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Supramolecular sensitizer: complexation of *meso*-tetrakis(4-sulfonatophenyl)porphyrin with 2-hydroxypropyl-cyclodextrins

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Abstract

5, 10, 15, 20-tetraphenyl-21 H, 23 H-porphine tetrasulfonic acid, tetrasodium salt (TPPS4) forms in ground state 1 : 1 supramolecular complexes with 2-hydroxypropyl-cyclodextrins (hp-CD) in aqueous neutral solutions. It was investigated with UV–VIS absorption and fluorescence spectroscopy. The complexing ability of CD's exhibits marked differences for hp-α-CD, hp-β-CD and hp-γ-CD. The hp-β-CD was found to bind the porphyrin the strongest with K_b about two order of magnitude greater when compared to native β-CD. It suggests that the binding site on the porphyrin moiety predominantly interacts with the primary face of the modified CD's. Complexation of TPPS4 significantly prevents protonation of pyrrole nitrogens, induce deaggregation of J-aggregates and slows down metalation indicating that the porphyrin moiety is shielded from bulk solution. The presence of hp-CD's considerably prolong the lifetimes of the triplet states of the title porphyrin; simultaneously, the rate constants of quenching of the triplet states by oxygen are decreased. In the presence of hp-β-CD the quantum yields of the triplet states and of singlet oxygen formation remain unchanged. The addition of hp-β-CD prevents staining effect of TPPS4 on the cellular membranes and greatly affects the uptake of TPPS4 by human Caucasian acute lymphoblastic leukemia CCRF-CEM cells. In conclusion, TPPS4 in a supramolecular complex represents an efficient sensitizer, which is due to shielding effect of hp-CD's (against protonization, aggregation, metalation, or interaction with membrane proteins) much less sensitive towards the influence of its environment.

Keywords: Photodynamic effect; Sensitizer; Triplet state; Singlet oxygen; Cyclodextrin; Porphyrin

1. Introduction

Numerous tetrapyrrole compounds as porphyrins and phthalocyanines photosensitize production of singlet oxygen ($^1O_2$) whose multiform oxidative effects are the basis for photodynamic therapy (PDT) of tumors [1–3]. Appropriate chemical and photochemical properties of a sensitizer such as spectral characteristics, fluorescence, quantum yields of the triplet states and singlet oxygen formation, are prerequisites for photodynamic action [4,5]. Still, their binding to biopolymers in the biological systems and/or to vectors transporting the sensitizer to tumor tissue is decisive for the final photodynamic efficiency since it can result in changes of physico-chemical, photophysical and photochemical properties [6–9].

Cyclic oligosacharides, cyclodextrins (CD) consist of 6, 7 or 8 α-1,4-D-glucopyranose units [10] arranged to a truncated cone thus giving rise to three readily available α, β, γ-CD's. The interior is lined by C–H groups and glycosidic oxygen bridges making the cavity of CD's nonpolar relative to the hydrophilic exterior with primary and secondary hydroxyl groups. This combination implicates high tendency of CD's to form noncovalent host-guest inclusion complexes as has been reviewed in detail by Connors [11]. As a result, complexation to CD prevents aggregation of a guest, facilitates transfer of hydrophobic substances to aqueous solutions [12,13], and directs electron transfer reactions [14,15]. CD's have been also used as carriers of pharmaceuticals or for improving their solubility and stability [16]. It is worth considering them as inert carriers in PDT as proposed by Ruebner et al. for cyclodextrin dimers [17]. However, the detailed ground state and photophysical data of sensitizer-CD complexes have not been collected so far.

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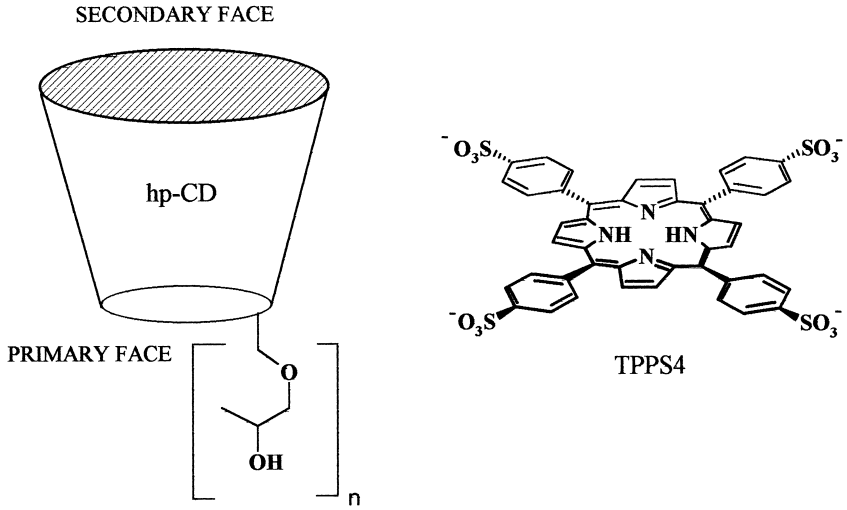

