An Exceedingly Long-Lived Fluorescent State as a Distinct Structural and Dynamic Probe for Supramolecular Association: An Exploratory Study of Host−Guest Complexation by Cyclodextrins

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Abstract: A novel fluorescent probe with n,π^* configuration, the azoalkane 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO), is responsive to complexation by supramolecular hosts. The n,π^* fluorescent probe serves to provide structural and, owing to its exceedingly long fluorescence lifetime (up to 1 μs), also kinetic information on host−guest complexation. The three cyclodextrins (CDs) were selected as prototypal hosts in aqueous solution, and the complexation, in both the ground and excited states, was followed by four techniques: time-resolved and steady-state fluorescence, UV absorption spectrophotometry, and NMR spectroscopy. The fluorescence quenching rate constants (k_q) of DBO by α-, β-, and γ-CD (1.9, 4.0, and 0.78 × 10^8 M^{-1} s^{-1}) were determined from the dynamic component of the biexponential time-resolved decay traces, while the static component was assigned to the fluorescence lifetimes of the complexes (τ_{CD}) of α-CD (ca. 33 ns) and β-CD (ca. 95 ns). Time-resolved and steady-state fluorescence measurements yielded consistent results. The shorter lifetimes in the complexes are attributed to the propensity of singlet-excited DBO to undergo fluorescence quenching by an "aborted" hydrogen abstraction with the labile glycosidic C−H bonds inside the cavity. Ground-state binding constants (K) could be determined by both UV spectrophotometry (for β-CD, ca. 900 M^{-1}) and, owing to the high water solubility, also by NMR spectroscopy to afford values of 50, 1100, and 6 M^{-1} for α-, β-, and γ-CD, respectively. The spectroscopic data support the formation of *inclusion* complexes in both the excited and ground states. The dynamic quenching is attributed to inclusion with subsequent quenching inside the shorter-lived complex. The examination of the complexation dynamics at high guest (DBO) concentration revealed an unprecedented behavior, which may be indicative of singlet energy transfer between the free DBO and the CD·DBO complex. The potential of DBO as a distinct and complementary fluorescent probe is discussed.

Introduction

The advantages of fluorescent probes or switches for sensing molecular, supramolecular, and biological events are well recognized.[1] They comprise high sensitivity of detection down to the single molecule, excellent temporal and spatial resolution, ease of application, a great variety of experimental techniques, and more than a century of scientific experience. In principle, the readily detectable fluorescent properties of the probe molecule (fluorophore), i.e., its fluorescence lifetime, intensity, spectral appearance, or polarization, are altered (in some cases switched on or off) by molecular or environmental interactions.

One timely challenge in the field of supramolecular chemistry comprises the development of fluorescent probes for monitoring host−guest complexation and association processes in compartmentalized systems. Besides *structural* probes, i.e., those which yield information on the chemical environment in the host and its cavity dimensions, there is also a considerable interest in *dynamic* probes, i.e., those which allow one to obtain information on the kinetics of association or complexation with the host or dissociation of the complex.[2] Although accurate association and dissociation rate constants are generally more difficult to obtain than thermodynamic data such as binding constants, they are invaluable for the development of structure−activity relationships in supramolecular chemistry, and their absolute magnitude has implications for the practical use of supramolecular systems for catalytic and analytical purposes.

The direct measurement of fast processes requires the use of techniques with high time resolution, such as T-jump experiments or laser flash photolysis. Not surprisingly, excited states, which can be readily generated with the latter technique, have been employed to explore this active area of supramolecular kinetics.[2,3] Unfortunately, the decay of most *singlet-excited states* is too fast (often less than 10 ns) to compete with dissociation and association, which precludes information on the kinetics. This restriction is particularly severe for the slow bimolecular association processes,[2] since the typically low concentrations of supramolecular assemblies result in small observed rate constants. Hence, the exceptional advantages of fluorescence have been employed rarely for probing association kinetics with supramolecular systems, but mostly for probing local structural or ground-state thermodynamic properties.[4] The longer lived *triplet-excited states* have been more successfully applied to

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(4) Fluorescence quenching of pyrene and other polyaromatics has been extensively employed, however, for the *indirect* determination of micellar kinetics by using the method of external quenchers (cf. ref 2).

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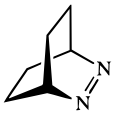
obtain dynamic information,[3,5−10] but the probing techniques, generally transient absorption, lack some of the above-mentioned advantages of fluorescence measurements, in particular sensitivity.[3] Moreover, while triplet states frequently allow the measurement of *dissociation* rate constants by a methodology which employs addition of external quenchers,[2,3] *association* rate constants have become accessible only in exceptional cases, e.g., for xanthone.[7,8]

Herein, we present a novel n,π* fluorescent probe, the azoalkane 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO), which is useful for obtaining both structural and dynamic information on inclusion phenomena. Excited DBO responds to the environment of supramolecular assemblies by interacting with abstractable hydrogens without significant product formation. Moreover, the very long fluorescence lifetime of DBO (up to 1 μs)[11,12] provides a large range for monitoring the dynamics of host−guest complexation, in particular for the assessment of association rate constants. Due to the potential use of DBO, not only in supramolecular chemistry, but also for the recently described biological applications (monitoring of antioxidant concentration or reactivity),[13] we have recently coined the name *fluorazophores* for fluorescent probes based on this azo fluorophore.[14]



2,3-diazabicyclo[2.2.2]oct-2-ene

(DBO)

In this exploratory study, cyclodextrins[5−8,15,16] have been studied as representative hosts in aqueous solution.[17] α-, β-, and γ-cyclodextrin (CD) are cyclic oligomers consisting of six, seven, or eight α-D-glucose units, which are well known to complex a number of suitably sized organic molecules in their cavities.[5−7,15,16]

Experimental Section

Materials. The azoalkane 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO) was synthesized according to the literature procedure.[18] It was purified by sublimation at reduced pressure and by subsequent 2-fold recrystallization from *n*-hexane. The commercial (Aldrich) α- and γ-CD were deuterated before use by three solvation−evaporation cycles with the 5-fold weight excess of D_2O. β-CD and L-ascorbic acid were used as received (Aldrich). D_2O (>99.9%, Glaser AG, Basel, Switzerland) was used as solvent for all measurements. All experiments were performed at ambient temperature (25 °C).

Spectroscopic Measurements. Samples were prepared by dissolving DBO (ca. 0.1−6 mM) and the appropriate amount of the CDs in D_2O. The samples were kept at ambient temperature under air (for NMR, UV, and steady-state fluorescence measurements) or degassed by two freeze−pump−thaw cycles (for time-resolved fluorescence measurements). Homemade quartz cells (4 × 1 × 1 cm^3) with high-vacuum Teflon stopcocks were used for degassing.

A XeF excimer laser pulse from a Lambda Physics COMPex 205 laser (351 nm, fwhm ca. 20 ns, pulse energy 40−175 mJ) or an LTB MNL 202 nitrogen laser (337 nm, fwhm ca. 400 ps, pulse energy ca. 0.2 mJ) was used for excitation to obtain the time-resolved fluorescence decays. The decays were monitored with a monochromator−photomultiplier setup at 420−500 nm, depending on signal intensity. The kinetic traces were registered by means of a transient digitizer and analyzed by nonlinear least-squares fitting of monoexponential or biexponential decay functions. Steady-state fluorescence spectra and quenching were measured with a Spex Fluorolog fluorimeter (λ_{exc} = 340−360 nm). UV spectra were obtained with a Hewlett-Packard 8452 diode array spectrophotometer (2 nm resolution) or with a Perkin-Elmer Lambda 19 spectrophotometer (0.1 nm resolution). The NMR spectra were obtained with a Varian Gemini 300 NMR spectrometer. GC−MS data were obtained with a 5890 Series II gas chromatograph (SE-52, 25-m column with 5% phenylmethylsilicone) coupled with a 5970A Series mass selective detector (Hewlett-Packard).

Quantum Yield Measurements. The quantum yield for chemical reaction of DBO (4.0 mM) in the presence of β-CD (16 mM, the solubility limit at 25 °C)[16] was determined in deaerated D_2O as solvent (ca. 5 mL) at the laser excitation wavelength of 351 nm. Solutions of DBO in deaerated D_2O [quantum yield of decomposition (Φ_r) ≈ 0.3%,[19] ϵ^{365}(max) = 48 ± 3 M^{-1} cm^{-1}, ϵ^{351} = 40 ± 2 M^{-1} cm^{-1}][13] served as references (actinometer). The decay of the absorbance (A) at the absorption maximum ($A_0 \approx 0.2$) was monitored as a function of incident laser pulses (≡ irradiation time) at constant pulse energy. Plots of $\log([10^{A_0} - 1]/[10^{A} - 1])$ versus irradiation time[20] were linear up to ca. 70% conversion of DBO. The ratio of the slopes of the logarithmic plots [after correction for the known values of Φ_r and the smaller (factor 0.86) ϵ^{351} of the complex][20] provided the desired quantum yield.

