Orientational Dynamics of β-Cyclodextrin Inclusion Complexes

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The structure and dynamics of organic dye molecules included in β-cyclodextrin are studied using spectroscopic measurements and theoretical models. The effect of the charge and the size of the guest molecule on the properties of the host−guest complex is probed by comparing complexes formed with three different chromophores: resorufin (anion), oxazine-118 (cation), and oxazine-725 (cation) in aqueous solutions of cyclodextrin. The binding is characterized using absorption and calorimetry titrations. The structure of the complexes is analyzed by molecular modeling using empirical force field and semiempirical quantum theory calculations. Time-resolved polarization spectroscopy is used to investigate the rotational dynamics of different chromophores bound to β-cyclodextrin. Internal motion of the guest and overall rotational tumbling of the complex are observed for resorufin and oxazine-118. Modeling the internal motion of the chromophore as diffusion in a cone provides the mean square diffusion angle inside the cavity. It is found that the relative host−guest size determines the character of intermolecular host−guest dynamics.

Introduction

The fast development of supramolecular chemistry over the past decade has resulted, in part, from the progress in understanding host−guest interactions.[1] The nature and structure of host−guest complexation is of fundamental interest in the field of molecular recognition and is of increasing importance to the design of host−guest systems for applications. For example, the nature of the binding forces, the structure and the geometry of the intermolecular complexes, and the intermolecular dynamics are important in the design and function of drugs and drug delivery systems in the pharmaceutical industry. Although structural features in such complexes have been widely studied, the dynamical behavior of the host−guest complexes has not been well explored. The goal of the present study is to combine static and time-resolved spectroscopic techniques to obtain information about the structural and dynamical features of the intermolecular complexes.

Cyclodextrin inclusion complexes are very attractive models for the investigation of host−guest interactions.[2,3] Cyclodextrins (CD) are cyclic oligomers of 1,4-linked-glucose monomers which form truncated cone-shaped compounds. A wide variety of organic molecules can be complexed in their hydrophobic interior. Cyclodextrins are used in different fields of chemistry ranging from analytical to synthetic chemistry. An advantage of these systems is that their properties (relative host−guest size, charge distribution, solvent polarity) can be systematically tuned to investigate their effect on the structure and dynamics of the complex.

The present study explores the effect of the charge and the size of the guest molecule on the complex's behavior. The influence of the charge is investigated by comparing the properties of the complex formed between resorufin (an anion) and β-cyclodextrin to that formed between the oxazine-118 (cation) and β-cyclodextrin. The resorufin anion and oxazine-118 cation have similar size and shape (see Figure 1). The influence of the size was studied by comparing the structural and dynamical properties of the complex formed between β-cyclodextrin and oxazine-118 with that formed between β-cyclodextrin and the larger in size oxazine-725.

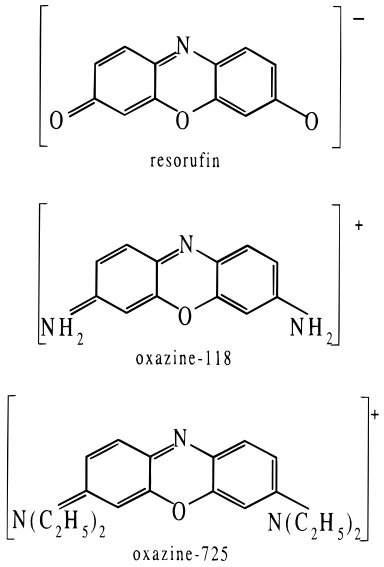

Figure 1. Molecular structures of resorufin, oxazine-118, and oxazine-725.

The inclusion geometry for a given molecule depends on the guest properties, e.g., a preference for one specific functional group or molecular fragment. It has been shown that the electronic absorption properties of chromophore−cyclodextrin complexes are very sensitive to the relative host−guest size and geometry of the complexes. The spectroscopic and photophysical behavior of these complexes was investigated to obtain information about the binding mechanism and the structure of the complex. The geometry of the host−guest complex is investigated by molecular modeling using semiempirical HF−SCF calculations to find the most favorable structure of the complex.[2−4]

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The orientational dynamics of the intermolecular complexes is probed by time-resolved polarization spectroscopy.[5−8] Time-resolved polarization spectroscopy provides direct information about the rotational dynamics of solute molecules in solution and therefore the solute−solvent interactions. The flexibility of the bound guest in the binding site is followed directly in time by measuring the second-rank orientational correlation function of the guest molecule's transition dipole moment. Changes in the probe molecule's environment changes its solvation energy, the diffusion coefficient, and the rotational relaxation time of the chromophore. A comparison of the chromophore's relaxation in solution to that bound in the cyclodextrin is used to elucidate these differences.[8d]

The outline of the paper is as follows. In the next section the experimental methods are described. Then the experimental results of the different measurements are presented. The analysis and molecular modeling of structure and dynamics of the host−guest complexes are provided in the Discussion section.