Fig. 1. Schematic representation of the structures of 2-hydroxypropyl-cyclodextrin and the porphyrin sensitizer TPPS4.

Meso-tetraphenyl porphyrins were reported to interact with CD's via encapsulation of the *meso*-phenyl substituents [10,12,13]. The same model was applied on complexation of water-soluble *meso*-tetrakis(4-sulfonatophenyl)porphyrin (TPPS4) (Fig. 1) with native CD's on the basis of NMR investigation [18,19]. However, the binding modes can be masked by high concentrations required by NMR measurements and therefore possible porphyrin aggregation that affects the values of binding constants [20]. Hydrophilic porphyrins can also form cofacial, 'cup-and-saucer' type complexes, attached by hydrogen bonding between the side polar groups and/or the tetrapyrrole nitrogens of porphyrin and the OH groups on the primary face of the CD ring. This geometry was proposed for *meso*-tetrakis(4-carboxyphenyl)porphyrin [21,22].

Despite growing amount of information about the interaction of pophyrins and other dyes with CD, a detail study of their complexation in the context of photodynamic effect is still missing.

The aim of this study was to find out, whether and how the presence of CD affects the physico-chemical, photophysical and photochemical properties of monomeric form of porphyrin sensitizers. Our selection of TPPS4 and 2-hydroxypropyl-cyclodextrins (hp-CD, see Fig. 1) was based on three factors. First, TPPS4 is well soluble in water with low aggregation tendency at concentrations of 1 μM. Second, TPPS4 has been suggested for application in PDT due to its proper photochemical properties [23]. Third, the modification of the primary face of CD's causes better solubility and suppresses aggregation of CD's in aqueous media, compared to native CD [12,13,24].

2. Experimental details

Native β-cyclodextrin (β-CD, Fluka), 2-hydroxypropyl-α-cyclodextrin (hp-α-CD, molar substitution $f_a = 0.6$; Aldrich), 2-hydroxypropyl-β-cyclodextrin (hp-β-CD, $f_a = 0.6$ and 1.0; Aldrich) and 2-hydroxypropyl-γ-cyclodextrin (hp-γ-CD, $f_a = 0.6$; Fluka) were used as received. TPPS4 was synthesized and purified as described elsewhere [25]. Incubation minimum medium RPMI 1640, fetal calf serum, antibiotics, Hank's solution and phosphate buffered saline solution PBS were purchased from the Gibco company.

UV–VIS absorption and fluorescence spectra were measured on a Varian Carry IE spectrophotometer and Perkin–Elmer LS 50B spectrometer, respectively, using 10 mm quartz cells. Unless otherwise stated, binding experiments were performed in 0.02 M phosphate buffer (pH 7.0) at room temperature (22°C).

The fluorescence quantum yield (Φ_F) was obtained by comparison of the total porphyrin fluorescence intensity in the absence (a reference with $\Phi_F = 0.06$ [26]) and in the presence of hp-β-CD (molar ratios TPPS4/hp-β-CD = 0.01) when excited at the isosbestic point of 417 nm.

Acido-basic properties were studied in Britton–Robinson buffers of various pH's. The concentrations of free, complexed, and the diprotonated form were calculated from absorbances at 413, 420 and 432 nm, respectively, under 100-fold molar excess of hp-β-CD. The apparent pK_a's were evaluated graphically from the concentration vs. pH plots.