Product Studies. Solutions of DBO (10 mM) in deaerated D_2O in the absence and presence (16 mM) of β-CD were subjected to laser photolysis (351 nm), and the resulting opaque photolysates (>85% conversion) were extracted with *n*-pentane (2 × 0.5 mL per 5 mL). A pentane solution of DBO (30 mM) was photolyzed analogously. GC−MS analysis of the pentane solutions revealed only 1,5-hexadiene and bicyclo[2.2.0]hexane, the known photoproducts from unimolecular loss of nitrogen from DBO.[21] The product distributions of the photolysis are given in Table 1. The photoproducts were identified by comparison with the products formed in the photolysis of DBO in other organic solvents, which has been extensively examined.[21−23] The formation of the hydrazine derived from photoreduction of DBO can be excluded on the basis of the GC−MS data. Moreover, the D_2O layer indicated no readily apparent resonances which could be ascribed to a chemically

Table 1. Photoproduct Distributions of DBO upon Direct Photolysis[a]

medium	1,5-hexadiene (%)	bicyclo[2.2.0]hexane (%)
D_2O	70	30
D_2O + β-CD[b]	82	18
benzene[c]	58	42
n-pentane	51	49
n-octane[d]	51	49
NaY zeolite[e]	65	35

[a] Relative product ratios from GC−MS analysis. [b] Under conditions of predominant (>90%) complexation of DBO, i.e., 10 mM DBO and 16 mM β-CD. [c] From ref 21. [d] From ref 22. [e] From ref 23.

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modified cyclodextrin. The NMR data also confirmed the absence of hexadiene or bicyclohexane in the aqueous phase, confirming their efficient removal through extraction with *n*-pentane.

Computational Studies. The AMBER-S force field[24] included in the HyperChem software[25] was chosen for molecular modeling of the inclusion complex of DBO and β-CD, which was examined as a representative case. Since bicyclic molecules are not well parametrized in AMBER-S, the DBO geometry was kept frozen in the AM1-optimized geometry, which closely resembled the experimental gas-phase structure.[26] The geometries and dipole moments of the DBO ground and triplet states were also calculated at (U)B3LYP/6-31G* levels of density functional theory by using the Gaussian 94 package.[27]

Results

Straightforward evidence for the formation of DBO·CD complexes was obtained by mixing saturated DBO and β-CD solutions in D_2O. A white precipitate was formed, which was revealed as a 1:1 complex by NMR spectroscopy (no evidence for higher association modes was obtained in any experiment). Complexation was studied in detail by employing four independent spectroscopic techniques in D_2O as solvent: time-resolved and steady-state fluorescence, UV spectrophotometry, and ^{1}H NMR spectroscopy. The consistent choice of D_2O as solvent, not only for the NMR investigations where its use is mandatory, was motivated by the recent observation of solvent deuterium isotope effects (1.3−3.4) on the kinetics and thermodynamics for association of triplet xanthone with β-CD.[8] Moreover, the longer fluorescence lifetime of DBO in D_2O (730 ns deaerated and 505 ns aerated) versus that in H_2O (420 ns deaerated and 325 ns aerated) results in larger quenching effects.[11,13]

NMR Spectroscopy. NMR spectroscopy[28,29] in D_2O was preferred for the quantitative analysis of the ground-state inclusion equilibria. The observed ^{1}H NMR shifts were qualitatively the same for all three cyclodextrins but largest for β-CD. Both inner H-3 and H-5 CD protons shifted ca. 0.1 ppm upfield upon addition of DBO to CD solutions. The shift of H-5 can be taken as evidence for a "deep" penetration.[16] These ^{1}H NMR changes of the CDs are accompanied by shifts of the DBO protons, most prominent for those in the anti position[30] (downfield by 0.14 ppm). The latter were also employed to determine the ground-state binding constants (K) of the CD·DBO complexes. The established "NMR titration" method was employed,[28,29] making use of a nonlinear fitting procedure. Namely, eq 1a was employed, where the ^{1}H NMR shift of the particular protons ($\Delta\delta_{obsd}$) at a constant guest concentration $[DBO]_0$ was plotted against the total CD concentration, $[CD]_0$. The fitting provided values for the ground-state binding constant K (Table 2).

Table 2. Kinetic and Thermodynamic Data for the Complexation of DBO by Cyclodextrins (CDs)

	α-CD	β-CD	γ-CD
τ_{CD}/ns[a]	33 ± 7 [40]	95 [80]	[90]
$k_q/10^8$ M^{-1} s^{-1}[b]	1.9 [1.8]	4.0 [5.0]	0.78 [0.8]
K/M^{-1}[c]	50 ± 10	1100 ± 300[d]	6 ± 3

[a] Obtained from time-resolved or steady-state (in brackets) fluorescence; 10% error unless stated. [b] Obtained from time-resolved or steady-state (in brackets) fluorescence; 10% error. [c] Obtained from NMR spectroscopy. [d] Independent values from UV spectrophotometric titration (eq 1b) and analysis of the preexponential factors of the biexponential time-resolved decay traces (cf. text) are 900 ± 100 and 700 ± 300 M^{-1}.

$$\Delta\delta_{obsd} = \left(1 - \frac{[DBO]}{[DBO]_0}\right)(\delta_{CD\cdot DBO} - \delta_{DBO}) \qquad (1a)$$

$$\Delta A^{\lambda}_{obsd} = \left(1 - \frac{[DBO]}{[DBO]_0}\right)(A^{\lambda}_{CD\cdot DBO} - A^{\lambda}_{DBO}) \qquad (1b)$$

with

$$[DBO] = \{K[DBO]_0 - K[CD]_0 - 1 + \sqrt{(K[DBO]_0 + K[CD]_0 + 1)^2 - 4K^2[DBO]_0[CD]_0}\}/2K$$

The highest binding constant (ca. 1100 ± 300 M^{-1}) was measured for β-CD, while those for α-CD (ca. 50 ± 10 M^{-1}) and γ-CD (ca. 6 ± 3 M^{-1}) were more than 1 or 2 orders of magnitude lower. While the binding constants for many guest molecules, e.g., benzophenone (1500 versus 50 and 170 M^{-1}),[5] follow a similar up-and-down trend,[16] the dramatic drop for γ-CD, which falls even 1 order below α-CD, is noteworthy. Apparently, the cavity of α-CD (4.7−5.2 Å)[15] is somewhat too small to allow a comfortable fit of DBO (diameter ca. 5.0 Å, from calculated and experimental[26] geometries), while for γ-CD the complex-driving interactions are much reduced due to the larger cavity diameter (7.5−8.3 Å)[15] and the possible co-inclusion of water molecules. Only for β-CD (6.0−6.4 Å)[15] are both size and binding requirements met to ensure a medium-to-large binding constant with the ground state of DBO.

UV Spectrophotometry. Since the UV absorption maxima of azoalkanes undergo a distinct bathochromic shift with decreasing solvent polarity,[31] which is accompanied by a sharpening of the absorption band and a concomitant increase in the extinction coefficient, the inclusion of DBO into CDs (which have a less polar interior) could be followed by UV spectrophotometry. The spectral changes, i.e., the bathochromic shift and the increase in absorbance, were observed for all three CDs, and were most pronounced for β-CD (Figure 1). A spectral component analysis was performed by comparing the experimental spectrum under conditions of predominant (>90%) complexation with those obtained from linear combinations of the DBO spectra in water and methanol. A 2:1 methanol/water combination provided a perfect match (Figure 1), which suggests that the DBO probe in the inclusion complex senses an environment which resembles that of methanol in its polarity. For comparison, the polarity in the CD cavity has been evaluated several times.[16,32] Depending on the probe, the polarity was qualified as being similar to that of dioxane, alcohols, or aqueous alcohols (e.g., a 3:1 methanol/water mixture).[32] Hence, DBO yields a consistent result.

