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Ternary Complexes of 4*H*-1-Benzopyran-4-thione with *β*-Cyclodextrin and Alcohols. Dramatic Effect of Complex Formation on Fluorescence and Phosphorescence Intensity

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The effect of coinclusion of alcohol (ROH) on the stoichiometry, stability, spectral, as well as photophysical properties of β-cyclodextrin (β-CD) complexes of 4*H*-1-benzopyran-4-thione (BPT) was studied. Although the binary complex was of 1:1 stoichiometry, the formation of ternary complexes of 1:2:2 BPT:ROH:β-CD stoichiometry was observed. The formation of the ternary complexes is revealed by a dramatic enhancement of fluorescence from the second excited singlet state and phosphorescence occurring from the two lowest thermally equilibrated triplet states. The increase in intensities of these emissions is discussed in terms of shielding the emissive molecule from bulk water molecules and expulsion (perhaps still partial) of water molecules coincluded with the BPT molecule in the binary complex. Even the coinclusion with a molecule that acts in solution as a very efficient quencher of BPT phosphorescence led to a very strong phosphorescence enhancement, which indicates that there is a spatial separation of reactive centers of the two coincluded molecules. The possible reasons for the changes in the shape in the phosphorescence spectrum and the appearance of vibrational structure in the S_2 band in the absorption spectrum are also discussed. The stability constants of the ternary complexes determined from absorption, fluorescence, and phosphorescence measurements depended strongly on the molecular size and structure of a coincluded alcohol.

Introduction

The formation of inclusion complexes between various guest molecules and cyclodextrins (CDs) is the most remarkable property of the latter. Apart from formation of two-component complexes by guest and CD molecules, the formation of ternary complexes in which coinclusion of a third component occurs have also been reported.[1−10] Recently, the formation of both binary and ternary β-cyclodextrin (β-CD) complexes of 4*H*-1-benzopyran-4-thione (benzopyranthione, BPT), whose structure is shown in Figure 1, has been reported.[11,12] BPT has proved to be a very convenient probe for studies of the microenvironment of the CD cavity because its spectral and photophysical properties strongly depend on its milieu.[13−15,11] The linearity of the Benesi−Hildebrand plot of $1/(A_0 - A)$ versus $1/[\beta\text{-CD}]_T$, where A_0 and A are the absorbances for the total β-CD concentration equal to 0 and $[\beta\text{-CD}]_T$, respectively, indicates that the stoichiometry of the binary complex is 1:1.[11,16,17] A similar plot based on the changes in phosphorescence intensity upon addition of β-CD confirms this stoichiometry.[17]

It has been shown that BPT fluorescence (occurring from the S_2 state)[18,14] is efficiently quenched by water molecules through their interactions with the reactive center of the thioketone molecule (namely, thiocarbonyl group) and that is why the fluorescence quantum yield (ϕ_{fl}) of aqueous solution of BPT is extremely low ($\sim 9 \times 10^{-5}$).[11,16] Addition of β-CD causes only a slight increase in fluorescence quantum yield (by a factor of 1.4) despite the fact that the high CD concentration ensures that the great majority of BPT molecules are complexed. The still very low value of ϕ_{fl} for binary CD complex of BPT is clear evidence for the interaction between water and BPT molecules incorporated into CD cavity leading to highly efficient fluorescence quenching. However, a dramatic increase in fluorescence intensity is observed when a small quantity of some alcohols is added to BPT solution containing β-CD. This increase evidences the formation of ternary complex between BPT, β-CD, and alcohols.[12]

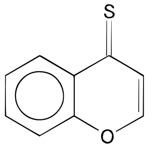

Figure 1. Structural formula of 4*H*-1-benzopyran-4-thione (BPT).

Although the binary inclusion complex of BPT has been found to have 1:1 stoichiometry, the 1:1 BPT:β-CD stoichiometry cannot be assumed a priori in the ternary complex. Hamai et al.[1] have found that despite 1:1 stoichiometry for β-CD complex of azulene, the guest:β-CD stoichiometry in the three-component systems is 1:2. Coincluding additives can also strongly influence the stability of the CD complexes, as has been found for several guest molecules.[1,3,5,6] The fact that coinclusion of a third component can trigger strong emission of some organic compounds in CD solution can be used in emission detection of these compounds[19] or coincluded molecules of the cosolvent.[9] The strength of ternary complexes has been found to depend on the size and geometry of coincluded molecules.[1,3,5]

Aromatic thioketones exhibit very interesting spectral, photophysical, and photochemical properties, including a very rich absorption spectrum, two relatively strong emissions (fluorescence from the second excited singlet state[18,14] and phospho-

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rescence from the two lowest thermally equilibrated triplet states[20-22]), as well as photochemical reactions both from the second excited singlet state and the triplet states.[13] Moreover, these processes strongly depend on the environment of a thioketone molecule. This situation provides an opportunity to investigate the interactions of guest molecules incorporated into a CD cavity with coincluded molecules of water and a cosolvent through absorbance, fluorescence, and phosphorescence measurements. The investigations of such interactions may prove to be interesting also from the viewpoint of the application of CDs as enzyme models.[23] The aim of this contribution is to describe the stoichiometry, stability, as well as spectral and photophysical properties of BPT ternary complexes and discuss the interactions between the molecules forming these complexes.

Experimental Section

BPT was synthesized from the appropriate ketone through a reaction with Lawesson's reagent and purified by repeated fractional crystallization from ethanol.[14] The purity of the thioketone was monitored by HPLC with photodiode array absorption (Waters 996) and emission (Waters 474) detectors as well as UV−vis spectrophotometry. Water was purified by triple distillation. β-Cyclodextrin (Fluka) was recrystallized three times from water. Methanol (Merck, for fluorometry), ethanol (Merck, p.a.), 1-propanol (POCh, p.a.), 2-propanol (Merck, for fluorometry), 1-butanol (Merck, for fluorometry), 1-pentanol (PSC, Qual Grade), and cyclohexanol (POCh, p.a.) did not show any significant emissive or absorptive impurities and were used as received. 2-Methyl-2-propanol (Loba Chemie) and cyclopentanol (POCh, pure) were distilled prior to use. The concentration of the thione was kept low ($[\mathrm{BPT}]_\mathrm{T} < 3 \times 10^{-5}$ M) because otherwise the solutions containing BPT, β-CD, and alcohols were turbid.