The stoichiometry of the TPPS4 complexes with CD's was evaluated by Job's method of continuous variations [27]. The solutions of TPPS4 and each of the corresponding CD's were mixed to standard volume while the total concentration of both components remained constant, i.e. 1 μM. Job's plots were constructed from the absorbance differences at the absorption maxima of the complexes at 420 nm. The same results were obtained for wavelengths throughout the Soret band. The determination of the binding constants K_b between TPPS4 and CD's is based on the equilibrium expression

$$K_b = \frac{[\text{TPPS4-CD}_n]}{([\text{CD}]^n[\text{TPPS4}])}, \quad (1)$$

where [TPPS4-CD_n], [TPPS4], [CD] are the equilibrium molar concentrations of the complex, the free porphyrin and CD, and n expresses the stoichiometry. Two wavelengths, 413 and 420 nm, were selected and absorbances were recorded for a set of about 15 solutions containing 1 μM TPPS4 and variable concentrations of CD's. The molar absorption coefficients of TPPS4 and that of the complex at both wavelengths were calculated from the absorption spectra of TPPS4 alone and of the TPPS4/CD solutions under complete porphyrin complexation. The concentrations of [TPPS4] and [TPPS4-CD_n] were obtained by two-component analysis of the absorption spectra [28] and were subjected to a least squares routine. The linear relationship was obtained when Eq. (1) was plotted as the ratio [TPPS4]/[TPPS4-CD_n] vs. $1/(CD_T - n\,[\text{TPPS4-CD}_n])^n$, where CD_T is the total analytical concentration of CD. The absorbance changes were also plotted in a double-reciprocal form known as the Benesi–Hildebrand treatment [29].

Laser flash photolysis experiments were performed with a Lambda Physik FL 3002 dye laser (417 nm, output energy 1–3 mJ/pulse, pulse width ~28 ns) [8]. Transient spectra were recorded within 300–600 nm on a laser kinetic spectrometer (Applied Photophysics, UK). The time profiles of the triplet states were probed at 450 or 460 nm using a 250 W Xe lamp equipped with a pulse unit and a R928 photomultiplier. The triplet lifetimes were measured at 460 nm in oxygen-free solutions saturated with argon. The bimolecular constants of quenching of the triplet state of the sensitizer by oxygen were calculated from the known oxygen concentrations [30] and the measured rate constants of deactivation of the triplet states for aqueous solutions saturated with oxygen, air and argon.

To calculate the molar absorption coefficients of the triplet states ε_T, solutions were prepared with identical absorbance at the excitation wavelength ($A_{417} = 0.50$, in 10 mm quartz cell). The ε_T's were estimated by the complete conversion method [25]. The quantum yields of the triplet states Φ_T at low excitation energy density were calculated by the comparative method (TPPS4 as a standard, $\varepsilon_{460} = 1.3 \times 10^5\ \text{l mol}^{-1}\ \text{cm}^{-1}$, $\Phi_T^{ref} = 0.76$) [31] using the relationship:

$$\Phi_T = \Phi_T^{ref} \frac{(\varepsilon_T^{ref}\ \Delta A_T)}{(\varepsilon_T\ \Delta A_T^{ref})} \quad (2)$$

Singlet oxygen quantum yields Φ_Δ were determined using time-resolved near-infrared emission at 1270 nm. Emission was monitored at right angle to the excitation beam with a germanium diode (Judson J16-8SP-R05M-HS, USA) after being selected by a 1270 nm band-pass filter (Laser Components, Olehing, FRG). All traces were accumulated 256 times. Optically matched solutions at the excitation wavelength of the isosbestic point were prepared in phosphate buffer containing 90% D_2O to increase the singlet oxygen lifetime. The Φ_Δ's were calculated by comparing the maximum amplitude of the signals for a reference – TPPS4 solution ($\Phi_\Delta = 0.62$ [32,33]) – and the working solutions. The linear dependence of the signal amplitude on the laser fluence was verified.

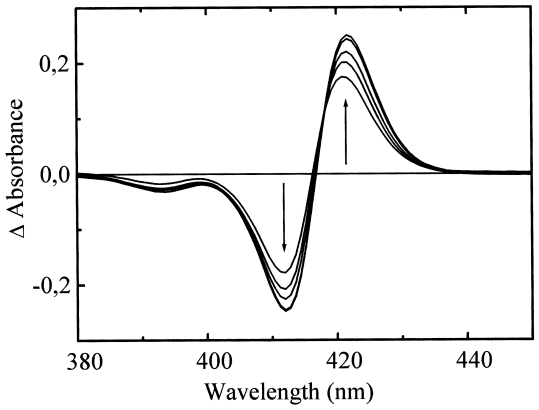

Fig. 2. Difference absorption spectra of TPPS4 in the presence of 0, 5, 10, 20, 100 and 500 μM hp-β-CD. Porphyrin concentration is 1 μM in 0.02 M phosphate buffer, pH 7.0. Arrows designate increasing concentration of hp-β-CD.