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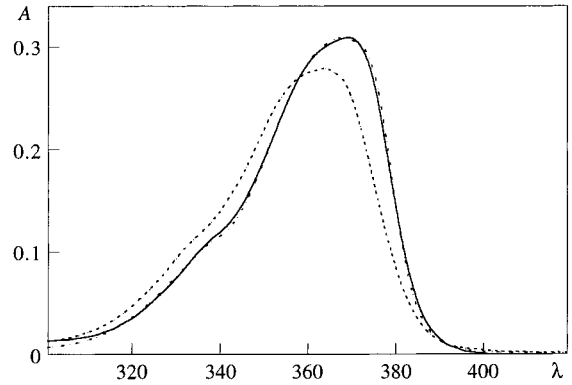

Figure 1. Bathochromic UV shift of DBO (6 mM) upon addition of β-CD (10 mM, dotted → solid line, nm scale); the dashed, hardly visible line refers to a spectral simulation obtained by adding the spectra in methanol and water in a 2:1 ratio.

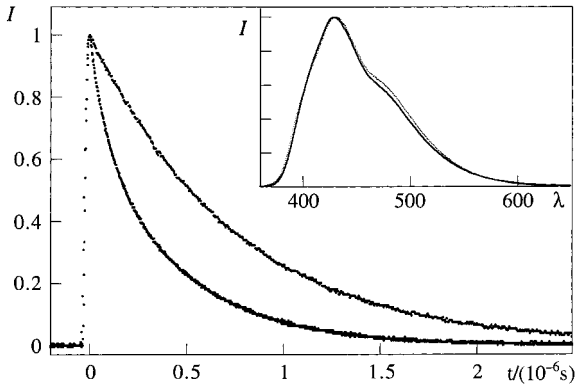

Figure 2. Change of the fluorescence decay behavior of DBO (2 mM, 351 nm excitation) from a slow, monoexponential kinetics (upper trace) to a faster, multiexponential one (lower trace) upon addition of β-CD (2 mM). Inset: Changes of the structure and shape of the fluorescence band of DBO (0.9 mM) upon addition of β-CD (12 mM, solid → dotted line); both spectra were normalized to unit intensity (linear scale) at the maximum, using a 6.5 times larger normalization factor for the spectrum in the presence of β-CD.

In the case of β-CD, the UV spectral shift was also employed to obtain an independent estimate of the ground-state binding constant with DBO. A nonlinear fitting procedure (eq 1b) analogous to that used for the NMR data was employed, using the changes in absorbance in the region of maximum variation (at 376 nm, cf. Figure 1) as characteristic property. The resulting binding constant from UV absorption (900 ± 100 M^{-1}) agreed reasonably well with the NMR value (1100 ± 300 M^{-1}).

Time-Resolved Fluorescence Spectroscopy. The time-resolved fluorescence experiments ($\lambda_{exc} = 351$ nm) revealed a monoexponential decay of singlet-excited DBO in degassed D_2O solution ($\tau_0 = 730$ ns), which became clearly multiexponential in the presence of α-CD (up to 46 mM) and β-CD (up to 10 mM, Figure 2). Two exponential decay functions were sufficient for fitting. One kinetic component was independent of the host concentration (ca. 33 ns for α-CD and 95 ns for β-CD), while the second decay function was first order in the *total* CD concentration, with bimolecular quenching rate constants (k_q) of 1.9×10^8 and 4.0×10^8 M^{-1} s^{-1} (Table 2, Figure 3b). When the concentration of *free* β-CD (calculated from the known ground-state binding constant) was used for plotting, one obtained kinetic plots which varied with the total concentration of DBO and which displayed pronounced curvature at high DBO concentration (Figure 3a). Quenching was also observed upon addition of γ-CD, but in this case the effect could be adequately accounted for by a single (dynamic) quenching component

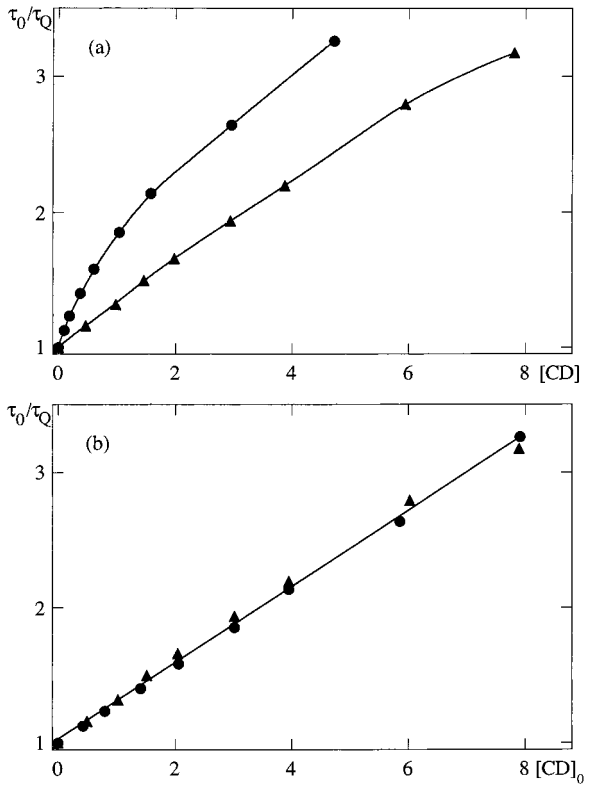

Figure 3. Plots of the dynamic component for fluorescence quenching (τ_0/τ_Q) at two different total concentrations of DBO, $[DBO]_0 = 0.101$ (▲) and 3.87 mM (●). (a) Plots versus [CD]/mM, the calculated concentration of uncomplexed β-CD (taking $K = 1000$ M^{-1}, Table 2), and (b) plots versus $[CD]_0$/mM, the total concentration of β-CD.

(monoexponential decay) with an apparent quenching rate constant of 7.8×10^7 M^{-1} s^{-1} (up to 60 mM). Note that the quenching rate constants of the CDs are 1−2 orders of magnitude larger than those for α-D-glucose in D_2O (1.3×10^6 M^{-1} s^{-1}). Evidently, the faster quenching by CDs is related to their specific oligocyclic geometry.[5]

Steady-State Fluorescence Spectroscopy. Steady-state fluorescence spectra with excitation at the isosbestic point ($\lambda_{exc} = 358$ nm, Figure 1) provided complementary information to the time-resolved experiments. The structure of the fluorescence band of DBO displayed little variation upon addition of CD, except for a somewhat earlier onset, perhaps a small shift of the emission maximum from 430 to 431 nm, and an apparently stronger bathochromic shoulder (cf. inlay in Figure 2). These changes are less characteristic than those of the absorption spectra, and further interpretations are difficult since the broad and structureless appearance of the DBO fluorescence is, itself, not well understood.[11,33,34] However, the Stern−Volmer quenching plots (I_0/I versus $[CD]_0$) in D_2O were nonlinear in all cases, and even when linear fitting was applied to the data points at low concentration, the apparent quenching rate constants differed significantly from those obtained in the time-resolved experiments (except for γ-CD). Second, for β-CD (but not significantly for the others), the overall appearance of the Stern−Volmer plot changed dramatically upon varying the concentration of DBO (Figure 4). These experimental findings are obviously due to the interference from complexation.

Effect of External Quenchers. Fluorescence quenching of the cyclodextrin complexes of DBO (ca. 0.5 mM) by external

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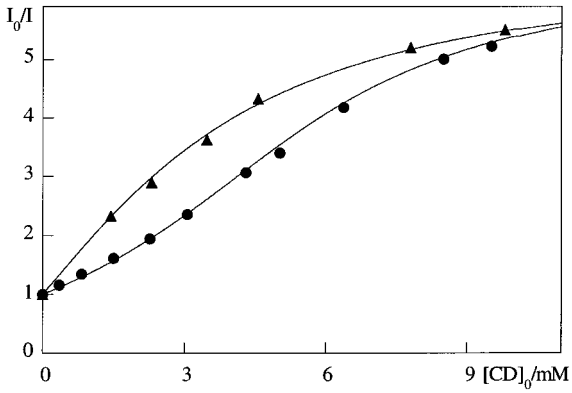


Figure 4. Dependence of the Stern−Volmer plot for steady-state fluorescence quenching by β-CD (I_0/I versus $[CD]_0$) at two different total concentrations of DBO, $[DBO]_0 = 0.136$ (▲) and 4.85 mM (●). The interpolated lines refer to the fits for model 1 and eq 3, cf. parameters in Table 2.

quenchers can be studied at high concentration of β-CD (10 mM), since under these conditions more than 90% of the DBO molecules are complexed due to its high binding constant. The effect of L-ascorbic acid, which is a potent quencher of DBO fluorescence due to its readily abstractable O−H bonds,[13] on the time-resolved fluorescence of DBO in D_2O in the presence of β-CD was measured at four different concentrations. The quenching rate constant was 1 order lower for the complexed DBO (0.22×10^9 M^{-1} s^{-1}) than for the free DBO (ca. 2×10^9 M^{-1} s^{-1}).[13]

