

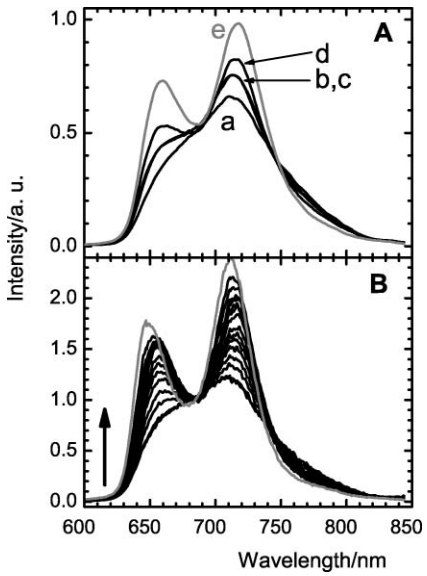

Fig. 2 (A) Fluorescence emission of 2×10^{-6} M TMPyP/Cl (a) with 2×10^{-3} M native βCD (b), triMeβCD (c), SO_3βCD (d), and di$MeSO_3$βCD (e, grey line); $\lambda_{exc} = 519$ nm. (B) Fluorescence emission of 2×10^{-6} M TMPyP in the presence of SO_3βCD. The arrow indicates increasing concentration of SO_3βCD up to 5.0×10^{-3} M (grey line); $\lambda_{exc} = 518$ nm.

overall increased intensities (Fig. 2c–e). In particular, sulfonated CDs had substantial influence, implying their strong binding with TMPyP (Fig. 2B.). We have already reported similar effects induced by 2-hydroxypropylated β- and γ-cyclodextrins (hpβCD, hpγCD).[27] A plausible explanation is based on the fact that the rotation of the methylpyridinium groups is restricted after binding, and intramolecular charge transfer within the TMPyP molecule responsible for broad emission features is eliminated. Surprisingly, similar fluorescence effects were observed after binding of TMPyP with cucurbit[7]uril where the *N*-methylpyridinium groups are partially included into the cucurbit[7]uril cavity.[39]

In acid aqueous solutions of TMPyP, pyrrole nitrogen atoms are protonated to form diprotonated $TMPyPH_2^{2+}$ that has the characteristic Soret band located at 444 nm. The absorption spectra at various pH values showed an equilibrium between the TMPyP base and $TMPyPH_2^{2+}$ in both the absence or presence of CDs (1000-fold molar excess) indicated by an isosbestic point

(Fig. 3). The corresponding pH-titration curves revealed that the binding with CDs affects the acidity of TMPyP (Fig. 3, inset). The p$K_{3,4}$ value of TMPyP is p$K_{3,4}$ = (1.1 ± 0.1) in agreement with reported values of 1.4 and <1 for pK_3 and pK_4, respectively.[40] The apparent p$K_{3,4}$ values in the presence of CDs are presented in Table 1. Two different trends can be noticed. First, the binding with sulfonated CDs decreases the acidity of the porphyrin moiety probably due to the charge compensation of electron-withdrawing *N*-methylpyridinium groups on the TMPyP periphery by the sulfonate groups of functionalized CDs. Second, native CDs slightly shift the acid–base equilibrium in favor of nonprotonated TMPyP at a given pH. This increase of pyrrole nitrogen acidity, however, more profoundly, was also reported for TPPS that forms inclusion complexes with CDs.[18] The behaviour can be attributed to shielding effects of the electron-rich CD cavity.

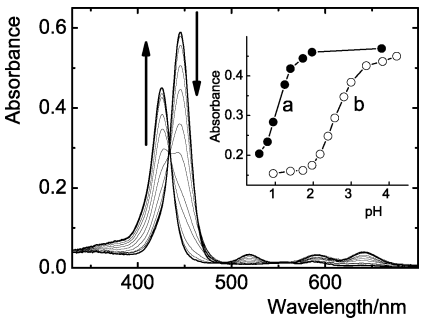

Fig. 3 Absorption spectra of TMPyP (2×10^{-6}) in the presence of SO_3βCD (4×10^{-3} M) at different pH values. The arrows show changes with increasing pH. Inset: The pH-titration curves demonstrating a shift of apparent pK_a values. The absorbances were recorded at 422 nm (a) and 427 nm (b) in the absence and presence of SO_3βCD, respectively.