Methods and Materials

The time-resolved optical heterodyned polarization spectroscopy technique (OHPS) was used to measure the rotational relaxation time of the chromophore in water and in the presence of the cyclodextrin. The optical heterodyned polarization spectroscopy technique is a modification of classic polarization pump probe technique.[5,6] The principles of the method have been described earlier.[6−8] Briefly, a linearly polarized pump beam excites molecules of a particular orientation and creates an orientational anisotropy in the solution. This anisotropy results in a dichroism and a birefringence in the sample. Relaxation of the anisotropy as a function of time is detected by a polarized probe beam which senses the sample dichroism and birefringence. The sensitivity and selectivity of polarization spectroscopy are significantly improved in optical heterodyned polarization spectroscopy.[8] The heterodyned signal is generated by rotating the analyzer polarizer's transmission axis a small angle δ from the null position. This procedure causes a large increase in the signal level (proportional to δ) for the dichroic signal but does not enhance the birefringent signal. By measuring decay curves at $+\delta$ and $-\delta$ and subtracting them, the contribution of the nonheterodyned signal can be eliminated.

The measured relaxation represents the superposition of two processes: the anisotropy decay which occurs by rotational relaxation (τ_{or}) and the population decay of the excited state (τ_f), such that

$$\frac{1}{\tau_m} = \frac{1}{\tau_{or}} + \frac{1}{\tau_f} \qquad (1)$$

By measuring τ_m and τ_f independently, eq 1 can be solved to determine τ_{or}.

The fluorescence lifetime τ_f was measured in a separate experiment using the time-correlated single-photon counting method.[9] The spectra were collected at an emission wavelength of 615 nm and an excitation wavelength of 590 nm.

The time-resolved polarization spectrometer has been described previously.[8] The instrument consists of a picosecond laser system (CW mode-locked Nd:YAG laser (Spectra Physics series 3000) and a home-built dye laser, operated at $\lambda_{max} = 590$ nm), a Michelson interferometer for performing pump/probe experiments, and a data acquisition system.

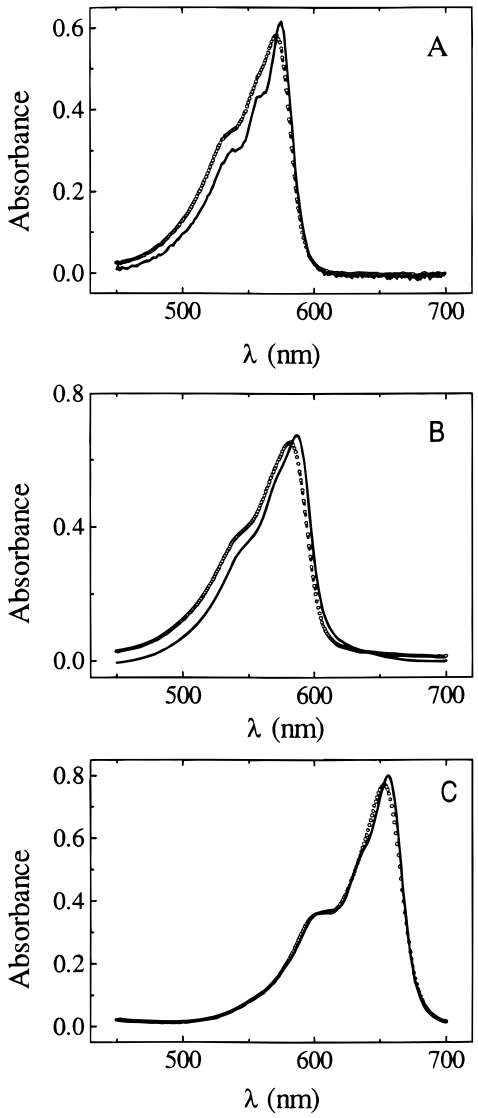

Figure 2. Absorption spectra for resorufin (A), oxazine-118 (B), and oxazine-725 (C) in water (dotted line) and aqueous solutions of β-cyclodextrin (solid line).

The steady-state absorption spectra of the chromophores in water and in the presence of cyclodextrin were measured on a Perkin-Elmer 559A UV−vis spectrophotometer.

The NMR spectra of the samples were recorded using a 300 MHz NMR Bruker spectrometer. The data were collected for the free guest and host in water as well as for the solutions with host/guest ratios of 1:1 and 100:1.

Resorufin (sodium salt) and oxazine-725 (perchlorate salt) were obtained from Aldrich and Exciton. The β-cyclodextrin was purchased from TCI. Samples were used as received in solutions of deionized water. Oxazine-118 was prepared in the manner described previously.[10]

Results

Measurement of the Binding Constant of the Host−Guest Complexes. The binding constant of the solute molecule with β-cyclodextrin was measured by two methods: electronic absorption spectroscopy and isothermal titration calorimetry.

Figure 2 presents the absorption spectra of resorufin (A), oxazine-118 (B), and oxazine-725 (C) in water and in aqueous solutions of β-cyclodextrin. The absorption spectra of the

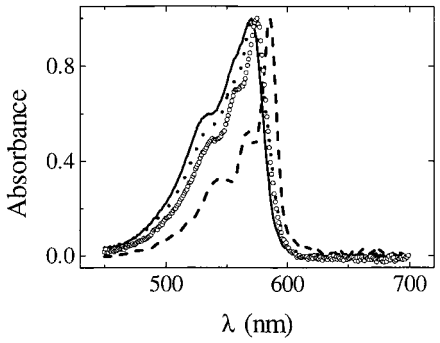

Figure 3. Normalized absorption spectra of the resorufin in different solvents: solid line, water; dotted line, methanol; open circles, aqueous solution of β-CD; dashed line, acetonitrile.