Any decrease in the diffusion coefficient of the CD·DBO complex relative to that of free DBO should be effectively counterbalanced by the increase in the reaction diameter. In fact, the two terms cancel in a first approximation. Hence, the observed reduction of the quenching rate constant by 1 order of magnitude cannot only be related to a reduced diffusion rate constant. Rather, it should be due to the fact that collisions of ascorbic acid with the outside walls of the CDs cannot contribute to quenching of the included DBO. This provides evidence for a protection of the complexed singlet-excited DBO against external quenchers. A similar protection toward quenching by ascorbic acid has been documented for an organic nitroxide complexed with β-CD.[35]

Quantum Yields. The reaction quantum yield of DBO (4.0 mM) in the presence of β-CD (16 mM) was found to be virtually the same (ca. 0.3%) as that for DBO in D_2O, the actinometer system. Since more than 90% of DBO is complexed under these conditions, the measured quantum yield is taken as the quantum yield of chemical reaction in the complex. It becomes evident that the fluorescence quenching of DBO by the CDs leads nearly quantitatively (>99%) to deactivation to the ground state, not to the formation of photoproducts.

Product Studies. Product studies (NMR and GC−MS) for the photolysis of the β-CD·DBO complexes revealed only the known decomposition products of DBO, 1,5-hexadiene, and bicyclo[2.2.0]hexane. However, the ratio of the photoproducts displayed a significant deviation, namely increased 1,5-hexadiene formation (Table 1), which may be indicative of an environmental effect (photoreaction in the CD cavity). Such differences in photoreactivity in constrained media are well established.[36,37] Note also that the product distribution of DBO in zeolites as host structures has been previously analyzed (Table 1).[23] The very low quantum yield for chemical reaction of DBO with CD, the competitive unimolecular photochemistry of DBO, and the general difficulties of identification and detection of photooxidized CDs[38] rendered the search for *intermolecular* reaction products difficult.

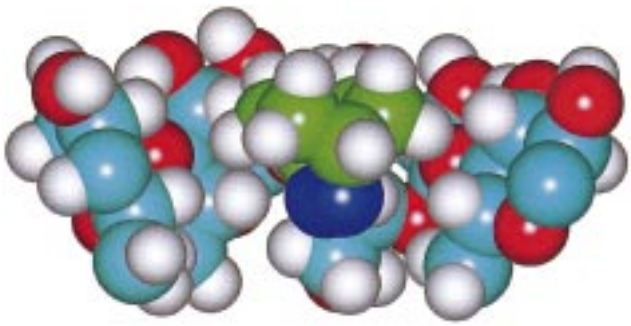


Figure 5. Intersection through the AMBER-S calculated structure of the β-CD·DBO inclusion complex.

Molecular Modeling. The formation of a 1:1 inclusion complex in the ground state is supported by the results from molecular modeling using the AMBER-S force field.[24,25] When the guest (DBO) was placed at a ca. 3 Å distance from the upper cavity opening (vacuum, no solvation), spontaneous formation of the CD·DBO inclusion complex with a deep penetration of the guest occurred (Figure 5). This formation of an inclusion complex is in agreement with the NMR data. An unsymmetrical conformation with a g^- orientation[39] ($O_5-C_5-C_6-O_6$ dihedral ca. −58°) of all primary hydroxymethyl groups was found to be the lowest energy conformation of β-CD in both the complexed and uncomplexed forms (Figure 5). This CD geometry is similar to the "normal" β-CD structure numbered 2 in the nomenclature by Lipkowitz.[39] The DBO molecule preferred a relative conformation with both azo nitrogens directed toward the tighter cavity opening (Figure 5).

Discussion

From the Introduction, it should be obvious that there is a significant interest in developing and applying longer-lived fluorescent probes,[3] and from this end we have chosen the azoalkane 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO) as a novel structural and dynamic fluorescent probe for host−guest complexation. Although DBO is the organic molecule with the longest fluorescence lifetime of purely organic compounds[11,12,40] and, hence, provides a much improved range for monitoring the relatively slow association processes by fluorescence, it has not been previously employed for such purposes.[41,42]

Conceptual Background. The application of any fluorescent probe, whether for structural or dynamic purposes, requires some

(35) Ebel, C.; Ingold, K. U.; Michon, J.; Rassat, A. *Nouv. J. Chim.* **1985**, *9*, 479−485.

(36) Scheffer, J. R.; Garcia-Garibay, M.; Nalamasu, O. In *Organic Photochemistry*; A. Padwa, Ed.; Marcel Dekker: New York, 1987; Vol. 8, pp 249−347.

(37) Weiss, R. G. In *Organic Photochemistry and Photobiology*; Horspool, W. M., Song, P.-S., Eds.; CRC Press: Boca Raton, FL, 1995; pp 471−483.

(38) Chow, Y. L.; Michon, J.; Michon, P.; Morat, C.; Rassat, A. *Tetrahedron Lett.* **1992**, *33*, 3315−3318.

(39) Lipkowitz, K. B. *J. Org. Chem.* **1991**, *56*, 6357−6367.

(40) Becker, H. G. O.; Böttcher, H.; Dietz, F.; Rehorek, D.; Roewer, G.; Schiller, K.; Timpe, H.-J. In *Einführung in die Photochemie*; Becker, H. G. O., Ed.; Deutscher Verlag der Wissenschaften: Berlin, 1991; pp 103−114.

(41) DBO has been employed twice in the context of supramolecular chemistry, but the previous studies have nothing in common with the present application of DBO as a structural and dynamic fluorescent probe. In one study, singlet energy transfer from dimethylnaphthalene was examined in micelles (Aikawa, M.; Yekta, A.; Liu, J.-M.; Turro, N. J. *Photochem. Photobiol.* **1980**, *32*, 297−303), and in the second study photoproduct distributions in zeolites were analyzed (ref 23).

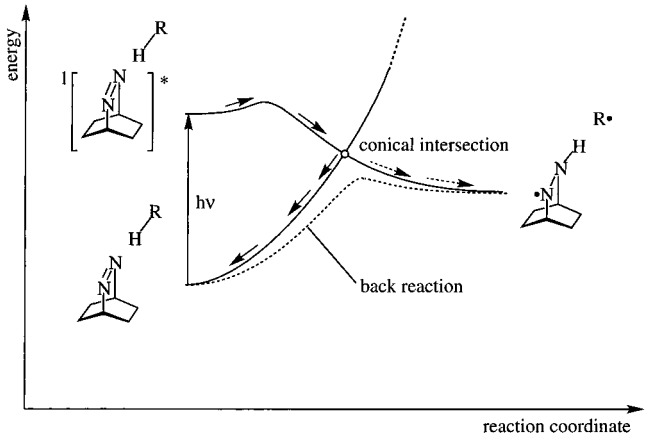


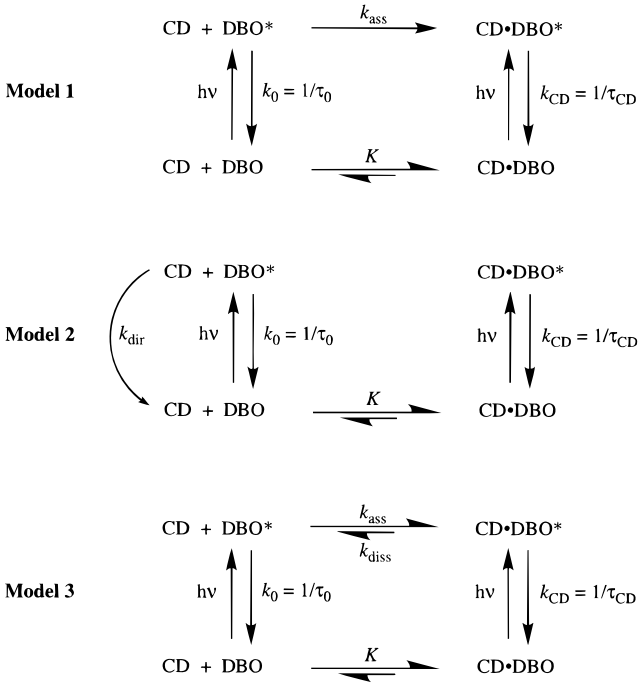
Figure 6. Reaction coordinate for hydrogen atom abstraction of n,π* singlet-excited DBO and possible deactivation through a conical intersection or back reaction of the radical pair.

kind of response (ideally readily and quantitatively predictable) of the fluorescent properties to environmental or molecular interactions. In other words, excited states whose fluorescence either is insensitive or responds unselectively to such interactions cannot be employed as fluorescent probes. In favor of the use of DBO as a potential fluorescent probe, we have recently discussed a distinct fluorescence quenching mechanism for azoalkanes.[11,43−46] This forms the basis for the present application.