The transient absorption spectra of TMPyP in the 450–550 nm region obtained after excitation into the Soret band exhibited the typical features of the TMPyP triplet states. The triplets ^{3}TMPyP were deactivated monoexponentially with a lifetime of $\tau_T = 153 \pm 31$ μs in argon-saturated solutions and were quenched by oxygen with a rate constant of $k_{O_2} = (1.5 \pm 0.2) \times 10^9$ M^{-1} s^{-1}. Both values are comparable with previously published data.[27,41,42] The addition of native βCD (up to 5×10^{-3} M) has no effect on the kinetics of ^{3}TMPyP relaxation because the measured values of k_{O_2} and τ_T are identical to those of free ^{3}TMPyP within the experimental error.

In contrast, the transients of TMPyP in the presence of SO_3βCD showed more complicated kinetics (Fig. 4a,b). It is obvious that the decay curves cannot be fitted to a monoexponential function, implying that a transient species other than ^{3}TMPyP is present. The transient absorption spectra recorded immediately after excitation matched the spectra of ^{3}TMPyP (Fig. 4d) while the spectra in microsecond time scale (*i.e.*, 3 μs after excitation; Fig. 4e) appear to be a superposition of the spectra of ^{3}TMPyP and diprotonated $^3TMPyPH_2^{2+}$ (Fig. 4f). The decay traces were recorded at 510 nm, *i.e.*, close to the absorption maximum of $^3TMPyPH_2^{2+}$. The traces show that the originally formed ^{3}TMPyP states are protonated fast even in neutral media (Fig. 4a) and that the relative concentration of produced diacid $^3TMPyPH_2^{2+}$ increases at lower pH (Fig. 4b). For the comparison, the decay curve in the absence of SO_3βCD did not exhibit an absorbance built-up at the beginning and it was purely monoexponential as a result of quenching of ^{3}TMPyP by dissolved oxygen (Fig. 4c). It

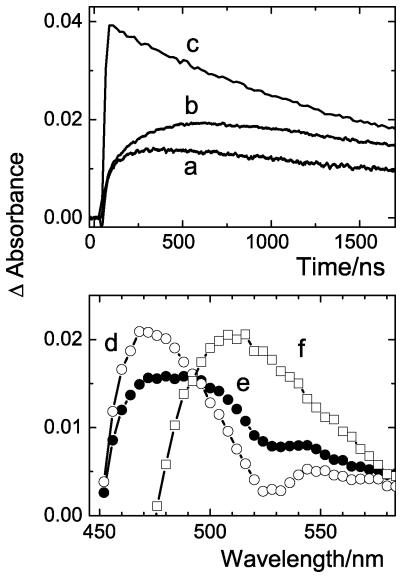

Fig. 4 Decay absorption traces recorded at 510 nm after excitation of TMPyP (5×10^{-6} M) in the presence of 1×10^{-3} M SO_3βCD in air-saturated Britton-Robinson buffers of pH 7.0 (a), pH 4.2 (b), and in the absence of SO_3βCD at pH 7.0 (c). Transient absorption spectra of the TMPyP–SO_3βCD system at pH 4.2 immediately after the excitation pulse (50 ns) (d) and after 3 μs (e) compared with the spectrum of the triplet states of diacid $^3TMPyPH_2^{2+}$ recorded at pH 0.7 (f).

has been described that water-soluble porphyrin TPPS is a stronger base in the triplet state than in the ground state.[43] This property can originate from the fact that four nitrogen atoms in the macrocycle are more strongly hydrogen-bonded with the surrounding water molecules in the excited state than in the ground state.[44] These effects are probably also important with regard to TMPyP having a p$K_{3,4}$ of 1.1 in the ground state.

The triplets ^{3}TMPyP in the presence of SO_3βCD (recorded at 460 nm) in argon-saturated solutions were deactivated monoexponentially with a lifetime of $\tau_T = 145 \pm 28$ μs, indicating that SO_3βCD has no effect on the lifetime of TMPyP.

Mass spectroscopy and capillary zone electrophoresis. The evidence provided by UV/Vis and fluorescence spectra on the binding of TMPyP with CDs was further confirmed by mass spectroscopy (MS) and capillary zone electrophoresis.

Under our experimental conditions, the mass spectra of TMPyP/(Cl, Tos) showed fragments corresponding to m/z = 169.5 $(TMPyP)^{4+}$, 221.0 $(TMPyP - CH_3)^{3+}$, and 324.3 $(TMPyP - 2CH_3)^{2+}$. Native and permethylated βCDs were detected at m/z = 1157.5 $(βCD + Na)^+$ and 1451.8 $(triMeβCD + Na)^+$, and tosylate anion was clearly detected at m/z = 170.9.