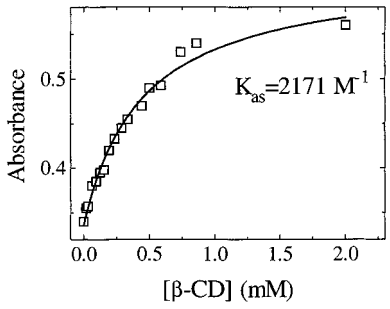

Figure 4. Absorption titration curve for resorufin with β-cyclodextrin. The solid line is a best fit to a 1:1 binding model.

chromophore in pure water has the main peak at 590 nm for resorufin, 580 nm for oxazine-118, and 640 nm for oxazine-725. Addition of β-cyclodextrin to the solution causes a slight red shift (4−5 nm) in this main feature of the spectrum. In addition, the spectrum of the resorufin with cyclodextrin has more clearly defined substructure as compared to the spectrum of resorufin in water. The small red shift found for each chromophore in the presence of β-cyclodextrin is commonly observed,[12,13,16,19,21] and it identifies the binding of the solute to the chromophore. The change in both the intensity and the position of the peak in each case demonstrates that all three chromophores bind to the β-cyclodextrin under the conditions of the experiment.

The nature of the red shift was explored by studying the absorption of the chromophores in different solvents. Figure 3 plots the absorbance of resorufin in water, methanol, β-cyclodextrin, and acetonitrile. The absorption spectra show that as one proceeds from water to acetonitrile solvent a red shift occurs, and the absorption spectrum has better defined substructure. The cyclodextrin−resorufin spectrum is an intermediate case between that of methanol and acetonitrile. This result demonstrates that binding of resorufin to β-cyclodextrin changes the local solvation environment in a way that is similar to changing the solvent from protic to polar aprotic.

The binding constants of the complexes were determined by a titration in which the complex formation was monitored using the electronic absorbance at 590 nm for resorufin and oxazine-118 and 640 nm for oxazine-725. Figure 4 shows the titration curve for the resorufin−cyclodextrin complex. The titration curve was fit to a 1:1 binding isotherm. The functional form used is given by eq 4.6 in ref 15b. A best fit to a model of 1:1 (cyclodextrin−chromophore) binding shown as the solid curve gives a reasonable fit to the experimental data, suggesting that the 1:1 binding is dominant for the studied systems. The values of the binding constant for the three complexes are reported in Table 1.

TABLE 1: Binding Constants for β-Cyclodextrin Inclusion Complexes

	resorufin	oxazine-118	oxazine-725
K_{as} (M^{-1})	2171	1976	1411

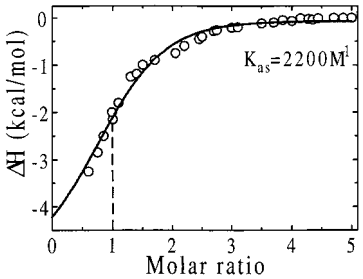

Figure 5. Calorimetry binding curve for the resorufin−cyclodextrin host−guest complex. The solid line is a fit of the data to a 1:1 binding model.

The thermodynamics of the binding was studied by isothermal titration calorimetry.[14] Each experiment involved two measurements: a dilution experiment and the titration. The dilution run measures the heat resulting from the addition of the concentrated resorufin solution (200 mM) to pure water. The titration run is performed by adding the resorufin solution to a solution of the cyclodextrin (2 mM). This experiment measures the heat resulting from complexation and dilution. When the heat measured in the dilution run is subtracted from that for the titration run, only the association enthalpy remains. The calorimetry data in Figure 5 show the complexation enthalpy for resorufin with cyclodextrin. These data are characteristic of a titration for an association that has a low binding strength.[15] The transition, i.e., the dramatic change in the produced heat, is clearly observed. The midpoint between maximal signal and no signal corresponds to 1 equivalent and indicates that 1:1 binding occurs. The curve goes to zero at 2:1 and higher molar ratio, indicating saturation of the binding sites and consequently no more heat produced. Hence, no transitions with 2:1 molar ratio are observed. The fit to these data was done using the software supplied for the calorimeter by Microcal Inc. A 1:1 model fit (solid line) demonstrates an excellent correspondence to the experimental data and supports the suggestion that 1:1 binding for resorufin/cyclodextrin is the most important. The value of the enthalpy where the fitted curve crosses the ΔH axis is taken to be the association enthalpy. For the data in Figure 5 one finds $\Delta H = -4.1$ kcal/mol and a binding constant K_{as} of 2200 M^{-1}. This value for K_{as} is in excellent agreement with that found from the absorption data. The calorimetry data demonstrate that 1:1 complex formation is the most significant for the studied systems, and association exhibits a large favorable exothermic enthalpy change.