While, in principle, the fluorescence quenching of DBO originates from hydrogen atom abstraction, i.e., a chemical reaction, the reaction efficiency is negligibly small due to the occurrence of a conical intersection ("aborted" hydrogen abstraction) and the spin-allowed back-reaction of the intermediate caged singlet radical pair (Figure 6).[11,43−46] A chemical selectivity on the environment (presence of abstractable hydrogens) results, which renders DBO also an interesting structural probe, in addition to its advantages for probing dynamic processes. To demonstrate, the fluorescence lifetimes of the azoalkane DBO in different solvents follow the order gas phase (930 ns), D_2O (730 ns), CH_3CN (690 ns), C_6F_{14} (605 ns), H_2O (420 ns), *n*-hexane (255 ns), 1,4-dioxane (155 ns), tetrahydrofuran (40 ns), and CH_3OH (22 ns).[11] Since the fluorescence quenching of DBO by solvents occurs along the reaction coordinate for hydrogen abstraction, the lifetimes do not simply reflect bulk properties of the solvent like polarity or acidity. Rather, they indicate the presence of particular bonds, since solvents with reactive C−H bonds (and some with O−H bonds) display short fluorescence lifetimes.

Conceptually, it must be noted that DBO is the first fluorescent probe with an n,π* electronic configuration,[47] while the established fluorescence-based detection methods rely on

Scheme 1



k_{ass}	association rate constant for excited state	k_{CD}	deactivation rate constant of excited-state complex
k_{diss}	dissociation rate constant of excited-state complex	K	equilibrium association constant in ground state
k_0	deactivation rate constant without CD	k_{dir}	rate constant for direct quenching

organic aromatic or organometallic molecules with either π,π* or CT electronic configurations.[1,48] Compared to the established fluorescent probes with π,π* configuration,[1,48] the n,π* fluorescent probe DBO displays a number of potentially useful and some uncommon features, which are contrasted in Table 3. They include a high fluorescence quantum yield; long fluorescence lifetime; chemical and thermal stability; sufficient solubility in all organic, aqueous, and fluorocarbon solvents; polarity-dependent long-wavelength absorption; UV transparency between 250 and 320 nm;[31] relatively small, spherical shape; and high photostability ($\Phi_r \approx 1\%$). These combine with the distinct fluorescence quenching mechanism.

Kinetic Models. The time-resolved fluorescence behavior of DBO upon addition of CDs (Figure 2), namely the observation of an interplay between a static (CD concentration independent) and dynamic (CD concentration dependent) quenching component, can be principally accounted for by three kinetic scenarios (Scheme 1). The "static" component (k_{CD}) corresponds to

(42) Although pyrene is one of the few molecules whose fluorescence lifetime (ca. 435 ns in hexane, ref 40) approaches that of DBO, its application for the *direct* measurement of association rate constants has several disadvantages. These include its low water solubility (ref 15), its competitive excimer emission at concentrations above ca. 10 μM (ref 40), and a significant drop of the fluorescence lifetime upon derivatization due to the elevation of symmetry constraints (Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley: London, 1970), e.g., to 120 ns in deaerated water for pyrene-1-butyrate (ref 15).

(43) Nau, W. M.; Adam, W.; Scaiano, J. C. *Chem. Phys. Lett.* **1996**, *253*, 92−96.

(44) Nau, W. M.; Greiner, G.; Wall, J.; Rau, H.; Olivucci, M.; Robb, M. A. *Angew. Chem., Int. Ed.* **1998**, *37*, 98−101.

(45) Nau, W. M.; Greiner, G.; Wall, J.; Rau, H.; Olivucci, M.; Robb, M. A. *Ber. Bunsen-Ges. Phys. Chem.* **1998**, *102*, 476−485.

(46) Nau, W. M. *Ber. Bunsen-Ges. Phys. Chem.* **1998**, *102*, 486-492.

(47) A short survey of the literature is in order to substantiate this point. First, although the fluorescence of n,π*-excited ketones is generally too short-lived and too weak to allow practical exploitation, the steady-state fluorescence of n,π*-excited xanthone has been employed in one case to determine binding constants with cyclodextrins (ref 5). However, the short fluorescence lifetime of xanthone (ca. 0.6−0.8 ns in water, ref 9) precludes dynamic information and, moreover, the solvent dependence is not well understood (Bohne, C., personal communication). Second, compounds with n,π* configuration have been previously employed as fluorescent probes (ref 1), but in the reported examples it is actually *not the n,π* state* which is fluorescent, but a close-lying π,π* state; the latter becomes the lowest excited state in polar environments ("state switching").

(48) Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*; Molecular Probes: Eugene, OR, 1996.

Table 3. Comparison of the Properties of the n,π^* Fluorescent Probe DBO with Typical Characteristics of Fluorescent Probes with π,π^* Configuration

	DBO (n,π^* configuration)	π,π^* configuration
quenching mechanism	aborted hydrogen atom abstraction	proton transfer or polarity-dependent photophysical processes
selectivity	specific bonds (C−H, O−H)	bulk acidity, bulk polarity
absorption	small ϵ (45 M^{-1} cm^{-1} in H_2O), polarity-dependent $\lambda_{max} \approx 370$ nm, UV "window" from 250 to 320 nm	large ϵ, not UV transparent below ca. 320 nm
fluorescence quantum yield	high (up to 50%)	high
fluorescence lifetime	very long (up to 1 μs)	<50 ns (except pyrenes)
oxygen quenching	$k_q = 2.1 \times 10^9$ M^{-1} s^{-1} (H_2O)	$k_q \approx 2 \times 10^{10}$ M^{-1} s^{-1}
solubility	also H_2O, fluorocarbons	substituent-dependent
shape, size	spherical, ca. 5 Å	flat, ca. 4−10 Å
photostability	high ($\Phi_r \approx 1\%$)	high

fluorescence from the CD·DBO complex, while the "dynamic" part (k_q) stems either from association of "free" excited DBO to the CD and subsequent quenching in the complex (k_{ass}, model 1) or from direct quenching (k_{dir}, model 2). In addition, the excited complex could undergo dissociation during its lifetime (k_{diss}, model 3).

While the two rate constants which are obtained from the biexponential decay traces cannot alone serve to differentiate between the association (model 1) or direct quenching pathway (model 2), it is important to note that the preexponential factors of the two decay functions have quite different meanings for the two kinetic models. For model 1, the combination of the kinetics for an irreversible consecutive reaction (DBO* + CD → CD·DBO* → CD·DBO) with that of a parallel reaction (CD·DBO* → CD·DBO)[49] yields eq 2 as an analytic expression of the normalized ($I_{t=0} = 1$) time-dependent fluorescence intensity, where the first and second exponents correspond to the static and dynamic components, respectively.

$$I_t = \left(\frac{[\text{CD·DBO}]}{[\text{DBO}]_0} - \frac{[\text{DBO}]}{[\text{DBO}]_0}C\right) e^{-(\tau_{CD})^{-1}t} + \frac{[\text{DBO}]}{[\text{DBO}]_0}(1 + C)\, e^{-(\tau_Q)^{-1}t} \quad (2)$$

with

$$C = \frac{k_{ass}\tau_0[\text{CD}]_0}{\tau_0/\tau_{CD} - k_{ass}\tau_0[\text{CD}]_0 - 1},$$

$$\tau_{CD}^{-1} = k_{CD} \qquad \text{and} \qquad \tau_Q^{-1} = k_0 + k_q[\text{CD}]_0$$

Hence, the first exponent (k_{CD}) is independent of the CD concentration and can be assigned to the lifetime of the excited complex, while the second, dynamic component is linearly related to the concentration of CD and provides a direct measure for the association rate constant, since k_q corresponds to k_{ass} for model 1.