The UV−vis absorption spectra were obtained on an M40 Specord (Carl Zeiss, Jenna), whereas fluorescence and phosphorescence spectra were recorded on a modified MPF-3 (Perkin-Elmer) spectrofluorimeter. The introduced modifications enabled single-photon counting detection, computer control, and data processing with a dual-photon counting set (Light Scan). The spectrofluorimeter was also equipped with a reference quantum counter, which measured the intensity of incident light directly in front of the sample. Measurements were carried out at room temperature on air-equilibrated samples unless otherwise stated. It is important for further discussion to note that the excitation wavelengths (within the $S_0 \rightarrow S_2$ band) were chosen so that the differences in absorption coefficients for various forms of the guest were negligible in emission experiments. All emission spectra given here were corrected for the photomultiplier tube response, and the background due to solvent impurities and scattering was subtracted. To avoid overestimation of Raman scattering in a sample, which is lower than in the pure solvent, the contribution due to Raman scattering in the solvent was multiplied by transmitance of the sample for the excitation wavelength. The error of the equilibrium constants determined for all alcohols larger than ethanol used as cosolvents from measurements of absorption changes upon addition of β-CD to aqueous solution of BPT containing alcohol (1% v/v) did not exceed 20%, and of those based on emission intensity were not higher than 15% and were slightly smaller for the values obtained from phosphorescence compared with the fluorescence studies. For ethanol, the absorption method proved to be not sensitive enough for determination of the equilibrium constants, and the error of emission methods were much larger than for larger alcohols (~40%).

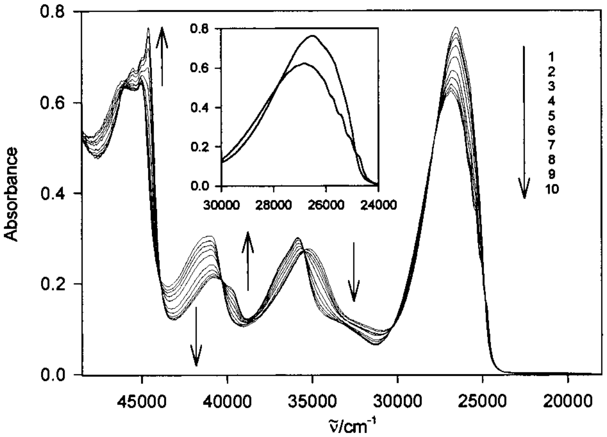

Figure 2. Absorption spectra of BPT ($[\mathrm{BPT}]_\mathrm{T} = 2 \times 10^{-5}$ M) in 1% (v/v) aqueous solution of 1-propanol with addition of β-CD: (1) $[\beta\text{-CD}]_\mathrm{T} = 0$ M; (2) $[\beta\text{-CD}]_\mathrm{T} = 2 \times 10^{-4}$ M; (3) $[\beta\text{-CD}]_\mathrm{T} = 3 \times 10^{-4}$ M; (4) $[\beta\text{-CD}]_\mathrm{T} = 5 \times 10^{-4}$ M; (5) $[\beta\text{-CD}]_\mathrm{T} = 8 \times 10^{-4}$ M; (6) $[\beta\text{-CD}]_\mathrm{T} = 1.4 \times 10^{-3}$ M; (7) $[\beta\text{-CD}]_\mathrm{T} = 2 \times 10^{-3}$ M; (8) $[\beta\text{-CD}]_\mathrm{T} = 3.5 \times 10^{-3}$ M; (9) $[\beta\text{-CD}]_\mathrm{T} = 6 \times 10^{-3}$ M; (10) $[\beta\text{-CD}]_\mathrm{T} = 1 \times 10^{-2}$ M; cell length = 2 cm.

Results and Discussion

The UV−Vis Absorption Spectra of Aqueous Solution of BPT in the Presence of *β*-CD and 1-Propanol. The UV−vis absorption spectrum of BPT consists of several well-separated bands. The intensity, shape, and position of these bands clearly depend on the medium. Because the environment of BPT molecules changes upon inclusion into a β-CD cavity, the BPT spectrum changes substantially when β-CD is added to the aqueous solution of BPT. In the absence of alcohols, the bands at 45 000 cm^{-1} and at 41 000 cm^{-1} become less intense with a very slight batochromic shift. The 35 100 and 26 400 cm^{-1} bands show hipsochromic shifts and, for the latter (the S_2 band), a substantial decrease in absorption coefficient is observed. In the presence of 1-propanol for the lowest β-CD concentration used (up to 5×10^{-4} M), the absorption changes are similar to those already described (Figure 2). For higher CD concentration, the effect of complexation on BPT spectra is dramatically different. The 45 000 cm^{-1} band moves to the lower wavenumbers with a strong increase in its intensity. The emergence of a sideband at ~40 000 cm^{-1} is observed. The band at 35 100 cm^{-1} experiences a strong hipsochromic shift together with a significant intensity increase. A very important feature of the BPT spectrum in these conditions is a vibrational structure appearing in the S_2 band. All these observations are indicative of ternary complex formation. Because these changes are observed only for higher β-CD concentrations, whereas the spectral changes for lower concentrations are similar to those corresponding to the formation of binary complex, it seems likely that the BPT:β-CD stoichiometry in the ternary complex is 1:2.

Emission Measurements of Aqueous Solution of BPT in the Presence of *β*-CD and 1-Propanol. Aromatic thioketones in solution show both fluorescence and phosphorescence.[13] However, for aqueous solutions of BPT, both emissions are weak. Addition of β-CD in the absence of alcohols causes only a slight increase in ϕ_fl. Also, the phosphorescence is slightly enhanced upon complexation (from $\phi_\mathrm{ph} = 3.6 \times 10^{-4}$ to $\phi_\mathrm{ph} = 6.2 \times 10^{-4}$ for air-equilibrated solutions, BPT concentration equal to 2.8×10^{-5} M, and the maximum concentration of β-CD of 0.015 M). A dramatic increase in fluorescence intensity (~14 times) was observed when β-CD (0.01 M) was added to an aqueous solution of BPT containing propanol (1% v/v) as shown

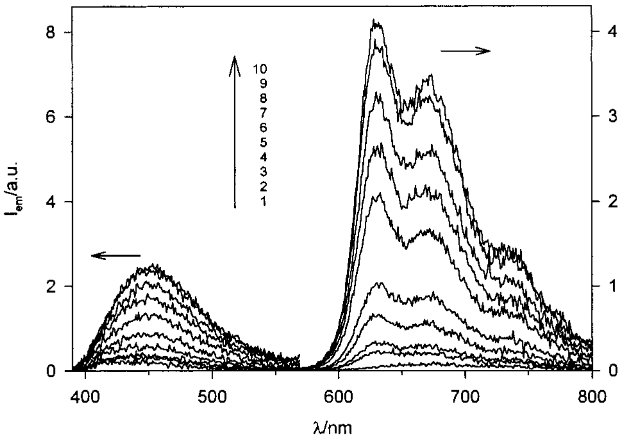

Figure 3. Changes in emission spectra of aqueous solution of BPT upon addition of β-CD in the presence of 1-propanol (1% v/v): $\lambda_{exc} = 359$ nm; $[BPT]_T = 1.2 \times 10^{-5}$ M; (1) $[\beta\text{-CD}]_T = 0$ M; (2) $[\beta\text{-CD}]_T = 2 \times 10^{-4}$ M; (3) $[\beta\text{-CD}]_T = 3 \times 10^{-4}$ M; (4) $[\beta\text{-CD}]_T = 5 \times 10^{-4}$ M; (5) $[\beta\text{-CD}]_T = 8 \times 10^{-4}$ M; (6) $[\beta\text{-CD}]_T = 1.4 \times 10^{-3}$ M; (7) $[\beta\text{-CD}]_T = 2 \times 10^{-3}$ M; (8) $[\beta\text{-CD}]_T = 3 \times 10^{-3}$ M; (9) $[\beta\text{-CD}]_T = 6 \times 10^{-3}$ M; (10) $[\beta\text{-CD}]_T = 1 \times 10^{-2}$ M.