NMR Results. The NMR spectra were recorded for free guest in D_2O and cyclodextrin in D_2O. These spectra were compared to the spectra of the bound guest and host in D_2O. Each spectrum of the complex showed an induced upfield chemical shift of 0.07−0.19 ppm for the inner protons of the cyclodextrin, as compared to the free host. The host−guest concentration ratio was 1:1. (Tables listing experimental $\Delta\delta$ values (in ppm) for the inner protons of the cyclodextrin and aromatic protons of the guest are given in the Supporting Information.) It is known that if a guest molecule penetrates into the cavity, then the host hydrogen atoms located in the cavity interior will be considerably shielded by the guest, and an upfield chemical shift will be observed.[1−4] The induced chemical shift observed for the β-cyclodextrin in the presence of each of the chromophores demonstrates that all three

TABLE 2: Rotational Relaxation Times of Chromophores in Water and in Aqueous Solution of β-Cyclodextrin (CD)

		resorufin	oxazine-118	oxazine-725
no CD	τ_{or} (ps)	63 ± 5	67 ± 6	146 ± 9
β-CD	τ_{or1} (ps)	59 ± 5	56 ± 9	406 ± 43
	τ_{or2} (ps)	301 ± 25	281 ± 29	
	A_{long}/A_{short}	1.6 ± 0.4	1.2 ± 0.4	

chromophores bind to the cyclodextrin under the conditions of the experiment. In addition, a relatively large (0.10−0.7) low-field chemical shift is observed for aromatic protons of the guest at 100:1 host:guest ratio. These data also support the conclusion that host−guest complexation occurs.

In all cases the NMR spectra are not split into the signals corresponding to uncomplexed and complexed β-CD and chromophore. This observation indicates that the association and dissociation processes between free and bound species occurs on a time scale faster than microseconds.

Rotational Relaxation. Optically heterodyned polarization spectroscopy is used to monitor the rotational dynamics of the chromophores. The rotational relaxation of each solute in pure water is well characterized by a single-exponential decay law. The rotational relaxation times of the resorufin anion and the oxazine-118 cation are very similar in aqueous solution (Table 2). This result correlates with their similar size and shape. Oxazine-725 relaxes more slowly than the smaller resorufin and oxazine-118. These observations are consistent with simple hydrodynamic predictions.[16]

Addition of β-cyclodextrin changes the relaxation behavior of the solute molecules significantly. Figure 6 shows the measured anisotropy decay for the resorufin (A), the oxazine-118 (B), and oxazine-725 (C) in water and in β-cyclodextrin solution. The data are presented on a log scale in order to illustrate the essential difference between the decay curves of the free and the complexed chromophore. In these data the concentration of cyclodextrin is 1000 times higher than the concentration of the dye. Taking the binding constant for the chromophore−cyclodextrin complex to be $\geq 10^3$ M^{-1}, more than 99% of the solute should be bound under this condition. One exponential (solid line) and two exponential (dashed line) fits are shown for the resorufin and oxazine-118 with cyclodextrin. A single-exponential fit to the anisotropy decay of the complex results in 216 and 230 ps rotational relaxation times for resorufin and oxazine-118, respectively. It is evident that a single-exponential fit does not represent the experimental data very well. A double-exponential fit improves the correspondence to the experimental data and significantly decreases the χ^2 value. The best fit parameters are 60% of a 59 ps component and 40% of a 301 ps component for the resorufin and 55% of a 56 ps component and 45% of a 281 component for the oxazine. The decay times of the short component are close to that of the free solute. Both the long and short time components of the resorufin/CD complex are similar to those of the oxazine-118/CD complex.

In contrast to the double-exponential behavior of resorufin− and oxazine-118−cyclodextrin inclusion complexes, the relaxation decay of oxazine-725 is well described by a single exponential with a time constant of 401 ps (Figure 6C). A double-exponential fit to these data does not improve the χ^2 value and gives similar values for the relaxation time. It is important to realize that the relaxation time measured by this method only probes an average of the host−guest conformations.

Fluorescence Lifetime Measurements. The inclusion of the chromophore into the cyclodextrin cavity also changes its fluorescence lifetime.[11,17−20] The lifetime of the first electronically excited state of each chromophore was measured in pure water and in β-cyclodextrin solution (Table 3). Addition of the cyclodextrin increases the fluorescence lifetime for the resorufin and oxazine-118 but does not significantly impact the lifetime of the oxazine-725. The increase in the lifetime is most pronounced for the resorufin.

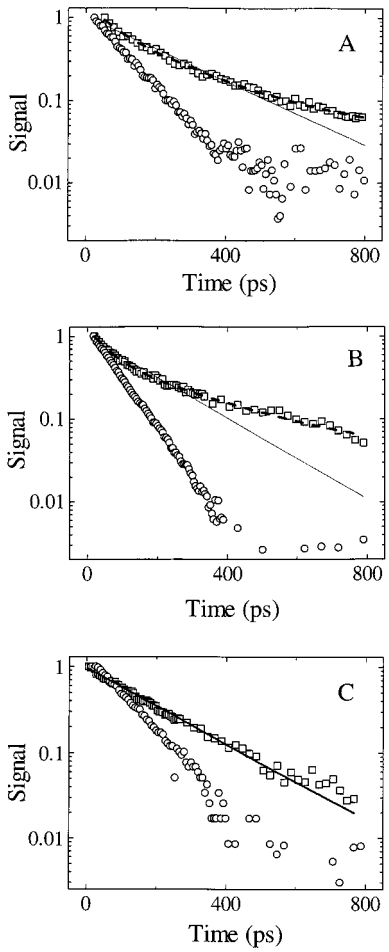

Figure 6. Anisotropy decays for resorufin (A), oxazine-118 (B), and oxazine-725 (C) in water (open circles) and in the presence of β-cyclodextrin (open squares). Single-exponential (solid line) and double-exponential (dashed line) fits are shown for the decay of chromophore in aqueous solution of CD.