In contrast, for model 2, the simpler kinetics of two independent parallel reactions (DBO* + CD → CD + DBO and CD·DBO* → CD·DBO)[49] applies, which causes C in eq 2 to vanish, since $k_{ass} = 0$. Moreover, k_q becomes synonymous with k_{dir}. Most importantly, according to model 2, the preexponential factors are a direct measure of the ground-state equilibrium concentrations [CD·DBO] and [DBO], while according to model 1, the apparent preexponential factors are smaller or larger than expected from ground-state complexation alone.[50]

Accurate preexponential factors are inherently more difficult to extract from biexponential time-resolved decay traces than rate constants, in particular when the conventional flash photolysis technique is employed, as in the present study. This is due to the necessary extrapolation to time zero of the laser pulse and the larger error range. Nevertheless, the analysis of our experimental data for β-CD (nine data points, from Figure 3) provided support for model 1. Namely, when [CD·DBO] and [DBO], i.e., the concentrations of complexed and uncomplexed DBO in the ground state, were calculated from the preexponential factors according to model 2, the resulting values for the binding constant K (230 ± 170 M^{-1}) were inconsistent with the NMR and UV data for K (ca. 1000 M^{-1}). In contrast, application of model 1 provided reasonable agreement (700 ± 300 M^{-1}).

Moreover, one must consider that the most probable mechanism for direct quenching of DBO would involve a direct hydrogen abstraction from the outside walls of the CDs. Hence, if model 2 applied, the dynamic quenching rate constant should show little dependence on the type of the CD and should not exceed the value for α-D-glucose by orders of magnitude.[3,5] These expectations, however, are not met, since k_q(β-CD) > k_q(α-CD) > k_q(γ-CD) ≫ k_q(α-D-glucose). Noteworthy, even for γ-CD, for which the driving force for complexation is much lower than those for the other two CDs (smallest K and k_q values), the observed k_q value is still 80 times larger than that for glucose, and even the consideration of an upper-limit statistical factor (eight glucose units) leaves 1 order of magnitude discrepancy. This provides a further, strong argument that quenching proceeds through association (model 1) and not directly (model 2). Note also that the absolute magnitude of the association rate constant of singlet-excited DBO with β-CD (4×10^8−5×10^8 M^{-1} s^{-1}, Table 2) is reasonable by comparison with the β-CD association rates previously determined for triplet-excited benzophenone,[5] xanthone,[7,8] and two naphthalene derivatives (all ca. 3×10^8−8×10^8 M^{-1} s^{-1}).[10]

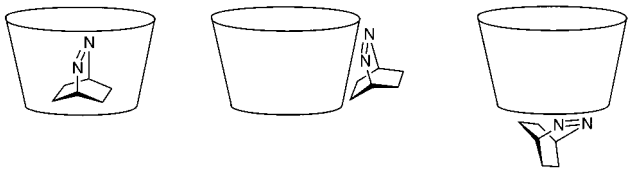
The consideration of dissociation of the excited-state complex (model 3) would further modify the preexponential factors in eq 2,[51] but due to the uncertainty in their determination with the present technique, this possibility could not be further evaluated. However, the satisfactory K values estimated according to model 1 and independent evidence from steady-state fluorescence data (see below) suggests that the excited state complex deactivates much faster than it dissociates; i.e., model 1 provides a satisfactory kinetic description. The observation

(49) Mauser, H. *Formale Kinetik*; Bertelsmann Universitätsverlag: Düsseldorf, Germany, 1974; p 88.

(50) Counterintuitively, although the association pathway for model 1 *increases* the concentration of CD·DBO* complexes, it *reduces* the apparent static preexponential factor associated with the decay of the CD·DBO* complexes.

(51) van Stam, J.; De Feyter, S.; Boens, N.; Schryver, F. C. D. *J. Chem. Soc., Chem. Commun.* **1995**, 2433−2434.

Scheme 2



of two distinct kinetic components certainly precludes the possibility that exit is much faster than deactivation, since in this case, a typical preequilibrium situation, only a single (dynamic) component, i.e., $k_q = k_{CD}(k_{ass}[CD]_0/k_{diss})$, would be expected. Note also that one component is independent, within error, of the CD concentration (static), which would not be expected when exit becomes significant.[51]

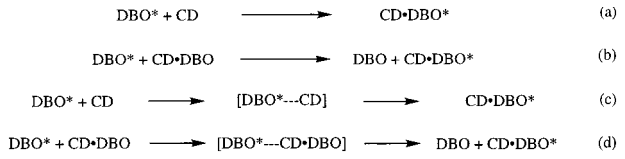
Mode of Association: Inside or Outside? The peculiar dependence of the quenching rate constant on the size of the CD (Table 2) and the discrepancy to the value for glucose provide compulsory evidence that the mode of excited-state complexation involves the inside of the CDs, i.e., their cavity, providing an example for an inclusion complex, not some sort of loose association with the outside wall or with the bottom hydroxyl rim (Scheme 2). Only for an association in the inside can one expect steric effects (e.g., cavity too small to allow deep penetration) to cooperate with the goodness of fit (e.g., cavity too large to allow ideal van der Waals interactions) and, thus, to result in the experimental up-and-down trend of the quenching rate constants. Moreover, quenching of the complexed DBO by an external additive (ascorbic acid) is slower than that for free DBO (cf. Results). This indicates a protection against quenching, which can be best rationalized by the formation of a geometrically shielded inclusion complex.

Since the formation of an inclusion complex in the *ground state* is rigorously proven by the specific chemically induced NMR shift (cf. Results), the characteristic shift of the UV absorption band (Figure 1), the size dependence of the binding constants (Table 2), and the medium effect on the photoproduct distribution (Table 1), and also suggested by the molecular modeling results (Figure 5), a different mode of complexation in the *excited state* appears highly unlikely. In fact, there is no precedence that the modes of association (inside versus outside) vary in the ground and excited states of a molecule, although the association constant may show some trend with excitation.[3,6,7] Note also that examples of competitive outside and inside association are extremely scarce[52] for ground-state complexes.

Quenching of Excited DBO by Ground-State CD·DBO Complexes. The interpretation of the dynamic component for fluorescence quenching deserves more detailed attention. Unlike several other excited-state probes for probing complexation dynamics, e.g., benzophenones[3,5] and xanthone,[3,6−9] DBO shows an excellent water solubility, which allows the examination of host−guest interactions at high guest concentrations (millimolar range). While this is, itself, advantageous, since it allows (i) the application of otherwise inaccessible spectroscopic techniques such as NMR titrations, and experimentally desirable, since (ii) the low extinction coefficient requires a relatively high minimum working concentration of ca. 0.1 mM to obtain sufficiently strong fluorescence, it also provides the possibility to examine (iii) concentration effects on the complexation dynamics. For example, at ca. 1−6 mM DBO concentration, a

(52) Gadre, A.; Rüdiger, V.; Schneider, H. J.; Connors, K. A. *J. Pharm. Sci.* **1997**, *86*, 236−243.

Scheme 3



significant or even the major amount of β-CD (solubility only ca. 16 mM at 25 °C)[16] may be present as a β-CD·DBO complex due to the high binding constant of β-CD (ca. 1100 M^{-1}). This means that the *total* concentration of β-CD differs significantly from the concentration of *uncomplexed* β-CD at high DBO concentration.[53]

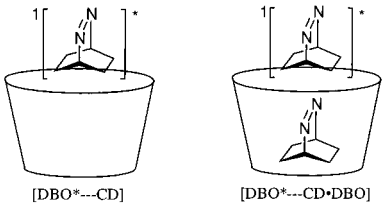
According to model 1, only the concentration of "vacant" cyclodextrin, [CD], should contribute to the dynamic fluorescence quenching. However, when the dynamic component (τ_0/τ_Q) is plotted against [CD], which can be calculated from the known binding constants (Table 2), inconsistent results are obtained (Figure 3a). On one hand, one observes a strong curvature of the kinetic plot for β-CD at high DBO concentration (4 mM), which is absent for the other CDs or less pronounced for β-CD at low DBO concentration (0.1 mM). On the other hand, the initial slope at high DBO concentration is much steeper than that at low concentration. Such a behavior, where the quenching rate constant would vary with both the CD and DBO concentrations, makes little sense from a kinetic point of view and contrasts models 1−3. Strikingly, when the *total* CD concentration is employed, $[CD]_0 = [CD] + [CD{\cdot}DBO]$, the two lines "merge", and linear plots with very similar slopes are obtained in both cases (Figure 3b). This is a novel finding. Obviously, the CD·DBO ground-state complex also contributes to dynamic fluorescence quenching.