The ESI-mass spectra of the mixtures of TMPyP/(Cl or Tos) with native or permethylated CDs had peaks corresponding to 1:1 TMPyP–CD complexes at m/z = 453.4 $(TMPyP + βCD)^{4+}$ and 527.0 $(TMPyP + triMeβCD)^{4+}$. Also, a 1:1 complex between Tos and βCD was found at m/z = 1305.6 $(Tos + βCD)^-$. In the case of sulfonated SO_3βCD and di$MeSO_3$βCD, no corresponding adducts were detected. All fragments found in ESI-MS suggest that TMPyP and Tos form complexes with βCD and triMeβCD (see ESI), even though a possibility that electrostatic adducts formed during the electrospray process cannot be excluded.[45]

Table 2 ^{1}H NMR chemical shifts δ_H (ppm) of native CDs, $TPPSH_2^{2+}$ at pD 2.2, TMPyP/(Cl or Tos), Tos and corresponding supramolecular complexes in D_2O solution

	CD			$TPPSH_2^{2+}$			TMPyP/Cl		TMPyP/Tos			Tos		
H	α	β	γ	—	βCD	γCD	—	βCD	—	αCD	βCD	—	αCD	βCD
1	5.05	5.05	5.12	—	5.07	5.02	—	4.71	—	4.77	4.71	—	5.05	5.06
2	3.63	3.63	3.66	—	3.65	3.58	—	3.34	—	3.39	3.34	—	3.60	3.62
3	3.98	3.94	3.94	—	3.95	3.90	—	3.73	—	3.79	3.69	—	3.92	3.87
4	3.58	3.56	3.60	—	3.59	3.54	—	3.22	—	3.30	3.22	—	3.58	3.56
5	3.83	3.84	3.86	—	3.87	3.68	—	3.59	—	3.67	3.55	—	3.84	3.70
6	3.87	3.86	3.88	—	3.85	3.40	—	3.59	—	3.66	3.57	—	3.86	3.81
6′	3.91	3.90	3.88	—	3.92	3.56	—	3.72	—	3.76	3.71	—	3.90	3.84
H-a	—	—	—	9.10	9.07	8.81	—[a]	—[a]	9.10	9.06	9.13	—	—	—
H-b	—	—	—	8.84	8.85	8.37	8.97	9.00	8.92	8.92	8.96	—	—	—
H-c	—	—	—	8.52	8.48	8.37	9.34	9.34	9.31	9.31	9.31	—	—	—
H-d	—	—	—	—	—	—	4.81	4.82	4.82	4.80	4.81	—	—	—
H-e	—	—	—	—	—	—	—	—	7.19	7.15	7.29	7.70	7.71	7.69
H-f	—	—	—	—	—	—	—	—	6.56	6.53	6.74	7.38	7.38	7.37
H-g	—	—	—	—	—	—	—	—	1.61	1.59	1.81	2.40	2.40	2.40

[a] Broad band. For other experimental conditions, see Experimental.

The capillary zone electrophoresis was used to uncover the interaction of TMPyP with CDs. As an example, the electropherogram of TMPyP/(Cl or Tos) clearly demonstrates the reproducible increase of a migration time in the presence of βCD. This is a result of the retention of TMPyP, migrating in an electrophoretic capillary towards the cathode located behind a diode array detector, by βCD (Fig. 5). The limiting mobility of several guest molecules was explained by the formation of supramolecular complexes with CDs.[46]

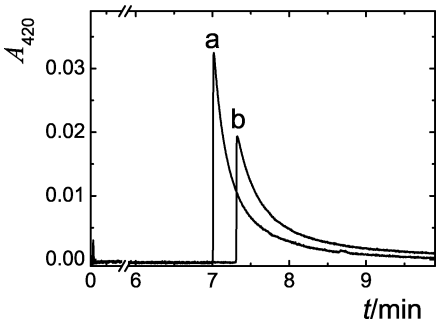

Fig. 5 Capillary zone electrophoresis of 2×10^{-4} M TMPyP/Cl in the absence (a) and presence (b) of 2×10^{-3} M βCD. 0.04 M acetate buffer, pH 4.7.