TABLE 3: Fluorescence Lifetimes of Chromophores in Water and in Aqueous Solution of β-Cyclodextrin (CD)

	τ_{fl} (ps)		
	resorufin	oxazine-118	oxazine-725
no CD	2750 ± 31	2419 ± 26	498 ± 14
β-CD	4016 ± 27	2742 ± 41	447 ± 17

Because of the significant change in lifetime for the resorufin system, its dependence on the β-cyclodextrin was measured at 1:1, 1:10, and 1:1000 resorufin-to-cyclodextrin ratio. At a 1:1 ratio the fluorescence decay curves were best fit by a double exponential with 20% of a short component (2.6 ns) and 80% of a long component (3.9 ns). These data correlate with the calculation of the relative fraction of free and bound guest based on the binding constants. The binding constant predicts that at a 1:1 concentration ratio 15% of the resorufin should be unbound and contribute to the short component. At higher concentrations of the cyclodextrin the relative statistical weight of the short component decreases, and the decay curve becomes single exponential with a lifetime of 4.1 ns.

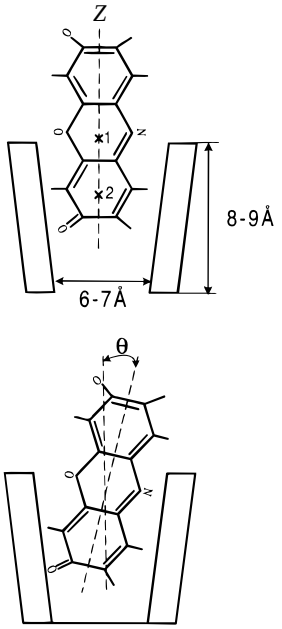

Figure 7. Size and possible structure of host−guest complex for the resorufin−β-cyclodextrin complex. 1 and 2 identify the centers of mass of resorufin and cyclodextrin, respectively. Axis *Z* connects the corresponding centers of mass.

Discussion

Photophysical and Spectroscopic Properties of the Complexes. The ability of the β-cyclodextrin to form inclusion complexes with resorufin, oxazine-118, and oxazine-725 is clearly demonstrated by the absorption data. These methods correlate well with the calorimetry measurements showing that 1:1 binding is dominant in all three cases with binding constants $\geq 10^3$ M^{-1}.

The red shift in the absorption spectra that is found for the complexed chromophore is usually attributed to a change in the structure of the hydrogen bonding around the chromophore upon its inclusion inside the β-cyclodextrin cavity. The effect of hydrogen bonding on the absorption of the resorufin was tested by measuring its absorption in a series of solvents with different hydrogen-bonding abilities (Figure 3). Resorufin is able to accept hydrogen bonds at its oxygen sites but is not able to donate hydrogen bonds; hence, it is expected to form hydrogen bonds in methanol and water but not in acetonitrile. The ability to form hydrogen bonds appears to be important for the structural features and the position of the absorption spectrum of the resorufin. Specifically, hydrogen bond formation appears to cause a blue shift and a broadening of the spectrum. The position and the shape of the cyclodextrin−resorufin complex indicates that inclusion of the resorufin into the cavity diminishes the extent of its hydrogen bonding, as compared to the case of pure water. Because the inner surface of the cyclodextrin is not a good hydrogen bond donor, the hydrogen bonding with resorufin is expected to be weak, in agreement with the spectral changes. These considerations indicate that resorufin binds inside the cyclodextrin cavity, at least partially. Simple geometrical considerations demonstrate that such binding is possible. The internal diameter of the β-cyclodextrin is 6.5−8 Å, and its length is 8−9 Å (see Figure 7). By comparison, the dimension of the resorufin is 12:7:3 Å. The internal diameter of the β-cyclodextrin allows partial inclusion of the resorufin, resulting in the modification of the structure of its local solvation shell.

The partial inclusion of resorufin is further supported by the fluorescence lifetime measurements. The fluorescence lifetime was found to increase from a value of 2.8 ns to 4.0 ns upon the addition of cyclodextrin and was double exponential at small host−guest ratio. The concentration dependence of the fluorescence lifetime of resorufin demonstrates that at low concentrations of cyclodextrin at least two different species are present in the solution: free resorufin and resorufin bound with cyclodextrin. At higher concentrations of β-cyclodextrin the statistical weight of unbound guest decreases and vanishes at a concentration ratio of 1 resorufin to 100 cyclodextrins. The dependence of the fluorescence lifetime of the resorufin on the environment is clearly demonstrated by the effect of the solvent. The lifetime ranges from 2.7 ns in water, which is able to form hydrogen bonds with resorufin, to 4.9 ns in DMSO,[8a] which is not expected to form hydrogen bonds with resorufin.[23] The excited-state lifetime of resorufin in the cyclodextrin inclusion complex is 4 ns, indicating that the environment of the resorufin changes upon complexation with β-cyclodextrin and involves less hydrogen bonding.