A tempting explanation for the participation of CD·DBO complexes in the fluorescence quenching would involve direct quenching by the outside CD walls (model 2), since this should be independent of whether the inside cavity of the CD is occupied or vacant. However, we have rigorously excluded this possibility by a number of other experimental findings, e.g., the quenching rate constants, which are 2 orders of magnitude higher for β-CD than for glucose. Alternatively, the encounter of free singlet-excited DBO by the CD·DBO complex could somehow lead to the formation of the excited complex CD·DBO*. As a tentative explanation for such an unprecedented process, we suggest that, besides entry into the uncomplexed CD (process a in Scheme 3), the formal energy transfer from an excited DBO molecule to the CD·DBO ground-state complex can take place (process b). In view of the bathochromic shift in the absorption maxima from 365 to 371 nm upon complexation (Figure 1), this process should, indeed, be energetically favored by ca. 1.3 kcal mol^{-1}. Finally, the formation of 2:2 or 2:1 complexes could interfere at high concentrations of DBO,[10] although no indications for higher complexation modes were obtained in any experiment. Unfortunately, DBO does not show excimer emission, which is frequently taken as evidence for double (2:1 or 2:2) occupation of aromatic guest molecules.[10,15]

Of course, it would be rather surprising if the two rate constants, i.e., the entrance of DBO into a vacant cavity (reaction

(53) This contrasts the situation for other excited-state probes, whose concentrations are usually much smaller than the employed CD concentration, e.g., only ca. 10 μM for xanthone (refs 6−9). Hence, $[CD] \approx [CD_0]$ holds in a very good approximation. Note that low guest concentrations are often adjusted on purpose to allow simplified (since linearized) quantitative treatments, e.g., the so-called double-reciprocal Benesi−Hildebrand plots (Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703−2710).

Scheme 4



a) and the singlet energy transfer from DBO to CD•DBO (reaction b), were exactly the same, as implied by the successful use of the total CD concentration as parameter in Figure 3b. However, possible differences could lie within the error range. Alternatively, the formation of a common precursor complex, e.g., with binding to the upper hydroxyl rim (Scheme 4), could be rate-determining for both processes, thus resulting in similar rate constants for occupation (process c) and energy transfer (process d). The possible formation of such precursor complexes with CDs, including examples of rate-determining formation of a precursor complex, is well-established.[16] Although the actual reasons for the observed nonlinear effects at high DBO concentrations are not yet fully understood, it is important to emphasize that other conclusions from the present work remain unaffected by this experimental aspect.

Steady-State Fluorescence. As a particular advantage of fluorescent versus triplet probes, steady-state measurements can be readily performed to complement the time-resolved data. The steady-state fluorescence quenching of DBO by CDs yielded curved Stern−Volmer plots, most pronounced for β-CD (Figure 4). The sigmoidal appearance of the Stern−Volmer plot for β-CD at relatively high DBO concentration (lower curve in Figure 4) can be qualitatively understood. On one hand, the consideration of a ground-state complexation equilibrium with fast quenching of the excited complex should result in an *upward* curvature of a Stern−Volmer plot,[40] which is observed at low CD concentrations. On the other hand, since the complex displays a shortened but significant lifetime, a *downward* curvature toward a limiting value of τ_0/τ_{CD} is expected and observed at high concentration, where DBO is nearly quantitatively complexed. Steady-state analysis of the models in Scheme 1 or integration of eq 2 yields eq 3 as a general analytic expression of the relative fluorescence intensities.[54]

$$\frac{I}{I_0} = \frac{[\mathrm{DBO}]}{[\mathrm{DBO}]_0}\frac{\tau_Q}{\tau_0} + \frac{[\mathrm{DBO}]}{[\mathrm{DBO}]_0} QR\frac{\tau_{CD}}{\tau_0} + \left(1 - \frac{[\mathrm{DBO}]}{[\mathrm{DBO}]_0}\right)R\frac{\tau_{CD}}{\tau_0} \quad (3)$$

with

$$Q = \frac{k_{ass}\tau_0[\mathrm{CD}]_0}{1 + k_{ass}\tau_0[\mathrm{CD}]_0}, \quad R = \frac{1 + k_{ass}\tau_0[\mathrm{CD}]_0 + k_{diss}\tau_0}{1 + k_{ass}\tau_0[\mathrm{CD}]_0 + k_{diss}\tau_{CD}}$$

and $$\tau_Q = \frac{1}{k_0 + k_q[\mathrm{CD}]_0}$$

Equation 3 includes the assumptions of identical extinction coefficients and natural emission quantum yields of the free and the complexed DBO. These are ensured by excitation at the isosbestic point (Figure 1) or reasonably justified in view of the relative constancy (±30%) of the natural fluorescence lifetimes[11] and the minor fluorescence spectral changes (cf. inset in Figure 2).

To extract quantitative information from the experimental quenching plots, nonlinear least-squares fitting was performed according to the logarithmic form of eq 3, i.e., $\log(I/I_0)$, since we assume that the relative errors of the fluorescence intensity measurements are constant.[55] τ_0, the lifetime in the absence of CDs, could be measured with high accuracy by time-resolved fluorescence, and the ground-state binding constant K can be independently assessed by NMR and UV titrations (cf. Results). Hence, these two parameters were kept fixed. The analysis was first performed according to model 1, where $R = 1$ and $k_q = k_{ass}$. As can be seen from Table 2 (values in brackets), the independent steady-state estimates for k_{ass} and τ_{CD} according to model 1 are in excellent agreement with those from the time-resolved fluorescence data. This means that the steady-state fluorescence of DBO is similarly suitable, while more readily accessible, as time-resolved fluorescence to monitor supramolecular association processes. The mutual consistency of the independent experimental results provides further, strong support for the kinetic model 1.

Exit from the Excited-State Complex. Exit from an excited-state complex within its lifetime is an important process for CD−ketone complexes.[3,6,7] Long lifetimes of the excited complex and small binding constant may facilitate exit, suggesting that this process is most likely for the γ-CD•DBO complex. When the exit from the excited complex is considered as an additional pathway (model 3), eq 3 applies with the condition that $k_q = k_{ass}$. The dissociation process increases fluorescence ($R > 1$ due to $\tau_0 > \tau_{CD}$), since some excited molecules leave the complex before they are quenched.

Nonlinear fitting of the steady-state quenching data according to model 3, taking the values for K and τ_{CD} from Table 2 and leaving the association and dissociation (entry and exit) rate constants as parameters, provided entry rate constants (k_{entry}) insignificantly different from the experimental quenching rate constants k_q. In other words, exit from the excited complex (k_{exit}) is not required to account for the experimental data, and the measured quenching rate constants (dynamic components in the time-resolved experiments) should correspond directly to the entry rate. Apparently, exit from the excited complex is prevented because the probe is deactivated in a reaction that is faster than exit.

The more than 2 orders of magnitude larger entry rate constant (from fitting) implies similar or larger association constants in the excited state relative to the ground state. This meets expectation, since the dipole moment of the DBO ground state (3.5 D with the B3LYP-6-31G* method, the same as found experimentally)[56] *decreases* slightly upon excitation (3.0 D for the triplet state, UB3LYP-6-31G* method). In contrast, the variation of the dipole moment upon excitation of several ketones is much larger (differences up to 3.5 D), and this was

(54) Equation 3 can be intuitively understood: that fraction of free DBO which is uncomplexed in the ground state ($[\mathrm{DBO}]/[\mathrm{DBO}_0]$) *and* which is not quenched during the excited-state lifetime (τ_Q/τ_0) fluoresces with a relative efficiency of unity (first term). That fraction of DBO which is uncomplexed in the ground state ($[\mathrm{DBO}]/[\mathrm{DBO}_0]$) *but* which undergoes complexation during the excited-state lifetime (Q) fluoresces with a reduced relative efficiency characteristic for the lower (τ_{CD}/τ_0) complex fluorescence (second term). Finally, the remaining fraction of DBO, which *is complexed* in the ground state ($1-[\mathrm{DBO}]/[\mathrm{DBO}_0]$), fluoresces also with the reduced relative efficiency characteristic for the lower (τ_{CD}/τ_0) complex fluorescence (third term). The contributions due to the fluorescence from excited-state complexes (last two terms) need to be further multiplied by the efficiency factor R when dissociation of the excited-complexes becomes important (model 3); otherwise, R is unity.