The biological tests were carried out using human Caucasian acute lymphoblastic leukemia cells (CCRF-CEM). The cells were grown up to a concentration of 10^6 cells/ml by incubation in RPMI 1640 medium enriched by 10% fetal calf serum and antibiotics at 37°C under air with 5% concentration of CO_2. The cell concentration was determined by a cytometer (Coulter counter, model ZM). The cell suspension was centrifuged (1000 rpm, 5 min) to remove the nutrient solution. A total of 5×10^6 cells were washed twice by PBS and incubated in the dark in 2 ml of Hank's solution (pH 7.2) containing (a) 1×10^{-5} M TPPS4, (b) 1×10^{-5} M TPPS4 with 1×10^{-3} M hp-β-CD for 15 min, 3, 6, 24, 48 and 72 h. After incubation, the cells were separated by centrifugation (1000 rpm, 5 min) and washed twice with PBS. Cell pellets were dissolved in 2 ml of 10 mM NaOH at $T = 60$°C (30 min in sonificator with heating). The absorbances of dissolved cell pellets were measured at 418 nm (isosbestic point in hydrolyzate) and the fluorescence intensities at 643 nm ($\lambda_{exc} = 418$ nm).

3. Results

3.1. Ground state interaction between TPPS4 and CD'S

The addition of CD's to the neutral buffered solutions of TPPS4 results in substantial changes in absorption and emission spectra. In absorption spectra, the Soret band of TPPS4 is bathochromically shifted from 413 up to 420 nm. The changes are best visualized in difference absorption spectra after subtracting the absorbances of free TPPS4 (Fig. 2). These results are attributed to the formation of a ground state complex between TPPS4 and CD and suggest the presence of only two absorbing species in equilibrium in the solutions

Table 1
Photophysical data of TPPS4 and of its complexes with CD's. In the column of triplet states, λ_{max} stands for absorption maxima of T–T spectra, ε_T for absorption molar coefficients at 460 nm, k_q for bimolecular rate constants of oxygen quenching, τ_T for lifetimes in the absence of oxygen and Φ_T for quantum yields of the triplet states. In the column of singlet oxygen τ_Δ stands for lifetimes and Φ_Δ for quantum yields of singlet oxygen formation. All measurements were performed in 0.02 M phosphate buffer (pH 7.0) or in Britton–Robinson buffer (pH 4.2) using 1 μM TPPS4 and 100 μM CD's

System	pH	$(\Phi_F)^a$	Triplet states					Singlet oxygen	
			λ_{max} (nm)	$(\varepsilon_T)^a$ (M^{-1} cm^{-1})	$k_q \times 10^{-9}$ (M^{-1} s^{-1})	$(\tau_T)^a$ (μs)	$(\Phi_T)^a$	$(\tau_\Delta)^a$ (μs)	$(\Phi_\Delta)^a$
TPPS4	7.0	0.060[b]	444	(1.3×10^5)[c]	1.8	290	0.76[d]	20	0.62[e]
TPPS4	4.2	–	–	–	–	–	–	23	0.58
TPPS4 + hp-α-CD	7.0	–	450	–	1.6	290	–	–	–
TPPS4 + hp-β-CD	7.0	0.053	450	1.14×10^5	0.3	2000	0.76	26	0.60
TPPS4 + hp-β-CD	4.2	–	–	–	–	–	–	23	0.67
TPPS4 + hp-γ-CD	7.0	–	450	–	1.0	340 (40%) 2100(60%)	–	–	–

[a] Estimated error 15%.
[b] [26].
[c] [31].
[d] [33].
[e] [32].

– the free porphyrin and the porphyrin supramolecular complex. Examination of the absorbance changes as a function of CD concentration reveals the high complexing strength of CD since the most marked changes were found for molar ratios TPPS4/CD above 0.10. Fluorescence emission bands are red shifted from 648 to 654 nm and from 708 to 720 nm, thus copying the shift of the Soret band, whereas the fluorescence quantum yield remains nearly unaffected (Table 1). Due to low porphyrin concentrations of 1 μM, the association takes place between the porphyrin monomer and cyclodextrin.