in Figure 3. The strong increase in fluorescence quantum yield corroborates the conclusion about ternary complex formation between BPT, β-CD, and 1-propanol. A similar effect has been shown for inclusion complexes of α-naphthyloxyacetic acid,[8] pyrene,[2] fluorene,[24] and a series of coumarins.[25] The fluorescence enhancement can be interpreted in terms of repulsion of water molecules from the vicinity of thiocarbonyl group of the complexed guest molecules by alcohol molecules. Because the very low quantum yield of fluorescence for the binary complex of BPT is determined by interactions between complexed BPT and water molecules, the removal of water from the vicinity of the reactive group of the guest by coincluded alcohol molecules must lead to the increase in fluorescence quantum yield. The increase in ϕ_{fl} observed upon formation of the three-component complex is one of the greatest ever reported for CD inclusion complexes. Even stronger enhancement of phosphorescence was noted upon the addition of β-CD to an aqueous solution of BPT containing 1-propanol (~60 times). We expect that in the case of phosphorescence the removal of water molecules from the proximity of the thiocarbonyl group of the guest molecule is also responsible for the high emission intensities.

The shape of phosphorescence spectra of aromatic thioketones is determined by the configuration of the states from which it originates. In nonpolar solvents (e.g., perfluoroalkanes, alkanes) the T_1 and T_2 state are of the (n, π*) and (π, π*) configuration, respectively.[21,13] The energy gap between the two lowest thione triplets is 600−2400 cm^{-1}. In more polar solvents, the energy difference decreases. In solvents of medium polarity (e.g., methanol and benzene), the energies of the triplet states are similar and the states are strongly coupled. In highly polar solvents (e.g., acetonitrile and water), an inversion of the two triplet states occurs.[11,13,21] The difference in the configuration of the lowest triplet state for thioketones dissolved in solvents of various polarity manifests itself in the shape of the phosphorescence spectra (Figure 4). The shape of the spectrum of BPT in aqueous solution of β-CD resembles that measured in methanol, which indicates that the polarity of the vicinity of BPT molecules, forming together with a CD molecule the binary complex, is similar to that of methanol.[11,16] However, the shape of the phosphorescence spectrum is changed substantially on addition of 1-propanol to the two-component system and it becomes more similar to the spectrum measured in alkanes. The contribution of the emission from the $^3(n,\pi^*)$ state to the phosphorescence emission of the ternary complex is much bigger than that for the binary complex. This result clearly indicates that the polarity of BPT microenvironment in a three-component system is much lower than that in a binary system. A comparison of the phosphorescence spectrum of the ternary complex and those of BPT in alcohols indicates that the polarity sensed by the BPT in the ternary complex is lower than that of methanol (dielectric constant $\epsilon = 32.6$), ethanol ($\epsilon = 24.3$), 1-propanol ($\epsilon = 20.1$), and even 1-butanol ($\epsilon = 17.1$). This result, however, cannot by treated as general because the apparent polarity of the interior of the CD cavity has been shown to depend strongly on the position of the guest molecule in the complex and its contact with other molecules in the CD cavity, namely, those of water or a cosolvent.[2,26,27] The shape of the fluorescence spectrum is only little changed when BPT is involved in the ternary complex formation, however a discernible red shift of the maximum in that spectrum is observed.

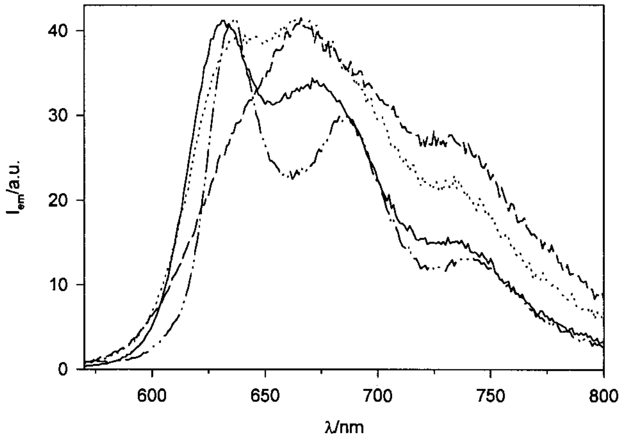

Figure 4. Normalized phosphorescence spectra of BPT in water (- - -), 0.012 M aqueous solution of β-CD (···), 0.012 M aqueous solution of β-CD with 1% v/v addition of 1-propanol (—), and in hexane (—··—).

Stoichiometry and Equilibria. Changes in absorbance, fluorescence, and phosphorescence intensity upon addition of β-CD and/or alcohol can be used for the determination of the stoichiometry and binding constant of the ternary complex. Knowledge of the BPT:β-CD stoichiometry can be gained from the Benesi−Hildebrand treatment of the changes in absorbance and emission upon addition of β-CD to a solution keeping the concentrations of the guest (BPT) and the alcohol constant. In Figure 5 the plots of the reciprocal of the changes in absorbance against the reciprocal of the β-CD concentration and against the reciprocal of the square of the β-CD concentration are presented. Because the former plot is bent upward and the latter downward, we can conclude that complexes of the BPT:β-CD stoichiometry 1:1 and 1:2 are formed. However, the plots of the reciprocal of the changes in emission (fluorescence and phosphorescence) intensity versus the reciprocal of the square of the β-CD concentration are linear. This result indicates that only the formation of the complex with the BPT:β-CD stoichiometry 1:2 is associated with a strong enhancement in emission intensity and the formation of the complex with the BPT:β-CD stoichiometry 1:1 accompanied only by a very slight change in the emission properties of the guest is obscured by the formation of the strongly emissive complex of the BPT:β-CD stoichiometry 1:2. Molecular modeling showed that the interactions between the reactive center of a complexed guest molecule and water molecules can be inhibited also in the case of a ternary complex with 1:1:1 stoichiometry and, therefore, the quantum yield of emission of such a complex can be significantly