The fluorescence lifetime measurements provide information on the stability of these chromophore/β-cyclodextrin complexes. Single-exponential decays are observed for the excited-state decay law of the free guest and for the excited-state decay law of the host−guest complex. When a mixture of free and bound guest is present in the solution, the decay is double-exponential. This result indicates that the individual species are well-defined on the nanosecond time scale, and on average, the time for dissociation of the complexes is slow compared to a few nanoseconds. In addition, the NMR spectrum of the resorufin/CD complex indicates that the exchange with the solvent is rapid on the NMR time scale. These observations are consistent with the temperature jump measurements of the dissociation rate constant of cyclodextrin complexes, which ranges from 10 to10^5 s^{-1}.[22]

The change in the spectra and photophysics of the cations is less clear. They can have more subtle behavior. The oxazine-118 can both accept and donate protons; hence, it could interact with the glucose oxygen and form hydrogen bonds with the cyclodextrin. In this case the inclusion of the oxazine into the cavity might not substantially change the structure of the hydrogen bonding around the chromophore. One expects that the oxazine-725 could accept hydrogen bonds but would be a poor hydrogen bond donor. Nevertheless, both species have similar spectral changes and a similar insensitivity of their excited-state lifetime. For example, the fluorescence lifetime of oxazine-118 changes from 2.4 to 2.8 ns, which is only 16% (compared to 46% for resorufin).

The change in the photophysical and photochemical properties of resorufin upon addition of cyclodextrin clearly demonstrates its possible incorporation into the cyclodextrin cavity. The smaller change in the oxazines properties likely results from its different chemical functionality and its larger size in the case of oxazine-725. For comparison, the dimensions of the oxazine-118 are 15.0:5.4:4.0 Å and that of oxazine-725 are 18.3:9.3:5.6 Å. These considerations reveal that oxazine-725 could bind in the cavity, but its larger volume would restrict its range of possible geometries. The important conclusion to draw from these data is that all three chromophores bind to cyclodextrin under the conditions of the experiment. Resorufin is partially included into the cavity while it is less clear how the oxazines bind.

The Host−Guest Reorientational Dynamics. The rotational relaxation data look very similar for both the resorufin and the

oxazine-118/β-cyclodextrin complexes. They are both well described by a double-exponential decay law. Their time constants are similar, but the relative amplitudes of the components are different. To determine whether the double-exponential character results from the existence of two species, free and bound chromophore, the rotational relaxation decay of the resorufin and oxazine-118 was measured as a function of the host/guest concentration ratio. Figure 8A,B plots the dependence of the A_{long}/A_{short} on the chromophore/CD concentration ratio. For the case when the concentration of the host is 10 (and less) times more than the concentration of the guest, the amplitude of the short component is higher than that of the long component. This concentration range corresponds to 50% (and less) of unbound chromophore. The percentage of the long component increases as the concentration of cyclodextrin increases. At high concentration ratios of 1:100 (chromophore/CD ratio) and higher, the relative amount of the short and long component does not change. These considerations support the conclusion that the decay characteristics do not depend on the concentration of the host if it is taken at 1:100 and higher molar ratio. The rotational relaxation times reported here are taken at 1:400 (guest:host) concentration ratio so that more than 99% of the guest is bound. The fast component of the relaxation decay remains the same at higher host-to-guest concentration ratio and reflects the internal dynamics of the complex. In particular, it is related to the internal angular motion of the chromophore in the cyclodextrin cavity. This internal motion is discussed in more detail later. The similarity in the time constants of oxazine-118 and resorufin is not surprising since they have similar rotational relaxation times in water.

The rotational relaxation times of the long components are also similar for both resorufin and oxazine-118. These time constants are attributed to the overall rotational motion of the complexes. The relatively high statistical weight of the long component shows that both the cation and the anion are "caged" inside the cavity. In addition, the similar relaxation times for the long component suggest similar geometries for the complexes (vide infra). It appears that the opposite charge of the guest does not significantly impact the dynamics of the host−guest complex.

In contrast to the similar behavior of the resorufin and oxazine-118, the rotational relaxation decay for oxazine-725 appears to be single exponential with a relaxation time of 401 ps.

This behavior could have multiple origins. First, the rotational relaxation time of the oxazine-725 in water is significantly longer than that of the other solutes, and the fluorescence lifetime of oxazine-725 is only 500 ps. These factors make it more difficult to distinguish the short and long components from each other (see eq 1). Second, since the diameter of the oxazine-725 is very close to the internal diameter of the β-cyclodextrin, the tight inclusion of the oxazine-725 into the cavity of the cyclodextrin would be expected, and the 401 ps time constant could be explained in terms of a rigid inclusion complex in which anisotropy decays by rotation of the entire complex.

The rotational relaxation data show that both internal and overall motion of the host−guest complex is not affected by the electrostatic properties of the guest. Both resorufin and oxazine-118 have similar dynamical behavior in the complex. Moreover, while the oxazine-118 can form hydrogen bonds in both water and the cyclodextrin cavity, the resorufin is unlikely to form hydrogen bonds inside the cyclodextrin cavity. It appears that the ability to form hydrogen bonds does not change the dynamics of the solute for these systems. This suggests

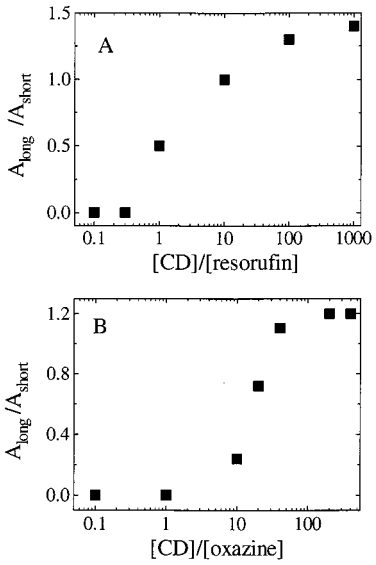

Figure 8. Dependence of the ratio of amplitude of the long component A_{long} to the amplitude of the short component A_{short} of the double-exponential fit plotted as a function of the CD/chromophore concentration ratio (A, cyclodextrin/resorufin; B, cyclodextrin/oxazine-118).