The stoichiometry of the complexes was assessed by Job's method of continuous variations. For all CD's studied, the maximal difference of absorbance appears at the porphyrin molar fraction of 0.5 (Fig. 3, inset). It obviously indicates a 1 : 1 TPPS4-CD complex (Table 2). The equilibrium equation is given as follows

$$\text{TPPS4} + \text{CD} \overset{K_b}{\rightleftharpoons} \text{TPPS4-CD}$$

The binding constants K_b of the TPPS4-CD complexes were determined (see Table 2) from the changes in absorption spectra at 413 and 420 nm of TPPS4 upon titration by CD's. The best fit to the experimental data is obtained assuming a 1 : 1 stoichiometry (Eq. (1), Fig. 3) confirming that binding in 1 : 1 ratio prevails in the studied systems. When the Benesi–Hildebrand treatment is applied, the values of estimated K_b's are the same within experimental error of 20%. The complexing ability of CD's exhibits marked differences for hp-α-CD, hp-β-CD, hp-γ-CD. The strongest complexing ability to bind the porphyrin was found for the hp-β-CD with K_b about two order of magnitude greater when with native β-CD. It suggests that the binding site on the porphyrin moiety predominantly interacts with the primary face of the modified CD (see Fig. 1).

We found that complexation of TPPS4 affects protonation of the porphyrin nitrogens. The detailed investigation was

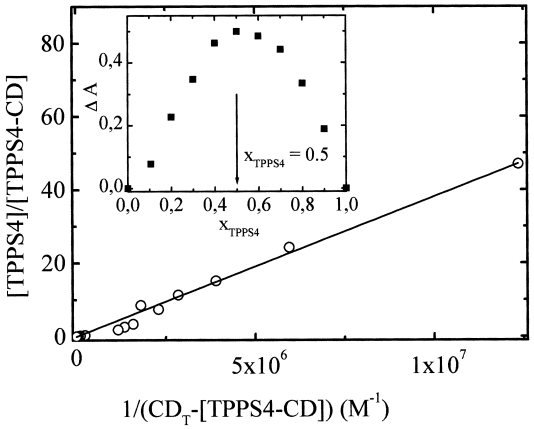

Fig. 3. Determination of K_b for the TPPS4–hp-β-CD complex. The concentrations of respective components were calculated using two-component spectral analysis. The solid line indicates the least-squared fit to the experimental data assuming the 1 : 1 complex. Inset: Job's plot in 0.02 M phosphate buffer, pH 7.0 at 420 nm. The total concentration was 1 μM. Molar fraction is given by $x_{TPPS4} = c_{TPPS4}/(c_{TPPS4} + c_{CD})$.

Table 2
Binding constants K_b of TPPS4 with studied CD. All measurements were performed in 0.02 M phosphate buffer at pH 7.0

Complex	Molar substitution	TPPS4 : CD	K_b [M^{-1}][a]
TPPS4–hp-α-CD	0.6	1 : 1	4.5×10^3
TPPS4–β-CD	0	1 : 1	5.6×10^3
TPPS4–hp-β-CD	0.6, 1.0	1 : 1	2.6×10^5
TPPS4–hp-γ-CD	0.6	1 : 1	1.3×10^4

[a] Average range calculated from all independent experiments was 20%. Data were treated by two-component and by the Benesi–Hildebrand analysis from the absorption spectra (see Section 2).

carried out with the strongest complex TPPS4–hp-β-CD. Qualitatively similar results can be concluded for other CD's studied, however, higher molar ratios CD/TPPS4 have to be used to bring about the equivalent effects. TPPS4 forms the diprotonated H_2TPPS4^{2+} in acidic aqueous media

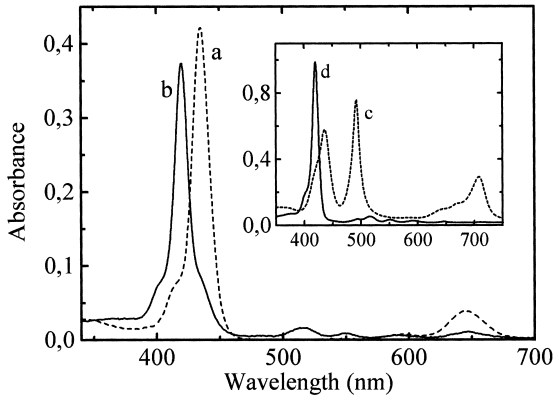

Fig. 4. Absorption spectra of 1 μM TPPS4 in the absence (a) and presence (b) of 1×10^{-4} M hp-α-CD. Curve (a) is the spectrum for the diprotonated H_2TPPS4^{2+}. 0.02 M acetate buffer, pH 3.6. Inset: absorption spectra of the J-aggregate (c); the same solution after addition of 5×10^{-4} M hp-β-CD (d). 5 μM TPPS4 in 0.02 M acetate buffer, 1 M NaCl, pH 3.6.