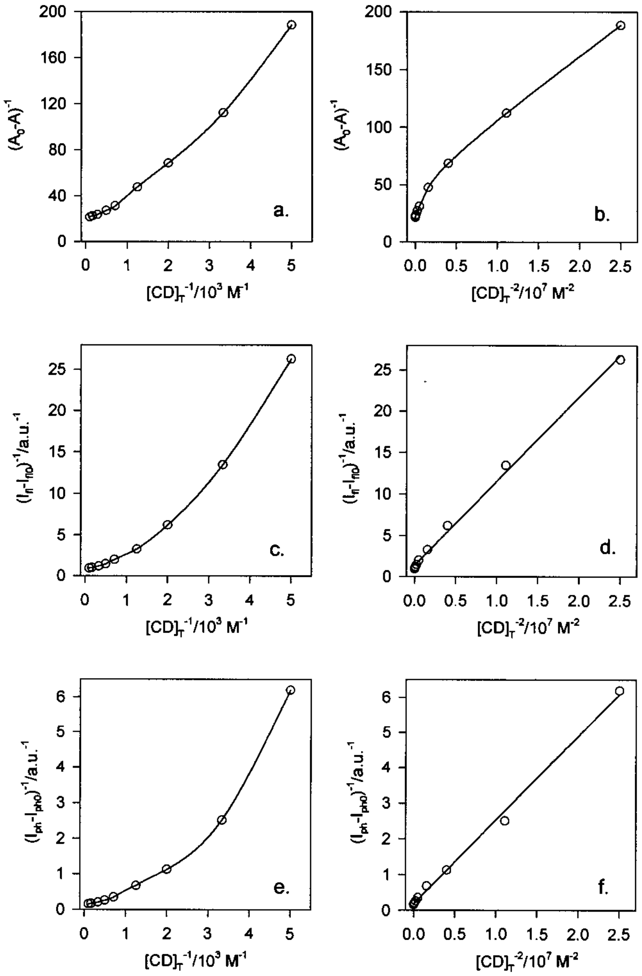

Figure 5. Benesi–Hildebrand plots for β-CD complexes of BPT with 1-propanol constructed using absorption for $\tilde{\nu} = 41\,000$ cm^{-1} (a and b), fluorescence (c and d), and phosphorescence (e and f) data, assuming two different BPT:β-CD stoichimetries: 1:1 (a, c, and e) and 1:2 (b, d, and f); $[BPT]_T = 1.2 \times 10^{-5}$ M; $C_{1\text{-propanol}} = 1\%$ v/v.

different from that of a free guest even when the quantum yield of emission for a binary complex is similar to that of the uncomplexed guest. Thus we can expect the formation of the ternary complex with the BPT:β-CD stoichiometry 1:1 would be connected with a significant emission enhancement and thereby it would markedly affect the shape of the plots shown in Figure 5d,f. Because these plots are linear, we can conclude that the formation of the ternary complex with the BPT:β-CD stoichiometry 1:1 is at most a minor process and the complex with the 1:1 stoichiometry whose formation in the presence of 1-propanol is revealed by absorption study is the binary BPT:β-CD complex.

The formation of the ternary complex of a BPT:β-CD stoichiometry different from 1:1 can also be deduced from the shape of the curves shown in Figures 6b and 6c. It can be proved that the plots of the relationships between emission intensity (or its change) and CD concentration cannot have a point of inflection if the guest:CD stoichiometry is 1:1 because, for such a stoichiometry, the second derivative of the functions describing these relationships[5] is never equal to zero. For the guest:CD 1:2 stoichiometry, the appearance of a point of inflection is possible and the curves can be "S-shaped". The presence of an inflection point in the plots representing the relationships just mentioned (for 5×10^{-4} M $< [CD]_T < 1 \times 10^{-3}$ M) indicates that a complex of a guest:CD stoichiometry different from 1:1 is formed. The presence of a point of inflection on the curve presented in Figure 6 d testifies to a more complex BPT:1-propanol stoichiometry than 1:1. The dependence of phosphorescence intensity of phenantrene on the concentration of β-CD in the presence of 1,2-dibromoethane[19] suggests that the stoichiometry of the complexes described there may be 1:2 rather than 1:1.

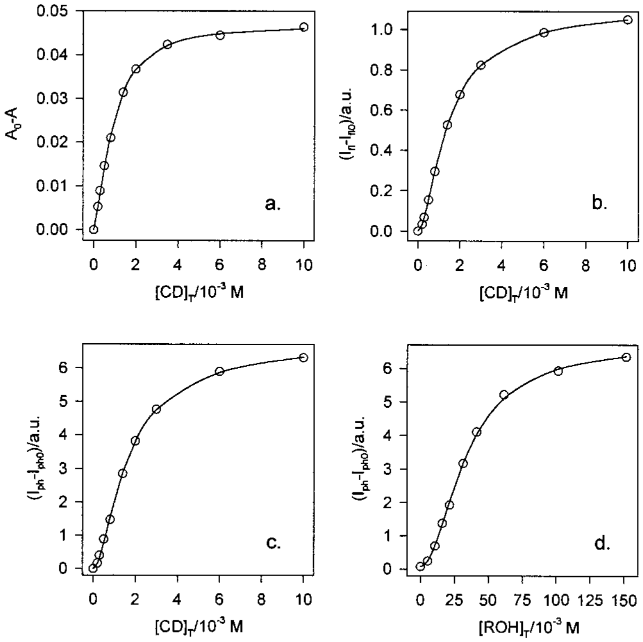

Figure 6. Changes in absorption for $\tilde{\nu} = 41\,000$ cm^{-1} (a), integrated fluorescence (b), and phosphorescence (c) intensity upon addition of β-CD to aqueous solution of BPT containing 1-propanol (1% v/v) and changes in integrated phosphorescence upon addition of 1-propanol to aqueous solution of BPT containing β-CD (0.01 M) (d), $[BPT]_T = 1.2 \times 10^{-5}$ M.

If the β-CD:1-propanol stoichiometric ratio in the ternary complex is assumed to be 1:1 (vide infra) as has been found for other three-component systems,[1–3,9,24] it is sufficient to consider the following equilibria to quantitatively explain the observed changes in absorbance and intensities of fluorescence and phosphorescence

$$\text{BPT} + \text{CD} \rightleftharpoons \text{BPT·CD} \qquad K_1 = [\text{BPT·CD}]/[\text{BPT}][\text{CD}] \tag{I}$$

$$\text{ROH} + \text{CD} \rightleftharpoons \text{ROH·CD} \qquad K_2 = [\text{ROH·CD}]/[\text{ROH}][\text{CD}] \tag{II}$$

$$\text{BPT·CD} + 2\text{ROH} + \text{CD} \rightleftharpoons \text{BPT·(ROH)}_2\text{·(CD)}_2$$
$$K_3 = [\text{BPT·(ROH)}_2\text{·(CD)}_2]/[\text{BPT·CD}][\text{ROH}]^2[\text{CD}] \tag{III}$$

In all experiments in this investigation, the total guest concentration ($[BPT]_T$) was much smaller (at least ~6 times) than the total concentrations of the other two components of the ternary complex ($[CD]_T$, $[ROH]_T$) and thus we can write the conditions:

$$[\text{CD}]_T = [\text{CD}] + [\text{ROH·CD}] \text{ and } [\text{ROH}]_T = [\text{ROH}] + [\text{ROH·CD}] \tag{1}$$

where $[CD]_T$ and $[ROH]_T$ denote total concentrations of CD and an alcohol, respectively. Then, the concentration of the binary complex ROH·CD can be found from the quadratic equation

$$K_2[\mathrm{ROH{\cdot}CD}]^2 - (K_2[\mathrm{ROH}]_\mathrm{T} - K_2[\mathrm{CD}]_\mathrm{T} + 1)[\mathrm{ROH{\cdot}CD}] + K_2[\mathrm{CD}]_\mathrm{T}[\mathrm{ROH}]_\mathrm{T} = 0$$