Mode of TMPyP–CD interaction. The mode of interaction between CD and a guest molecule can be inferred from ^{1}H NMR based on the chemical shift variations of in-cavity H-3 and H-5 protons. Typically, if only H-3 undergoes a shift in the presence of a guest then the cavity penetration through the secondary face is shallow, while if H-5 is also shifted the penetration is deep.[1]

First, the ^{1}H NMR data on TMPyP/Cl and TMPyP/Tos (2.4×10^{-4} to 6×10^{-3}M) were complemented (Table 2). While porphyrin protons were not practically affected by the substitution of the Cl^- counteranion by Tos, the chemical shifts of Tos protons were significantly shifted upfield by 0.51 to 0.82 ppm when compared with pure Tos. These shifts show an interesting temperature dependence, *i.e.*, when temperature of a TMPyP/Tos solution was increased (up to 90 °C), the chemical shifts of Tos protons revert to the values of pure Tos. This behaviour is probably due to a lower extent of Tos stacking and of ion pairing between Tos and TMPyP at higher temperatures. It does not complicate the analysis of measured complexation-induced chemical shifts.

The interaction between TMPyP/Cl and βCD is corroborated by chemical shifts of all βCD protons, especially of external protons H-4, H-1, H-2 (Fig. 1, Table 2). These differences in conjunction with spatial contacts in the ROESY spectra indicate an external mode of interaction in which the porphyrin moiety is attracted to the external CD surface (Table 2 and Table 3). However, protons H-3, H-6′ and H-5, H-6 of β-CD in the complex are not resolved, and the contacts between inner cyclodextrin and porphyrin protons, *i.e.*, some contribution of TMPyP inclusion, cannot be excluded. Per-methylated triMeβCD interacts with TMPyP similarly. The corresponding ^{1}H NMR chemical shifts are presented in the ESI†.

The importance of porphyrin counteranion on binding can be documented by the results on TMPyP/Tos in the presence of αCD or βCD (Table 3). Similarly to TMPyP/Cl, the external mode is confirmed by the 2D-ROESY cross-peaks between TMPyP and external CD protons. In contrast, the inclusion of TMPyP into the cavity can be excluded because the significant differences in the chemical shifts of H-3 (0.21–0.25 ppm) and H-5 (0.25–0.29 ppm) (Table 2) are due to the inclusion of Tos. Indeed, the intensive cross-peaks corresponding to the contacts between inner H-3 and H-5 and Tos protons were observed in the ROESY spectra of both TMPyP/Tos-CD and Tos-CD systems. The contacts of TMPyP/Tos-CD are even more intensive than those of Tos-CD. All described observations document that when Tos is used as counteranion, Tos forms inclusion complexes in contrast to TMPyP. Based on above descibed observations we can speculate that Tos acts as a week mediator and slightly supports the external binding mode between TMPyP and CDs due to ion pairing between Tos in the CD cavity and TMPyP. *Vice versa*, the presence of TMPyP may cause deeper inclusion of Tos counteranion into the CD cavity as indicated by intensive cross-peaks (see above).

The ^{1}H NMR of SO_3βCD and di$MeSO_3$βCD were not completed because sulfonated CDs precipitated out at concentrations needed for NMR experiments as a results of the charge compensation.

Table 3 2D-ROESY cross-peaks between porphyrin (TMPyP/Cl, TMPyP/Tos), Tos and cyclodextrins (βCD and αCD) in D_2O

	Contacts with CD protons[a]				
TMPyP and Tos protons	TMPyP/Cl + βCD[b]	TMPyP/Tos + αCD[c]	TMPyP/Tos + βCD	Tos + αCD	Tos +βCD
H-a	—	—	—	—	—
H-b	1vw, 2 m, 3/**6′**w, 4vw, 5/**6**m	3w, 6′m	1m, 2w, 4w, 6m, 6′m	—	—
H-c	3/**6′**vw	5/**6**vw	6w, 6′w	—	—
H-d	—	—	—	—	—
H-e	—	3s, **5**/6m	4w, 5vs, 6s	—	3vs, 5s, 4w
H-f	—	3vs, **5**/6s	3m, 4vw, 5s	5s	3s, 5w
H-g	—	3s, **5**/6vs	3s, 5s, 2w	5m	3s

[a] Intensity of cross-peaks: vs, very strong; s, strong; m, medium; w, weak; vw, very weak. [b] Protons H-3, H-6′ and H-5, H-6 of βCD in complex are unresolved. [c] Protons H-5 and H-6 of αCD in complex are unresolved. The bold numbers indicate the more probable assignment. For other experimental conditions, see Experimental.