that electrostatic interactions and hydrogen bonding are relatively unimportant. Rather, the complex's geometry and rotational behavior are mainly determined by the relative size of the guest and the inside of the cyclodextrin cavity. This dominance of shape and size is consistent with the interactions being van der Waals and dispersion forces. This hypothesis is supported by the binding energy calculations and measurements,[24] according to which the complex formation is stabilized by dispersive or van der Waals forces and not by electrostatic and hydrogen-bonding interactions.

Modeling the Overall Motion of the Guest. The rotational relaxation of the overall host−guest complex can be described by a hydrodynamic model. The Debye−Stokes−Einstein (DSE)[16] model expresses the rotational relaxation time as

$$\tau_{or} = \frac{6CV\eta}{kT} \qquad (2)$$

where V is the hydrodynamic volume of the solute, η is the viscosity of the solution, and C is a parameter that describes the shape of the solute and the boundary conditions (slip or stick). The volume of a single molecule of β-cyclodextrin is estimated from its dimensions to be 1147 Å^3. The reorientational relaxation time calculated from the above equation ranges from 55 ps for a slip boundary condition to 280 ps for a stick boundary condition. The translational diffusion coefficient of β-cyclodextrin in water was measured in a recent light-scattering study,[25] and its hydrodynamic radius is found to be 6.4 Å. If one uses this radius to determine the hydrodynamic volume of an appropriate spherical ellipsoid, one finds a rotational relaxation time of 242 ps for β-cyclodextrin in water, which is in accord with the experimental number for the overall correlation time of β-cyclodextrin (200−300 ps).[26] These values are very close to that expected for a stick boundary condition.

Different geometries of the inclusion complexes were analyzed to identify its effect on the rotational relaxation time. Complete inclusion of the resorufin into the cyclodextrin cavity does not increase the size and relaxation time of the complex significantly over the case of pure β-cyclodextrin. The stick limit of the rotational relaxation time of the complex in this case was estimated to be 350 ps, which is very close to the

rotational relaxation of pure cyclodextrin and the experimental relaxation time of the resorufin− and oxazine-118−cyclodextrin complexes (310 and 280 ps, respectively).

Two extreme structures were considered for the guest complexed externally to the cyclodextrin: (1) the long axis of the guest was assumed to be parallel to the principal axis of the cyclodextrin, but the guest molecule was located outside the cavity, and (2) the long axis of the guest is perpendicular to the principal axis of the cyclodextrin and oriented across the larger opening of the cyclodextrin cup. For the parallel case the stick relaxation time was estimated to be 600 ps, and for the perpendicular case it was found to be 400 ps. In both cases the calculated rotational relaxation time of the complex is considerably larger than the measured one. These calculations reveal that the resorufin and oxazine-118 are likely to be partially inside the cavity of the cyclodextrin rather than completely outside. In other studies a small increase in the relaxation time of cyclodextrin was observed to result from inclusion of a guest.[26,27]

A similar analysis was performed for the case of oxazine-725 in β-cyclodextrin. For the guest included inside the cavity the relaxation time is estimated to be 450 ps. For the oxazine-725 outside the cavity the stick relaxation time was found to be 830 ps for the parallel geometry and 540 ps for the perpendicular geometry. Both of these values are significantly larger than the experimental value of 406 ps. Even an inclusion of the oxazine-725 into the cyclodextrin cavity increases the relaxation time of the complex considerably, over 40%, as compared to that of free cyclodextrin. This difference between oxazine-118 and oxazine-725 appears to arise from oxazine-725's larger size. These calculations suggest that the smaller guests (resorufin and oxazine-118) can go deeper inside the cavity than does the larger oxazine-725.

Internal Motion of the Guest. Since the guest can be included relatively deeply inside the β-cyclodextrin cavity, its internal motion is restricted by the cavity of the cyclodextrin. A diffusion in a cone model was used to describe the motion of the chromophore inside the cavity.[28] In the diffusion in a cone model the unit vector μ with orientation $\Omega = (\theta,\phi)$ diffuses freely in the angular region $0° \leqslant \theta \leqslant \theta_{max}$ and $0 \leqslant \phi \leqslant 2\pi$ with a diffusion coefficient D_ω. In this case the amplitude of the short component which characterizes the amount of the anisotropy loss from the internal motion can be used to estimate the angle θ_{max} of the cone. Assuming that the overall tumbling motion of the complex occurs on a time scale that is much longer than internal librational motion, it is possible to evaluate Szabo's expression for $r(\infty)$.[28a] Taking the transition dipole of the chromophore to be directed along the long symmetry axis of the guest and assuming that the long axis of the guest is perpendicular to the β-cyclodextrin's open end (Figure 7), one finds

$$\frac{r(\infty)}{r(0)} = \frac{A_{long}}{A_{short} + A_{long}} = \frac{1}{4}\cos^2\theta_{max}(1 + \cos\theta_{max})^2 \quad (3)$$

From the rotational relaxation data $A_{long}/(A_{long} + A_{short}) = 0.62 \pm 0.19$ for resorufin and 0.54 ± 0.18 for the oxazine-118. Using this model, one finds $\theta_{max} = 32 \pm 10°$ for the resorufin and $\theta_{max} = 35 \pm 11°$ for the oxazine. The mean-square angle θ_{rms} can be calculated from these values, resulting in $\theta_{rms} = 18°$ for the resorufin and $\theta_{rms} = 20°$ for the oxazine-118. These values are very close to each other, indicating that the average structures of both complexes are similar.