As a solution the quadratic equation we obtain

$$[\mathrm{ROH{\cdot}CD}] = (K_2([\mathrm{CD}]_\mathrm{T} + [\mathrm{ROH}]_\mathrm{T}) - \sqrt{1 + 2K_2([\mathrm{CD}]_\mathrm{T} + [\mathrm{ROH}]_\mathrm{T}) + K_2^2([\mathrm{CD}]_\mathrm{T} - [\mathrm{ROH}]_\mathrm{T})^2} + 1)/2K_2 \quad (2)$$

Obviously there is another solution of eq 1; namely, that with a plus sign before the square root. However, only the solution given in eq 2 fulfills the condition that [ROH•CD] decreases to zero when $[\mathrm{ROH}]_\mathrm{T}$ or $[\mathrm{CD}]_\mathrm{T}$ decreases to zero. It is apparent from eq 2 that when $[\mathrm{CD}]_\mathrm{T} + [\mathrm{ROH}]_\mathrm{T} =$ constant, the concentration of the binary complex of alcohol is the highest when the total concentrations of its both components are the same ($[\mathrm{CD}]_\mathrm{T} - [\mathrm{ROH}]_\mathrm{T} = 0$). Having calculated [ROH•CD] from eq 2, one can readily obtain concentrations of free CD and alcohol using the conditions given in eq 1. However, in many experiments, the concentration of alcohol was much higher than that of CD and then simpler expressions describing the concentrations of free CD and alcohol could be used:[1]

$$[\mathrm{CD}] = [\mathrm{CD}]_\mathrm{T}/(1 + K_2[\mathrm{ROH}]_\mathrm{T}) \qquad [\mathrm{ROH}] = [\mathrm{ROH}]_\mathrm{T} \quad (3)$$

The concentrations of all forms of BPT in a solution containing CD and alcohol can be calculated from equations analogous to those given in ref 1:

$$[\mathrm{BPT}] = [\mathrm{BPT}]_\mathrm{T}/(1 + K_1[\mathrm{CD}] + K_1K_3[\mathrm{ROH}]^2[\mathrm{CD}]^2)$$

$$[\mathrm{BPT{\cdot}CD}] = K_1[\mathrm{BPT}]_\mathrm{T}[\mathrm{CD}]/(1 + K_1[\mathrm{CD}] + K_1K_3[\mathrm{ROH}]^2[\mathrm{CD}]^2)$$

$$[\mathrm{BPT{\cdot}(ROH)_2{\cdot}(CD)_2}] = K_1K_3[\mathrm{BPT}]_\mathrm{T}[\mathrm{ROH}]^2[\mathrm{CD}]^2/(1 + K_1[\mathrm{CD}] + K_1K_3[\mathrm{ROH}]^2[\mathrm{CD}]^2) \quad (4)$$

Using this set of equations, one can obtain formulas for the change of emission intensity and absorbance as a function of alcohol and CD concentration:

$$I - I_0 = \frac{k_\mathrm{ex}K_1[\mathrm{BPT}]_\mathrm{T}[\mathrm{CD}](\Delta\phi_1 + \Delta\phi_2K_3[\mathrm{ROH}]^2[\mathrm{CD}])}{1 + K_1[\mathrm{CD}] + K_1K_3[\mathrm{ROH}]^2[\mathrm{CD}]^2} \quad (5a)$$

where the symbol with the subscript ‘0’ denotes the value at zero concentration of CD, $\Delta\phi_1 = \phi_{\mathrm{BPT{\cdot}CD}} - \phi_{\mathrm{BPT}}$, $\Delta\phi_2 = \phi_{\mathrm{BPT{\cdot}(ROH)_2{\cdot}CD_2}} - \phi_{\mathrm{BPT}}$, and k_ex is an experimental constant

$$A_0 - A = \frac{K_1[\mathrm{BPT}]_\mathrm{T}[\mathrm{CD}](\Delta\epsilon_1 + \Delta\epsilon_2K_3[\mathrm{ROH}]^2[\mathrm{CD}])}{1 + K_1[\mathrm{CD}] + K_1K_3[\mathrm{ROH}]^2[\mathrm{CD}]^2} \quad (5b)$$

where $\Delta\epsilon_1 = \epsilon_{\mathrm{BPT}} - \epsilon_{\mathrm{BPT{\cdot}CD}}$ and $\Delta\epsilon_2 = \epsilon_{\mathrm{BPT}} - \epsilon_{\mathrm{BPT{\cdot}(ROH)_2{\cdot}CD_2}}$. For [ROH] = 0, eqs 5a and 5b become nonlinear forms[28] of a well-known Benesi−Hildebrand[29] equation for binary complexes.

In this study, the effects of varying concentration of β-CD on the absorbance, fluorescence, and phosphorescence of a BPT solution containing 1% 1-propanol were investigated; [ROH] and [CD] denote here the actual concentrations of free alcohol and β-CD, respectively, which can be calculated from the equations just given. In the conditions of such experiments, the concentration of 1-propanol was always much higher than that of β-CD and thus [ROH] and [CD] could be obtained by using both more accurate but complex eqs 1 and 2 and simpler though approximate eq 3, and in both cases the value of K_3 obtained from the fit of functions given by eqs 5a and 5b to experimental points was the same. The good fits shown in Figure 6 indicate that the model of complexation of BPT already given is appropriate. From the fits to absorption, fluorescence and phosphorescence data using $\Delta\epsilon_1$ and $\Delta\phi_1$ obtained from the experiments for the two-component BPT:β-CD system and the literature values of K_1 [11,16] and K_2,[30] the values of K_3 equal to 9.6×10^4 M^{-3}, 1.1×10^5 M^{-3}, and 9.8×10^4 M^{-3}, respectively, were obtained. Taking into account errors that these values are charged with, we can conclude that there is a good agreement between the values of K_3 obtained from absorption, fluorescence, and phosphorescence measurements.

Also the change in phosphorescence emission upon addition of 1-propanol to a solution of BPT containing β-CD at a constant concentration ($[\mathrm{CD}]_\mathrm{T} = 0.01$ M) can be used for determination of K_3. The function given by eq 5a was fitted to the experimental data obtained in such an experiment (Figure 6.) However, it should be remembered that I_0 in eq 5a denotes intensity of phosphorescence in the absence of CD. Therefore, phosphorescence changes upon addition of 1-propanol to a solution containing β-CD must be corrected by adding an increment in the phosphorescence enhancement due to the binary complex formation present prior to the addition of the alcohol. The concentration of β-CD was 0.01 M and the concentration of 1-propanol employed ranged from 0.005 to 0.15 M. In this case, it is no longer justified to use eq 3 to calculate the actual concentration of β-CD because this equation has been derived on the assumption that the concentration of alcohol is much higher than that of CD. Therefore we used the accurate method of calculating [CD] based on eqs 1 and 2. The value of K_3 obtained from this experiment (1.15×10^5 M^{-3}) is consistent with that obtained from measurements of phosphorescence enhancement upon addition of β-CD to a solution of BPT containing 1-propanol at constant concentration.