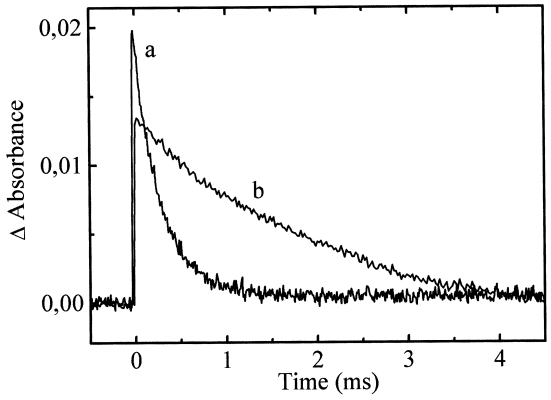

Fig. 5. Transient absorption traces of ^{3}TPPS4 at 450 nm in the absence (a) and presence (b) of 1×10^{-4} M hp-β-CD. Argon-saturated solutions, 1 μM TPPS4, 0.02 M phosphate buffer.

with a Soret band at 432 nm and a Q-band at 645 nm (Fig. 4a). After addition of hp-β-CD, the spectrum of complexed free-base TPPS4 appears with a Soret band at 420 nm and four Q-bands; the shoulder at 432 nm belongs to small amount of the remaining H_2TPPS4^{2+} (Fig. 4b).

Similarly, fluorescence emission spectra of the free diprotonated porphyrin (λ_{exc} = 400–650 nm, pH 3.6) show only the band of H_2TPPS4^{2+} at 674 nm. In the presence of hp-β-CD, the spectrum displays the typical emission bands of complexed free-base TPPS4 at λ = 654 and 721 nm. Excitation at 432 nm yields the low-intensity emission band of H_2TPPS4^{2+}.

The apparent pK_a values were graphically evaluated from the pH-titration curves, giving $pK_a^F = 4.8 \pm 0.1$ for TPPS4, which is in good agreement with reported pK_a^F of 4.8 [34], and $pK_a^B = 2.1 \pm 0.1$ for the supramolecular complex. Evidently, binding to hp-β-CD shifts the acid–base equilibrium in favor of the free-base TPPS4. Because the protonated H_2TPPS4^{2+} forms readily J-aggregates [35], the complexation also exerts a strong influence on this process under aggregation conditions (pH 3.6, 1 M NaCl). The absorption spectrum in Fig. 4c represents a mixture of the J-aggregate (absorption bands at 492 and 708 nm) and the diprotonated H_2TPPS4^{2+}. After addition of hp-β-CD (Fig. 4d) both diprotonated form and J-aggregates are converted into the supramolecular complex.

Strong association between TPPS4 and hp-β-CD (100-fold molar excess) considerably reduces the rate of insertion of zinc(II) and copper(II) into TPPS4 [36]. On the contrary, hp-α-CD with much lower complexing ability exerts under the same conditions almost no influence.

3.2. Photophysical properties of the complexes

Photophysical quantities characterizing TPPS4 in solution and upon complexation with CD's are summarized in Table 1. When the flash photolysis experiments are performed with argon-saturated solutions at pH 7.0, under the given conditions (TPPS4/hp-CD = 0.01), the presence of hp-β-CD significantly prolongs the lifetime of the triplet states of TPPS4 (Fig. 5). At the same molar ratio, in the presence of hp-γ-CD, there are two decay components: a fast one decaying as the triplet states of free TPPS4 and a slower one with lifetime corresponding to the complex. The time profiles of the ^{3}TPPS4 in the presence of hp-α-CD are best fitted to one exponential curve indicating that the lifetime remains unaffected. The complexation also influences the quenching of ^{3}TPPS4 by oxygen. Typically, the TPPS4–hp-β-CD complex has the bimolecular rate constant k_q almost 1 order of magnitude lower than TPPS4 in solution (Table 1). No effect of hp-β-CD was found on the quantum yield of the triplet state and consequently on the sensitizing ability of TPPS4 characterized by the quantum yields of singlet oxygen formation. The lifetime of singlet oxygen is independent of the CD presence implying that neither chemical nor physical quenching of singlet oxygen by CD's can occur.

The lifetimes and the k_q values reflect formation of the complex. In the case of hp-β-CD, the photophysical data in Table 1 are given by predominantly complexed TPPS4 (about 96%) differing from those of free TPPS4. Since hp-γ-CD exhibits intermediate K_b, both complexed (about 60%) and free TPPS4 forms contribute to the kinetic profiles of the triplet states. The hp-α-CD appears to have a marginal effect since only about 30% of TPPS4 is bound.