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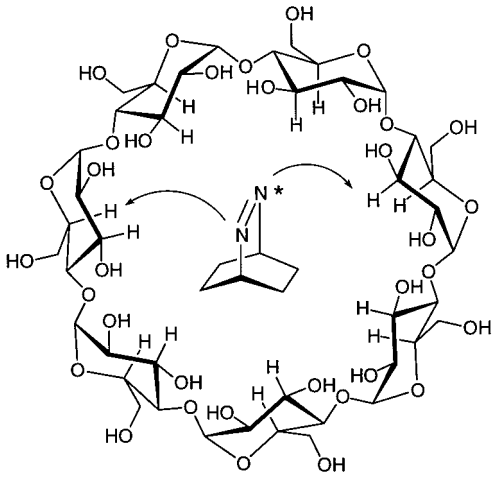
Scheme 5



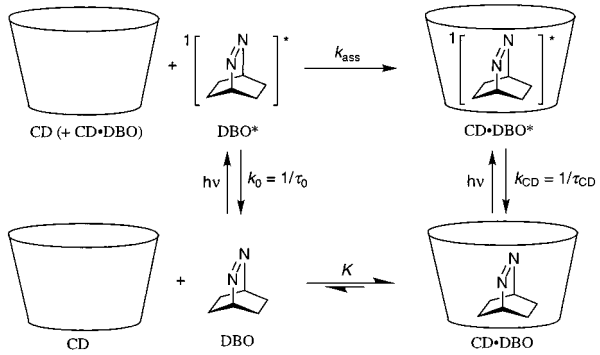
held responsible for the significant differences between ground-state and excited-state binding constants with CDs,[3,6,7] e.g., a factor of 20−100 decrease is observed upon excitation of xanthone.[6−8]

Quenching Mechanism and Structural Information. The application of DBO as a dynamic fluorescent probe is made possible by the high affinity for complexation combined with the fact that the lifetime in the particular supramolecular environment (inside of CDs) is short compared to the lifetime in solution (in the present case, D_2O). To understand the shorter lifetime of the excited-state complex, and to exploit this characteristic of the fluorescent probe for other supramolecular host systems, one must consider the nature of the quenching by the CDs. From a structural point of view, the fluorescence quenching of DBO by CD (characterized by the value of τ_{CD}) signals the presence of abstractable C−H bonds in the CD interior.

Presumably, since fluorescence quenching of DBO occurs along the reaction coordinate for hydrogen abstraction (Figure 6),[11,44,45] the included DBO molecules undergo quenching by interacting with the H-3 or H-5 CD hydrogens, cf. Scheme 5.[57] These point toward the inside of the cavity and resemble the α C−H bonds of cyclic ethers or alcohols. In fact, it has been previously established that n,π*-excited *triplet* states undergo hydrogen abstraction inside the CD cavity.[3,5,6] In the reaction of benzophenones with CDs in solution, ketyl radicals can be detected by transient absorption spectroscopy,[3,5] and in the reaction between *p*-nitroacetophenone and β-CD in water,[38] oxidized CDs were isolated as photoproducts, with an indication for H-3 abstraction.[15] The reactivity of the cyclodextrin C−H bonds toward abstraction by electrophilic radicals and n,π*-excited states (which are known to display a radical-like reactivity)[13,46,58,59] is akin to the reactivity of other saccharides, e.g., desoxyribose.[60]

In contrast to the triplet-excited ketones, which are known to undergo such hydrogen abstraction (photoreduction) reactions

Scheme 6



with high efficiency,[46,61] the quantum yields for product formation are very low for singlet-excited DBO due to the deactivation pathways in Figure 6. This holds for many solvents, which may also serve as quenchers of DBO fluorescence,[11,44] and also for the photoreaction of DBO included in β-CD. In fact, the quantum yield for photoinduced decomposition of DBO under conditions of nearly quantitative complexation by β-CD (>90%) amounts to only ca. 0.3%. The very low quantum yield supports the proposed quenching mechanism and provides the essential feature of photostability for the practical use as a fluorescent probe. To illustrate, on average, each DBO molecule can go through more than 100 absorption−fluorescence−probing cycles before it decomposes.

The low quantum yield is also held responsible for the failure to detect new intermolecular reaction products between β-CD and DBO, since unimolecular decomposition of DBO competes. Interestingly, the selectivity of the intramolecular photoreactions of DBO *increases* in the CD complex, since the product ratio 1,5-hexadiene:bicyclo[2.2.0]hexane increases from 70:30 to 82:18 in the presence of β-CD (Table 1). The reduced bicyclohexane formation in cyclodextrins is unexpected in terms of polarity arguments, since unpolar solvents (benzene, pentane, octane) tend to give *lower* ratios (Table 1). We tentatively attribute this variation to the constraints of the cyclodextrin cavity.

The proposed quenching mechanism (aborted hydrogen abstraction by DBO, Figure 6) is supported by the fluorescence lifetimes of the inclusion complexes (30−95 ns), which lie between those of alcohols such as methanol and cyclic ethers such as 1,4-dioxane and tetrahydrofuran (ca. 20−160 ns).[11] This rough correspondence is expected from the similarity of the reactive C−H bonds. Also, the trend in the fluorescence lifetimes of the inclusion complexes (τ_{CD}), i.e., α-CD (ca. 35 ns) < β-CD (ca. 80−95 ns) ≤ γ-CD (ca. 90 ns) can be rationalized in this train of thought since the distance to the reactive C−H bonds (and co-inclusion of water molecules) increases with the cavity diameter and, thus, should reduce the quenching rate. Alternatively, different modes of inclusion may be responsible.

Summary of the Results for Cyclodextrins. The combined experimental evidence in favor of model 1 rather than model 2 and in support of inside complexation (inclusion) rather than outside association by CDs in both the ground and excited states of DBO leads us to advance Scheme 6 as an appropriate kinetic and structural description. CD is depicted as a truncated cone. The quantitative data are contained in Table 2, where k_q corresponds to k_{ass} in Scheme 6. Alternative pathways for dynamic quenching, e.g., by direct hydrogen abstraction from the outside CD walls, or additional reaction pathways, e.g., exit

(57) The hydroxyl hydrogens of cyclodextrins could also serve as quenchers of DBO fluorescence in the excited complex, akin to the hydroxyl groups of alcohols, cf. refs 11 and 44. While the participation of the cyclodextrin hydroxyl groups cannot be excluded, we note that they are deuterated under the experimental conditions (D_2O solvent) and, thus, should display a significantly reduced reactivity. Moreover, the hydroxyl groups are located at the rim of the cavity, while DBO is deeply included in the cavity, as suggested by the NMR data.

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from the excited-state complex, might well occur but appear to be insignificant within the experimental error and, hence, are not required for a mechanistic interpretation.

Conclusions

(1) The use of DBO as a fluorescent probe to monitor supramolecular association phenomena presents a novel photochemical application, made possible by the recently reported quenching mechanism (aborted hydrogen abstraction).[11,43−46] The n,π* electronic configuration differentiates DBO from the established, mostly aromatic fluorescent probes with π,π* or CT electronic configuration.[1,48]

(2) The exceedingly long fluorescence lifetime (up to 1 μs, the longest among purely organic compounds) renders DBO a promising *dynamic* fluorescent probe, i.e., one which allows one to obtain direct information on supramolecular kinetics. DBO has been shown to be the first fluorescent guest molecule to date for which the complexation dynamics with cyclodextrins can be followed directly with a spectroscopic method, and it is the second dynamic probe to allow this, besides xanthone.[9]

(3) DBO appears to be particularly suitable for the determination of *association* rate constants, which are quite difficult to measure directly with alternative techniques.[2] The probe may thus provide complementary information to triplet probes, which are particularly suitable for assessing *dissociation* rate constants.[2] When comparing the fluorescent probe DBO with the alternative triplet probes, the higher sensitivity of fluorescence and the possibility to perform steady-state experiments deserve notice.

(4) The use of DBO as a dynamic probe requires different fluorescence lifetimes in the associated and unbound states. In general, the presence of abstractable hydrogen atoms, e.g., in ethers, amines, and sulfides, results in a shortening of the fluorescence lifetimes (fluorescence quenching), which can be used for signaling. The lifetime of the complexed fluorescent probe may thus provide *structural* information on the chemical environment in the supramolecular assembly. This *chemical selectivity* toward specific C−H or O−H bonds differentiates DBO from many π,π* fluorescent probes (e.g., aminonaphthalenesulfonic acid and pyrene derivatives, or β-naphthol),[1,15,48] which respond to the polarity or acidity of the environment. This could be exploited, for example, to monitor the migration between environments even if the polarity remains very similar, e.g., from a protiated to a deuterated, or from a hydrocarbon to a fluorocarbon environment.

(5) Further advantages include the excellent solubility in water and organic solvents and the small, spherical shape, which contrasts the shape of the established aromatic probes. Disadvantages include the low extinction coefficient due to the n,π* electronic configuration and the marginal shift in the fluorescence spectra. We contend that the UV window from 250 to 320 nm may be useful for specialized purposes, e.g., a second probe with absorption in this region could be selectively sounded out.

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