The continuous variation method[31] has been used to reveal the stoichiometry of both binary and ternary complexes.[2,3] This method depends on monitoring a property (absorbance, emission intensity) of a solution while varying the concentrations of CD and alcohol, keeping the sum of their concentrations constant. If the observed change in the emission intensity or absorption can be predominantly attributed to the formation of a ternary complex, these changes are the biggest for the solution containing the ternary complex at the highest concentration. The formation of the ternary complex of a guest G assuming its 1:1 alcohol:β-CD stoichiometric ratio can be described by the following equation:

$$\mathrm{G} + n\mathrm{ROH{\cdot}CD} \rightleftharpoons \mathrm{G{\cdot}(ROH)}_n\mathrm{(CD)}_n$$

$$K_4 = [\mathrm{G{\cdot}(ROH)}_n\mathrm{(CD)}_n]/[\mathrm{G}][\mathrm{ROH{\cdot}CD}]^n \quad (\mathrm{IVa})$$

It is apparent from the expression for K_4 that if the concentration of the free form of BPT did not change significantly or its change was predominantly due to the formation of the ternary complex, then it would be true that the higher the concentration of the binary ROH•CD complex, the higher the concentration of the ternary complex. As we have shown on the basis of eq 2, under the condition of $[\mathrm{CD}]_\mathrm{T} + [\mathrm{ROH}]_\mathrm{T} =$ constant, [ROH•CD] is the highest for $[\mathrm{ROH}]_\mathrm{T} = [\mathrm{CD}]_\mathrm{T}$. Thus, if the alcohol:CD stoichiometric ratio in a ternary complex is indeed 1:1, one could expect to obtain the highest concentration of the ternary complex and thereby the highest change in emission intensity or absorption for the concentration of β-CD and a cosolvent equal to each other (regardless of the stoichiometry between the guest G and CD). However, there are two factors that can disturb the picture just described. One is associated with a significant contribution of the binary complex formation in the changes in absorption or emission under the conditions of the continuous variation method experiment. It should be remembered that this method implies a low concentrations of a cosolvent, which does not exceed the highest concentration of CD, whereas for other experiments, much higher cosolvent concentrations can be used. That is why in this particular type of experiment the formation of the ternary complex can be inefficient and often the binary complex is the dominant form of the complexed guest. If $\Delta\phi_1$ and $\Delta\phi_2$ or $\Delta\epsilon_1$ and $\Delta\epsilon_2$ are of the same sign, the maximum changes in emission or absorption, respectively, can be obtained for $[\mathrm{CD}]_\mathrm{T} > [\mathrm{ROH}]_\mathrm{T}$ even if the maximum concentration of the ternary complex is obtained for $[\mathrm{CD}]_\mathrm{T} = [\mathrm{ROH}]_\mathrm{T}$. This phenomenon can be overcome in absorption experiments by monitoring absorbance change for the wavelength for which absorption coefficients of the free guest and that forming the binary complex is the same[32] or subtraction of the contribution to the change in emission intensity or absorption due to a binary complex formation.[2] Another factor affecting the relationship between $[\mathrm{CD}]_\mathrm{T}/([\mathrm{CD}]_\mathrm{T} + [\mathrm{ROH}]_\mathrm{T})$ and the change in a solution property is the reaction of the binary complex formation, which competes with reaction IVa, making the formation of the ternary complex less efficient. If the free guest concentration varies significantly in the continuous variation method experiment because of the formation of its binary complex, the continuous variation plot will become asymmetric and the maximum concentration of the ternary complex will be obtained for $[\mathrm{CD}]_\mathrm{T} < [\mathrm{ROH}]_\mathrm{T}$. The two factors just described can make the continuous variation method not always easily applicable for three-component systems, which might have been the reason this method gave inconsistent results in the studies of some such systems.[33,34]

To confirm the 1:1 alcohol:β-CD stoichiometric ratio in the ternary complex formed by BPT, 1-propanol, and β-CD, we measured the intensity of phosphorescence of aqueous solution of BPT with addition of β-CD and 1-propanol under the conditions of $[\mathrm{CD}]_\mathrm{T} + [\mathrm{ROH}]_\mathrm{T} = 0.012$ M. Because the value of K_1 for β-CD complex of BPT is relatively high and K_2 for 1-propanol is rather low, we can expect that the formation of the binary complex will influence the relationship between $[\mathrm{CD}]_\mathrm{T}/([\mathrm{CD}]_\mathrm{T} + [\mathrm{ROH}]_\mathrm{T})$ and the change in phosphorescence emission intensity. To be able to properly interpret the results of the experiment, we calculated the change in the emission intensity for the continuous variation method experiment using eqs 2 and 5a with the values of K_3, $\Delta\phi_1$, and $\Delta\phi_2$ obtained from other experiments (vide supra). The observation of dependence of emission intensity on $[\mathrm{CD}]_\mathrm{T}/([\mathrm{CD}]_\mathrm{T} + [\mathrm{ROH}]_\mathrm{T})$ similar to the calculated one confirms the 1:1 stoichiometric ratio between β-CD and 1-propanol (Figure 7), although the maximum phosphorescence intensity was obtained for $[\mathrm{CD}]_\mathrm{T} < [\mathrm{ROH}]_\mathrm{T}$. Taking into account the 1:2 BPT:β-CD stoichiometry, we can write the equilibrium IVa substituting n with 2:

$$\mathrm{BPT} + 2\mathrm{ROH{\cdot}CD} \rightleftharpoons \mathrm{BPT{\cdot}(ROH)_2(CD)_2}$$

$$K_4 = [\mathrm{BPT{\cdot}(ROH)_2(CD)_2}]/[\mathrm{BPT}][\mathrm{ROH{\cdot}CD}]^2 \quad \mathrm{(IV)}$$

We can conclude that all experiments described here have confirmed the 1:2:2 BPT:1-propanol:β-CD stoichiometry.

Results for Other Alcohols. Changes in the absorption and emission spectra of aqueous solutions of BPT containing other alcohols upon addition of β-CD have been also measured. In the case of methanol, no changes either in fluorescence or phosphorescence intensity were observed, which indicates that the ternary complex is not produced in detectable amounts. For ethanol used as a cosolvent, the spectral changes upon addition of β-CD are not much different from those observed without any alcohol. Only a slight increase in the 45 000 cm^{-1} band is noticeable for the highest β-CD concentrations ($>4 \times 10^{-3}$ M). A Benesi–Hildebrand plot based on the absorption changes for ethanol is linear. However, the distinct changes in fluorescence and phosphorescence clearly show that the ternary complex with ethanol is formed. The plots of $1/(I - I_0)$ (for both fluorescence and phosphorescence) against $1/[\mathrm{CD}]_\mathrm{T}$ and $1/[\mathrm{CD}]_\mathrm{T}{}^2$ are curved for this alcohol, which can be explained by a nonnegligible contribution due to the binary complex formation in the increase in the intensity of both emissions. All the observations show that the efficiency of the ternary complex formation in the case of ethanol is low and thus absorption changes are mainly due to the formation of the binary complex. On the other hand, for some alcohols (e.g., 2-methyl-2-propanol and cyclopentanol), the formation of the ternary complex is discernible even for the lowest β-CD concentrations used. This result indicates that the molecular size and structure of an alcohol is an important factor determining the contribution of binary and ternary complexes in the overall complexation.