Stoichiometry and K_b of the TMPyP–CD complexes. The experiments described above reveal that TMPyP form noncovalent complexes with CDs based mostly on the interaction of TMPyP with the exterior of the CD cavity. The unanswered question is if the complexes are stoichiometrically well-defined. In this respect, the Job plots yielded the stoichiometric TMPyP:CD ratio of 1:1 for native βCD and 2:1 for both TMPyP/Tos-diMeSO_3βCD and TMPyP/Tos-SO_3βCD systems (Table 1, Fig. 6, inset).

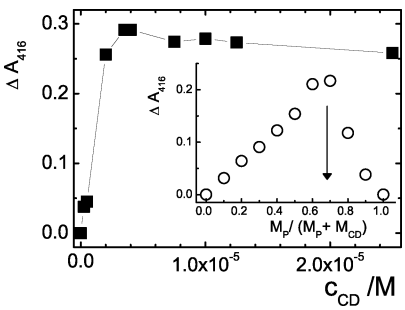

Fig. 6 Binding isotherm of 5×10^{-6} M TMPyP/Tos with SO_3βCD. The data points are connected for clarity. Inset: Corresponding Job plot with the constant total concentration of 8.3×10^{-6} M.

The binding constants (K_b) of TMPyP–CD complexes can be determined using a non-linear analysis of corresponding binding isotherms (Eq. 2). Neither counteranion of TMPyP has any absorbance above 300 nm; therefore, the spectral changes in absorption and fluorescence spectra can be attributed only to the interaction of the porphyrin moiety with CDs. The absorbance changes induced by βCD were small, but they were reproducible, and did not exhibit a single isosbestic point in the Soret region of the porphyrin absorption spectra. The binding isotherms of the TMPyP/Cl-βCD system can be fitted to Eq. 2 supposing the 1:1 stoichiometry to give an upper limit of K_b of about 1000 M^{-1}. The large uncertainty is due to small absorbance changes and to the fact that we cannot preclude a contribution from several binding modes and stoichiometries to the overall absorption changes, especially at large molar excess of one of the component, *i.e.*, under conditions different from those used for the construction of the Job plots. For comparison, the binding affinity of TMPyP towards hpβCD is characterized by a K_b of 5.4×10^3 M^{-1} and TPPS forms an inclusion complex with βCD with a K_b of 5.6×10^3 M^{-1}.[18,27] The presented binding constants document the important role of the peripheral porphyrin and CD substituents on the effectiveness of binding.

Titration experiments with SO_3βCD or diMeSO_3βCD showed a gradual bathochromic shift of the porphyrin Soret bands by several nm with no clear isosbestic points, while the absorbance remained the same (Table 1). The binding isotherms exhibit a sharp saturation between the molar concentration ratios TMPyP:CD of 1:1 and 2:1, indicating the high affinity of TMPyP towards sulfonated CD (Fig. 6). In agreement with it, the overall stoichiometry TMPyP:CD assigned by the Job plot is 2:1 (Fig. 6, inset). None of these facts allowed the evaluation of binding constants. Evidently, the binding is governed by electrostatic attraction between positively charged TMPyP and negatively charged sulfonate groups on the primary face. The total stoichiometry of 2:1 can be explained by the maximum compensation of CD negative charge.

$TPPSH_2^{2+}$–CD interaction

UV/Vis, fluorescence and transient absorption spectra. The interaction of TPPS with CDs in neutral media is visualized by a small red-shift of the Soret band due to the formation of inclusion-type complexes (Fig. 7a,b).[16–19] The decrease of pH of TPPS solutions results in protonation of two pyrrole nitrogen atoms and in the formation of the corresponding diprotonated form $TPPSH_2^{2+}$. We have also studied the binding of $TPPSH_2^{2+}$

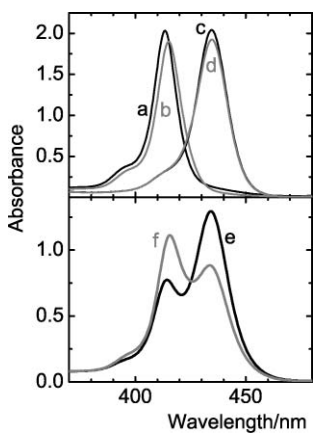

Fig. 7 Soret band of 2×10^{-6} M TPPS in the absence/presence of 2.5×10^{-3} M βCD (grey lines): pH 7.0 (a)/(b), pH 3.0 (c)/(d), and pH 4.6 (e)/(f). 0.01 M Britton-Robinson buffer.

in order to specify the effects of the positive charge location in the porphyrin molecule, *i.e.*, inner pyrrole *vs.* peripherally located charges of TMPyP.