The angle that is determined from the dynamical data is an average value obtained from the different conformations present in the solution. Both completely included guest molecules with very small flexibility and slightly included complexes with greater flexibility could exist in the solution. Moreover, different relative host−guest geometries will contribute to the observed relaxation times and, therefore, the diffusion angle. The maximum rotation angle was evaluated for different conformations of the complexes by assuming that the long axis of the resorufin is parallel to the axis of the cyclodextrin (Figure 7). The angle was evaluated to range from 2 to 4° for the completely included resorufin to 50° for more open structures in which the oxygen atom is at the CD boundary. The angle measured for the resorufin and oxazine-118 lies between these values, indicating that the most probable structure of the complex corresponds to the partial inclusion of the chromophore (ca. half of its volume). These data correlate with the quantum chemistry calculations which are described in the next section.

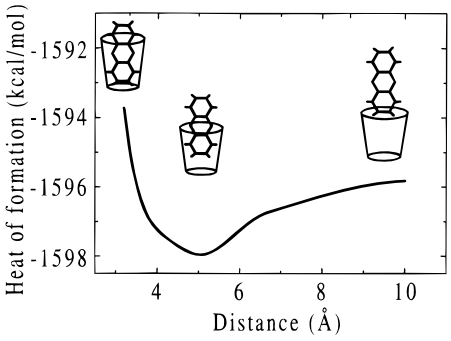

Figure 9. Dependence of the heat of the formation of the complex on the distance between the centers mass of the host and guest for the resorufin−β-cyclodextrin complex.

Figure 10. Relative host−guest geometry corresponding to the minimum of the heat of the formation of the complex.

Molecular Modeling. Molecular modeling studies were performed in a vacuum with empirical force fields[29] and semiempirical quantum theory using the AM1 Hamiltonian.[30] The initial geometry of the resorufin and β-cyclodextrin were obtained using a full geometry optimization at the AM1 level. The initial docked structure of the inclusion complex of resorufin and β-cyclodextrin was obtained using the simulated annealing method, with a rigid geometry and fixed partial charges for the individual molecules. The calculations were performed using HyperChem,[31] Mopac93,[32] and an in-house program for simulated annealing.[29] The centers of mass of the resorufin and β-cyclodextrin molecules were calculated for each molecule for the initial inclusion complex. The axis connecting the centers of mass was taken to be the *Z*-axis (see Figure 7).

Figure 9 plots the dependence of the energy of formation of the host−guest complex on the distance between the centers of the mass of two molecules. The minimum energy structure of the complex is found at the distance of 5.2 Å. This distance corresponds to the partial inclusion of the resorufin in β-cyclodextrin. Figure 10 shows the complex at this geometry. This

structure corresponds to the position of the guest in which one ring of the resorufin is completely inside the cavity. As the distance between the centers of mass of the host and the guest is reduced (i.e., inclusion of the resorufin increases), the energy of the complex increases. Further removal of the resorufin out of the β-cyclodextrin cavity requires 2 kcal/mol of energy. The partial inclusion found for the resorufin at the AM1 level of quantum theory provides good agreement with the experimental results and "diffusion in a cone" modeling of the internal dynamics of the guest.

Conclusions

A combination of static and time-resolved spectroscopic techniques was used to explore the structure and dynamics of β-cyclodextrin inclusion complexes. The optically heterodyned polarization spectroscopy method was applied to monitor the rotational relaxation dynamics of resorufin anion, oxazine-118 cation, and oxazine-725 cations bound to β-cyclodextrin. The binding constants and the thermodynamics of the binding were evaluated using calorimetry and the absorption measurements.

It was found that the dynamics of the resorufin−cyclodextrin and oxazine-118−cyclodextrin complexes are well characterized by a double-exponential decay law. The experimental data are taken at a host-to-guest concentration ratio where the percentage of unbound guest is less than 1%. Therefore, the fast component of the decay cannot be attributed to the rotational relaxation of free guest. Instead, the fast component reflects the internal motion of the guest inside the cavity of cyclodextrin. The slow component of the decay corresponds to the overall motion of the complex. In contrast, no internal motion was observed for the larger in size oxazine-725 chromophore. Modeling of the internal motion of the guest indicates that the chromophore can be partially included in the cyclodextrin, which is consistent with quantum chemistry calculations.

These studies demonstrate that the host−guest complex is bound for time scales in excess of a few nanoseconds but shorter than a few microseconds. It was shown that the electrostatic properties of the guest do not affect the internal rotational motion of the guest in the complex, but the relative host−guest size determines the character of intermolecular host−guest dynamics.

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Supporting Information Available: Tables of $\Delta\delta$ values for the protons of the cyclodextrin and guest and a figure showing a schematic representation of the guest−host complex (2 pages). Ordering and access information is given on any current masthead page.

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