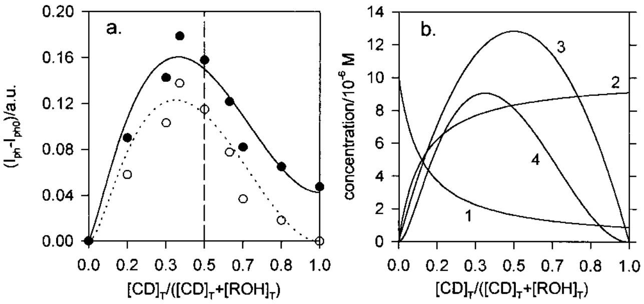

Figure 7. (a) Continuous variation plots of the integrated phosphorescence intensity change, uncorrected (full circles are solid line) and corrected for the contribution of the binary complex formation to the phosphorescence enhancement (open circles and dotted line) under conditions of $[\beta\text{-CD}]_\mathrm{T} + [\text{1-propanol}]_\mathrm{T} = 0.012$ M and $[\mathrm{BPT}]_\mathrm{T} = 1 \times 10^{-5}$ M. (b) Concentrations of various species in the continuous variation experiment: (1) [BPT]; (2) [BPT·β-CD]; (3) [1-propanol·β-CD] (multiplied by factor of 0.1); (4) [BPT·(1-propanol)$_2$·(β-CD)$_2$] (multiplied by factor of 50).

Table 1 shows the stability constants K_3 and K_4 of the ternary complexes with the alcohols obtained from the fits of the functions given by eqs 5a and 5b to the changes in absorption as well as fluorescence and phosphorescence intensity. The fact that the fits are good indicates that the equilibria I–IV describe well the formation of CD complexes of BPT in aqueous solutions containing β-CD and one of the alcohols from Table 1. Only for ethanol did the absorption method fail, not being sensitive enough to reveal the appearance of the ternary complex formed at a low efficiency. The values of K_3 (as well as K_4) are large when compared with the binding constants for binary constants, but the direct comparison between these constants is meaningless because the units of the constants are different and only comparison between constants of the same type can be done.

It is apparent from Table 1 that although K_3 increases monotonically with the molecular size of alcohol, K_4, which better describes the ability of binary CD complexes of alcohols to include a BPT molecule, has its maximum for the alcohol of a relatively small molecular size (i.e., 1-propanol). However,

TABLE 1: Binding Constants and Emission Quantum Yields for Ternary β-Cyclodextrin Complexes of 4*H*-1-Benzopyran-4-thione (BPT) with Various Alcohols[a]

alcohol	K_2 (M^{-1})	K_3 (M^{-3})	K_4 (M^{-2})	ϕ_{fl} ($\times 10^{-3}$)	ϕ_{ph} ($\times 10^{-2}$)
ethanol	1.07	$\sim 700^{c,d}$	$\sim 5.8 \times 10^{5\ b,c}$	1.0	1.3
1-propanol	3.72	$9.6 \times 10^{4\ b}$	$6.2 \times 10^{6\ b}$	1.5	2.6
		$9.8 \times 10^{4\ c}$	$6.7 \times 10^{6\ c}$		
		$1.1 \times 10^{5\ d}$	$7.5 \times 10^{6\ d}$		
2-propanol	3.8	$8.0 \times 10^{4\ b}$	$5.3 \times 10^{6\ b}$	1.6	2.5
		$7.6 \times 10^{4\ c}$	$5.0 \times 10^{6\ c}$		
		$8.5 \times 10^{4\ d}$	$5.6 \times 10^{6\ d}$		
1-butanol	16.6	$3.4 \times 10^{5\ b}$	$1.2 \times 10^{6\ b}$	1.5	2.2
		$4.5 \times 10^{5\ c}$	$1.5 \times 10^{6\ c}$		
		$4.7 \times 10^{5\ d}$	$1.6 \times 10^{6\ d}$		
2-methyl-2-propanol	48	$4.1 \times 10^{6\ b}$	$1.7 \times 10^{6\ b}$	1.4	2.7
		$5.0 \times 10^{6\ c}$	$2.1 \times 10^{6\ c}$		
		$5.2 \times 10^{6\ d}$	$2.1 \times 10^{6\ d}$		
1-pentanol	63	$1.5 \times 10^{6\ b}$	$3.7 \times 10^{5\ b}$	1.4	2.2
		$1.4 \times 10^{6\ c}$	$3.4 \times 10^{5\ c}$		
		$1.3 \times 10^{6\ d}$	$3.1 \times 10^{5\ d}$		
cyclopentanol	120	$4.0 \times 10^{7\ b}$	$2.6 \times 10^{6\ b}$	1.3	2.5
		$3.4 \times 10^{7\ c}$	$2.2 \times 10^{6\ c}$		
		$3.3 \times 10^{7\ d}$	$2.2 \times 10^{6\ d}$		
cyclohexanol	501	$9.1 \times 10^{7\ b}$	$3.4 \times 10^{5\ b}$	1.4	2.9
		$8.3 \times 10^{7\ c}$	$3.1 \times 10^{5\ c}$		
		$7.2 \times 10^{7\ d}$	$2.7 \times 10^{5\ d}$		

[a] $K_1 = (950 \pm 90)\ M^{-1}$, refs 11 and 16; superscripts *b*, *c*, and *d* indicate obtained from absorption, phosphorescence, and fluorescence measurements, respectively; K_2 is from ref 30; $K_4 = K_1K_3/K_2^2$ (from ref 1).

TABLE 2: Phosphorescence Quantum Yields of 4*H*-1-Benzopyran-4-thione (BPT) in Various Systems

system	ϕ_{ph}
3-methylpentane solution	$3.2 \times 10^{-2\ a,b,c}$
2-propanol solution	$1.4 \times 10^{-4\ c}$
aqueous solution	3.6×10^{-4}
β-CD binary complex	6.5×10^{-4}
β-CD ternary complex with 2-propanol	2.5×10^{-2}

[a] From ref 13. [b] Extrapolated to infinite dilution. [c] Measured for deoxygenated sample.

it should be noted that neither K_3 nor K_4 alone characterizes the overall equilibrium properly. For example, even though the value of K_4 is lower for 2-methyl-2-propanol than for 1-propanol, the formation of the ternary complex is more efficient for the former. This result indicates how important the value of K_2 is in overall equilibrium. The high value of K_2 causes the shift of equilibrium I to substrates as well as produces a substrate for equilibrium IV, which can make the formation of a ternary complex efficient even if K_4 is not high (e.g., for cyclohexanol). On the other hand, although K_4 for ethanol is larger than for some other alcohols, the formation of the complex is inefficient due to a low value of K_2. We can expect that the extremely low value of K_2 for methanol ($0.32\ M^{-1}$)[30] is an important reason the formation of ternary complex for this alcohol is not observed at all. We can conclude that it is important to separate the two factors determining the efficiency of ternary complex formation; namely, the value of K_2 and the value of K_4.