3.3. The effect of hp-β-CD on uptake of TPPS4 into leukemia CCRF lymphoblastic cells: preliminary dark experiments

Fig. 6 summarizes the results obtained for TPPS4 in the absence and in the presence of 100-fold excess of hp-β-CD after incubation with cells in the dark. For incubation with TPPS4, the most profound increase in absorption of the cell hydrolyzate occurs within first 15 min. Similarly, the fluorescence emission increases within the same time period,

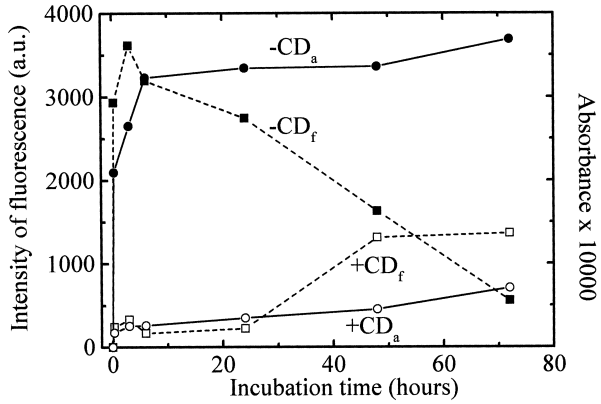

Fig. 6. Time course of uptake of TPPS4 (10 μM) by leukemia lymphoblastic cells CCRF-CEM in the absence (-CD) and presence of 1 mM hp-β-CD (+CD). Absorbance at 418 nm (index a) and fluorescence intensities at 643 nm (λ_{exc} = 418 nm, index f) were measured after hydrolyzing 5×10^6 cells in 10 mM NaOH.

however, further incubation is accompanied by sharp fluorescence quenching. The microscopic analysis indicated that these results corresponded to fast staining of the cell membranes that leads to high local porphyrin concentrations. This is consistent with phenomena occurring at high TPPS4/protein molar ratios, i.e. extensive TPPS4 aggregation after binding to a protein. The aggregate formation considerably reduces fluorescence quantum yields, quantum yields of the triplet states, and consequently production of singlet oxygen upon irradiation [6].

The cyclodextrin complex prevents the immediate staining effect. As the incubation period is prolonged, both absorbance and fluorescence of the cell hydrolyzate increase – the sensitizer after the uptake remains in a fluorescent form. The fact that fluorescence is not quenched leads to an assumption that hp-β-CD protects TPPS4 against binding to organelle and/or cytoplasmic proteins (or other substances) and that hp-β-CD may maintain the photosensitizing ability of TPPS4. Monitoring the number of disturbed cell structures under a fluorescence microscope led to the qualitative information that the slight 'dark' cytotoxicity of TPPS4 decreases in the presence of hp-β-CD.

4. Discussion

The TPPS4 forms 1 : 1 complexes with title CD's in neutral aqueous solutions. The binding constants K_b obtained for these systems depend strongly on the structure of the porphyrin counterpart. The highest K_b values are those for hp-β-CD, the cyclodextrin consisting of seven glucose units with the hydroxypropylated primary hydroxyl groups. This modification leads to about two order of magnitude increase of binding affinity when compared to native β-CD under the same experimental conditions. Dramatic influences on the complex strength are also seen when we compare hp-α-CD, hp-β-CD and hp-γ-CD, the modified CD differing only in the number of glucose units and consequently in the inner cavity diameter. The hp-β-CD is a much stronger binder, whereas binding constant for hp-α-CD is comparable to that of β-CD and hp-γ-CD has only moderately higher K_b. From these results we can conclude that 2-hydroxypropyls on the primary side (for both molar substitutions of 0.6 and 1.0) play a major role in the formation of these complexes. Since the K_b values are similar for β-CD, hp-α-CD and only two times higher for hp-γ-CD regardless of modification, the proper size of the CD molecule is the second important factor for the binding process.