The absorbance changes of $TPPSH_2^{2+}$ induced by βCD were small and in the cases of αCD and γCD could not be even evaluated. The Soret band of diprotonated $TPPSH_2^{2+}$ was red-shifted by 0.5–1 nm accompanied by a small hypochromicity (Fig. 7c,d). The effect of βCD is also expressed in the fluorescence emission spectra where the band of free $TPPSH_2^{2+}$ ($\lambda_{exc} = 400$ nm, pH 2.0) at 670 nm is slightly shifted to 673 nm. In addition, the presence of βCD caused the increase of TPPS acidity as shown by higher concentrations of TPPS over $TPPSH_2^{2+}$ at given pH (Fig. 7e,f). As reported, βCD induces a pK_a decrease by 1.2 as a result of a complex formation.[20,25] A more significant shift $\Delta pK_a = 2.7$ occurs in the presence of hpβCD that has larger affinity towards TPPS than βCD.[18] Because of low TPPS concentrations employed, the all described spectral changes can be attributed to the effects of CDs on porphyrin monomers.

The lifetime of the triplet states of $TPPSH_2^{2+}$ was 70 ± 10 μs in argon-saturated solutions as followed from transient absorption experiments at pH of 0.67. This value is in good agreement with previous measurements.[47] The addition of βCD (1×10^{-3}M) led to a significant prolongation of the triplet lifetime ($\tau_T = 230 \pm 32$ μs) similarly to the effect in neutral pH.[15] The effect can be attributed to the different microenvironment of porphyrin molecules after the inclusion of the part of the molecule into the CD cavity.

The mode of $TPPSH_2^{2+}$–CD interaction. The affinity of native CDs towards $TPPSH_2^{2+}$ was investigated by ^{1}H NMR titration experiments using different $TPPSH_2^{2+}$:CD ratios at pD 2.2. The addition of βCD or γCD shifts $TPPSH_2^{2+}$ protons, indicating complexation under fast exchange conditions (Table 2). More informative ROESY spectra show well-resolved cross-peaks corresponding to the spatial contacts between cyclodextrin protons H-3 and H-5 located inside the cavity and porphyrin protons H-a and H-c (Table 4, Fig. 8). In addition, protons H-3 and H-5 are in weak contact with H-b. The cross-peaks indicate the formation of a host–guest complex with the deep inclusion of a porphyrin sulfonatophenyl substituent into the βCD cavity *via* the secondary face. This binding model was suggested earlier on the basis of ^{1}H NMR experiments both for $TPPSH_2^{2+}$ and free-base TPPS (Table 4).[16,17,19] However, some differences can be noticed. The observed additional contact between pyrrole proton H-a and cyclodextrin proton H-5 might indicate a contribution of a host–guest complex with a sulfonatophenyl substituent included *via* the cyclodextrin primary face.

Table 4 2D-ROESY cross-peaks between porphyrin (TPPS, $TPPSH_2^{2+}$) and cyclodextrin (βCD, γCD) protons in D_2O

Porphyrin protons	Contacts with CD protons[a]		
	TPPS + βCD[b]	$TPPSH_2^{2+}$ + βCD	$TPPSH_2^{2+}$ + γCD[c]
H-a	3m	3s, 5m	5w, 6w
H-b	3m, 5m	3w, 5w	3m, 5m, 6m
H-c	5s	5s	3m, 5m, 6m

[a] Intensity of cross-peaks: s, strong; m, medium; w, weak; vw, very weak. [b] Published data.[19] [c] Protons H-b and H-c of $TPPSH_2^{2+}$ in the complex with γ-CD are not resolved. For other experimental conditions, see Experimental.