The Microenvironment of BPT Molecules. The fits of the function 5a not only provide the information about the complex stability, but also give differences ($\Delta\phi_2$) between fluorescence and phosphorescence quantum yields of BPT involved in the ternary complex formation and the free form and thus values of the quantum yields of fluorescence and phosphorescence of the ternary complex given in the Table 1. Because K_2 and K_4 constants for all alcohols studied here except methanol and ethanol ensured for the highest β-CD concentrations the incorporation of ~90% of BPT molecules into ternary complex, these values are only slightly higher than those measured directly. Spectral properties as well as the values of ϕ_{fl} and ϕ_{ph} of the ternary complexes show unambiguously that the binding of BPT to β-CD together with alcohol molecules renders the environment of the thione much less polar and much weaker interacting than the environment of bulk water and CD cavity without coincluded alcohols. In analogy to other β-CD complexes of the same stoichiometry,[1,3] we can expect that in $BPT{\cdot}(ROH)_2{:}(\beta\text{-CD})_2$ two β-CD cavities face each other with the wider openings, whereas spaces near the narrower ones are occupied by alcohol molecules with a hydrocarbon part buried in the cavity and hydroxyl group directed toward the bulk solution. The BPT molecule is positioned inside the cavity formed by the two CD rings. The increase of emission intensities can be explained only by removal of water molecules from the vicinity of a thiocarbonyl group. However, molecular modeling showed that there is still some space available for water molecule to be included together with BPT and two cosolvent molecules in the interior of the β-CD molecules involved in the ternary complex formation. Fortunately, the probe molecule used here exhibits two kinds of emissions: short-lived fluorescence and long-lived phosphorescence. A high value of fluorescence quantum yield in comparison with the value measured in water indicates that in the lifetime of the fluorescent S_2 state, BPT molecules do not interact with either bulk or coincluded water. This result is understandable in the light of reports of Liao and Bohne[5] on rates of exit of a guest molecule from a CD cavity, which is much slower than deactivation of the S_2 state of aromatic thioketones.[13,14,35] Very high quantum yields of phosphorescence from much longer-lived states[13,20,36] indicate that BPT triplets are also not quenched by water molecules either coincluded with BPT molecules or of bulk solution. This result suggests that the time needed for exit from the cavity is still longer than the lifetime of phosphorescence (which is perhaps similar to that extrapolated to infinite dilution in alkanes),[13,20] and the exit rate from ternary complexes with 1:2:2 guest:ROH:CD stoichiometry is much slower than the exit rate from the CD cavity in the case of a binary complex[37] and may be even slower than that measured for the ternary complexes characterized by the 1:1:1 stoichiometry.[5] Surprisingly, the quantum yields of fluorescence and phosphorescence of ternary complexes are much higher than those measured in pure alcohols. The most striking is the high quantum yield of phosphorescence of the complex with 2-propanol, which is an extremely strong quencher of phosphorescence, being a very efficient hydrogen atom donor in the reaction of hydrogen atom abstraction by a thioketone molecule in its triplet state.[38] Due to this interaction between ^{3}(BPT) and a molecule of 2-propanol, the phosphorescence in pure 2-propanol is even weaker than in water (Table 2). This result suggests that the BPT molecule (or at least its reactive center, namely, the thiocarbonyl group) and hydroxyl group as well as hydrogen atoms at the secondary carbon atom, which are the most reactive parts of alcohol molecules coincluded in the cavities of β-CD, are not brought into contact with each other, which would lead to the reaction of hydrogen abstraction and weaker phosphorescence than that actually observed. We can conclude that the restrictions imposed on the relative orientations of BPT, alcohol, and very likely also water molecules included in the CD cavities render their interactions inefficient. This result can be compared with that reported by Kano et al.[39] who studied fluorescence quenching of pyrene in aqueous solution of CDs containing various amines. They observed strong static quenching of pyrene fluorescence by secondary and tertiary aliphatic amines in solutions of β- and γ-CD, which was attributed to bimolecular process involving pyrene and amine molecules, both included in a CD cavity.

It is also interesting to note that the vibrational structure appearing in the S_2 absorption band of BPT for each alcohol used as a cosolvent larger than ethanol is as clear as that in the extremely weakly interacting perfluoroalkanes.[40] On the other hand, the fluorescence and phosphorescence quantum yields are much lower than those in perfluoroalkanes being rather similar to those measured in alkanes. The values of ϕ_{fl} and ϕ_{ph} for ternary complexes together with the shape of the phosphorescence spectra indicate that the interaction between complexed BPT molecules and their environment is at least as strong as that between BPT and alkane molecules. However, the vibrational structure is more pronounced for the ternary complexes than for alkane solutions of BPT. That is why a decrease in the energy of interactions between complexed BPT and its surrounding cannot be the only reason for appearance of the vibrational structure in absorption spectrum. The similar effect observed for the 1,4-dimethoxybenzene:$(\alpha\text{-CD})_2$ complex has been attributed to the restrictions imposed on intramolecular movements of the guest molecule.[41] Also, in the case of the ternary complexes of BPT, the main reason may be the restricted environment of BPT molecule leading to constraints on intramolecular movements as well as on the relative positions of molecules within the complex and thus to much more uniform distribution of the interaction energy than in the case of homogeneous systems characterized by similar interaction energy.

Conclusions

The results of this study showed that the dramatic effects on absorption spectra, fluorescence, and phosphorescence intensity upon addition of β-CD to aqueous solution containing alcohols is due to the formation of the ternary complex with the 1:2:2 BPT:ROH:β-CD stoichiometry. The study of the changes in absorbance and intensities of both emissions provided the information about the stability of the ternary complexes. The size and structure of alcohol is an important factor determining the strength of the complex. Phosphorescence quantum yields for ternary complexes with all alcohols larger than ethanol are similar to those extrapolated to infinite dilution measured in hydrocarbons, which are relatively unreactive toward BPT triplets. The emission (especially phosphorescence) study showed that the reactive center of a BPT molecule involved in the ternary complex formation is shielded not only from the interactions with solvent molecules acting as quenchers but also surprisingly from the interaction with coincluded molecules. This result indicates that there are some constraints imposed on orientations of coincluded molecules. The microenvironment of the ternary complex of BPT with β-CD and various alcohols appeared to be unique, being much different from both homogeneous media and β-CD cavity without any coincluded cosolvent molecules. The BPT molecule proved to be a very convenient probe in the studies of properties of environment formed by β-CD and coincluded molecules and, on the other hand, such an environment is a very interesting medium for investigation of the properties of BPT itself.

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