It is well established [11] that CD's bind aromatic compounds into the hydrophobic cavity. In this respect, experimental results based on NMR and circular dichroism are consistent with a model in which porphyrins of proper size and shape are incorporated within the interior of the cyclodextrin cavity [19,20,37–42]. The porphyrins with the anionic carboxy or sulfonato groups on the *meso*-phenyls are expected to bind similarly to native CD's [19,20,38–42] as proposed for anilinonaphthalenesulfonates [43] where the hydrophobic naphthalene slips into the cavity via the secondary face to form the 1 : 1 complex with the deprotonated sulfonato group oriented into bulk solution on the opposite side. Alternatively, cofacial, 'cup-and-saucer' complexes between 2-hydroxypropyl-CD's and *meso*-tetrakis(4-carboxyphenyl) porphyrin have been proposed [21,22], where the supramolecular assembly 1 : 1 is hold together via hydrogen bonding between the carboxy groups and/or pyrrole nitrogens of the porphyrin and OH groups of hydroxypropyls on primary face of hp-CD.

Our experiments demonstrate that much lower pH's are necessary for formation of diprotonated H_2TPPS4^{2+} upon addition of hp-CD's, i.e. complexation hinders protonation of the pyrrole nitrogens of TPPS4. An explanation was given by Lawrence et al. [38,39] for the host-guest model, stating that the hydrophobic interior of the cavity may preferentially bind a porphyrin with a neutral nucleus. It causes that the equilibrium concentration of the complexed, unprotonated form of porphyrin is shifted to higher value simply by binding to CD's.

The protonation of TPPS4 at lower pH's in the presence of hp-β-CD has interesting consequences with regards to formation of extended J-aggregates occurring at high ionic strength. Deaggregation of J-aggregates can be used as a measure of TPPS4 complexation with CD's. Because hp-β-CD forms much stronger complex than native CD's measured by Ribó et al. [19] lower excess of hp-CD's is needed for deaggregation. The TPPS4 in the supramolecular complex is metalated very slowly compared to TPPS4 itself that again confirms changes in availability of the pyrrole nitrogens.

Considering these effects the reasonable conclusion is that the assembly at *low concentrations* used here is hold together via hydrogen bondings or combination of hydrogen bondings and hydrophobic interactions. The formation of

the complex shields the porphyrin moiety from bulk solution. Although most of the existing studies (typically using NMR and therefore high concentrations of analyte required by the method) suggest host–guest type of interaction, the 'cup-and-saucer' arrangement can similarly explain hindering of pyrrole nitrogens against protonation and metalation as well as deaggregation of J-aggregates and moreover elucidate the 1 : 1 stoichiometry. Generally, we suppose that the highest affinity of hp-β-CD is given by the proper size of the β-CD cavity with major contribution of flexible hydroxypropyl groups probably allowing fine adjustment for hydrogen bonding.

The changes in the absorption spectra of the complexes reflect the electronic perturbations arising from solvent effects and from changes in the solvent–solute dipole interactions due to reduced exposure to water. The net result would be a red shift in the Soret region of complexed TPPS4 as compared to TPPS4 in aqueous solution [44]. Similarly, a small red shift is observed also for triplet–triplet absorption. In contrast, kinetic properties of the triplet states are quite different. We observed considerable prolongation of the lifetimes of the triplet states in the complex. This is expected since complexation rigidize the system, excludes water molecules from the solvation shell of the porphyrin and reduces collisional quenching of the triplet states due to solvent molecules, thereby increasing the lifetimes. This is frequently found for noncovalently bound porphyrins to proteins and nucleic acids (e.g. [6,7]). Judging from the stiochiometry of the complexes, we reasoned that TPPS4 could still efficiently sensitize the formation of singlet oxygen even under condition of extensive J-aggregation. This supposition was borne out. The quantum yields of the excited singlet states and of the triplet states remain unaffected. The bimolecular rate constant of quenching of the triplet states by oxygen is nearly one order of magnitude lower than that of free ^{3}TPPS4. This is expected due to slower collisional quenching by oxygen. However, the quantum yield of singlet oxygen formation that is a measure of photosensitizing ability of any sensitizer remains unchanged in the complex. The ability of hp-β-CD to maintain the monomeric, photodynamically active form of TPPS4 is highly important with respect to porphyrin tendency to aggregate at higher concentrations and ionic strengths in aqueous solutions because aggregates have very low yields of singlet oxygen [6,32]. In our preliminary experiments with cells we found that complexation prevents sorption of TPPS4 on the cellular membranes and affects the uptake.

This is the first report of ground state complexation of TPPS4 by 2-hydroxypropyl cyclodextrins. Moreover, it can be stated on the bases of our experiments that the TPPS4 in supramolecular complex represent a efficient sensitizer of singlet oxygen. Due to the shielding effect of the cyclodextrin molecule against protonation, aggregation, metalation and interaction with membrane proteins the bound porphyrin is much less sensitive to the chemical composition of the environment.

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