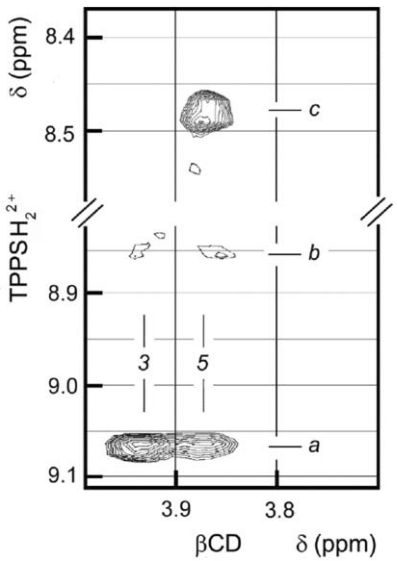

Fig. 8 2D-NMR (ROESY) spectrum of the host–guest complex $TPPSH_2^{2+}$ with βCD (400 MHz, D_2O, pD 2.2, 298 K). The proton assignment is in Fig. 1.

In contrast to Ribó *et al.*,[17] we have observed chemical shifts of γCD protons, especially of H-6,6′ and H-5, and of all porphyrin protons. This suggests that protonation of pyrrole nitrogens does not eliminate the formation of inclusion complexes. (Table 2). Unfortunately, porphyrin protons H-b and H-c of the sulfonatophenyl groups were not well resolved in either ^{1}H NMR or ROESY spectra. The ROESY spectrum contains cross-peaks corresponding to the interaction of these protons with both internal cyclodextrin protons H-3 and H-5 (Table 4). These results, and the fact that pyrrole protons H-a interact with H-5 and H-6, allow us to conclude that the binding mode of $TPPSH_2^{2+}$ is similar to that of free-base TPPS, *i.e.*, the inclusion of a sulfonatophenyl substituent into the cyclodextrin cavity *via* the primary face.

Summing up the spectroscopic data on TMPyP–CD and $TPPSH_2^{2+}$–CD, we can put forward following features:

i) Both external (TMPyP–CDs) and host–guest ($TPPSH_2^{2+}$–CDs) interactions lead to bathochromic shifts in UV/Vis and fluorescence spectra typical of less polar solvents. Both interactions induce changes in fluorescence emission spectra; external binding of TMPyP shows more dramatic changes due to hindered rotation of electron-accepting methylpyridinium groups.

ii) External interactions have no effect on the lifetimes of the triplet states of TMPyP in the absence or presence of oxygen, while host–guest complexation prolongs the lifetime of the triplet states of $TPPSH_2^{2+}$ due to the shielding effect.[15]

Stoichiometry and K_b of the $TPPSH_2^{2+}$–CD complexes. As described above, the absorbance changes were small and for these reasons the binding parameters were evaluated only for $TPPSH_2^{2+}$ with native βCD. Analyzing the isotherms and the corresponding Job plot indicate that both components form a 1:1 complex with K_b of about 1.6×10^3 M^{-1}. For comparison, TPPS has a larger affinity towards βCD, with the sulfonatophenyl substituents immersed into the CD cavity through the secondary face (1:1, $K_b = 5.6 \times 10^3$ M^{-1}).[18,19] The described variations reflect the effect of protonation of pyrrole nitrogens.

Conclusion

Cationic TMPyP with four positive charges on the periphery forms supramolecular complexes with native, per-methylated, sulfonated and dimethyl-sulfonated CDs. The measured stoichiometry is 1:1 or 2:1; however, it probably varies depending on the concentration of interacting components. The NMR results indicate that the interaction of the porphyrin moiety with the external surface of native CDs is the main binding mode. The strength of interaction is less sensitive to the cavity size, but significantly sensitive to the functionalization of CDs. The high affinity of TMPyP towards sulfonated CDs is due to ion pairing between positively charged TMPyP and negatively charged sulfonate groups of CDs. Native CDs do not affect the behavior of the TMPyP triplet states while sulfonated SO_3βCD increase their basicity. We have found that $TPPSH_2^{2+}$ forms inclusion complexes with βCD of the same stoichiometry (1:1), but with a lower binding constant compared to nonprotonated TPPS. Similarly to TPPS, the sulfonatophenyl substituents of $TPPSH_2^{2+}$ are included *via* the secondary face. In contrast to TPPS, the positive charge of the porphyrin moiety resulting from protonation of pyrrole nitrogens probably affects the equilibrium between the two binding modes, with inclusion through the secondary and primary faces. The binding mode of $TPPSH_2^{2+}$ with γCD does not appear to be affected by protonation of the porphyrin ring. Binding of $TPPSH_2^{2+}$ with βCD increases the lifetimes of the triplet states.

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