Picosecond Laser Studies on Photochemical Reactions in Restricted Environments: The Photoisomerization of *trans*-Stilbene Complexed to Cyclodextrins

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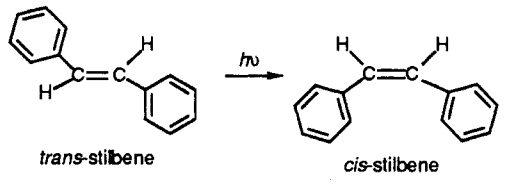
The effect on picosecond reaction dynamics due to complexation of a reactive molecule with cyclodextrins was examined, with the trans–cis photoisomerization of stilbene as an example. In the presence of α-cyclodextrin, a single, slow exponential decay of *trans*-stilbene fluorescence is observed, consistent with the formation of a single complex. In contrast, in the presence of β-cyclodextrin, the fluorescence of *trans*-stilbene showed a clear double-exponential decay, consistent with the formation of two complexes. In the latter case, a dynamic equilibrium between a loose and a more tightly bound conformation of the complex is proposed, resulting in different photoisomerization reaction rates of *trans*-stilbene.

Introduction

Cyclodextrins, zeolites, micelles, and other microheterogeneous structures have received increasing attention in recent years, since these systems have been found to provide so-called restricted microenvironments or cavities of molecular dimensions (of the order of 10 Å) capable of sequestering and controlling the chemistry of reactive molecules.[1] Indeed, these systems provide molecular environments that have been shown to modify both a variety of different chemical pathways as well as the dynamics of energy relaxation for the encapsulated guest molecules.[2-8] The effect on the energetics and on the dynamics caused by these microheterogeneous environments can often be rationalized by a simple "lock and key" hypothesis,[9] which is widely used to explain the nature of the guest–host interactions in enzymatic systems. Dynamic effects may result from geometric restrictions imposed on the guest molecule, resulting from the limited space in the host cavity. For example, cyclodextrins are cone-shaped oligosaccharides that have a hydrophobic cavity that varies from 5.6 Å for α-cyclodextrin up to 8 Å for γ-cyclodextrin.[10] This space limitation could affect the chemistry of a reactive guest molecule because it may restrict or encourage motions that are necessary for a reaction. Another factor that can affect energy relaxation of a guest molecule results from specific guest–host interactions. These interactions could provide a dramatic change in the energetics of a reaction. For example, the effective polarity around the guest molecule as it is bound inside the cavity may be substantially different than in the bulk.

In order to address the issue of how microenvironments can influence very fast reaction dynamics, we have investigated the photoisomerization of *trans*-stilbene complexed to cyclodextrins. The trans–cis photoisomerization of stilbene was chosen because it has been extensively studied in homogeneous solution.[11-22]



trans-stilbene *cis*-stilbene

It is known, for example, that the reaction occurs by a thermally activated twisting around the central double bond, which thus provides the principle mode of deactivation of the excited singlet state of stilbene. The inclusion of stilbene in various cycodextrins was previously examined by steady-state methods.[23] From picosecond time-resolved methods, we have been able to show for the first time that the nature of the complex is different for different cyclodextrins, being dependent mainly on the cavity size. Furthermore, we found that two distinctly different complexes are formed for the larger β- and γ-cyclodextrins, which yield vastly different kinetics for the photoisomerization process.

Experimental Section

Fluorescence Lifetime Measurements. An active–passive mode-locked Nd:YAG laser (Quantel Model 501C) with pulses 30 ps in duration was used to excite the *t*-stilbene at 266 nm, and the fluorescence was detected with a Hadland Imacon Model 675 streak camera. A polarizer oriented at 54.7° was placed in the collection optics in order to avoid the effects of time-dependent depolarization of fluorescence. The apparatus and data handling procedures have been described.[24] Temperature control (±0.1 °C) was attained by using a Neslab circulation cooler that was connected to a copper block into which the sample was placed. Depending on the intensity of the sample signal and on the number of exponential functions (one or two) required for a satisfying fit of the data, between six and twenty shots were averaged. The time profile of the laser pulse used for the deconvolution of the fluorescence lifetime was recorded simultaneously, appearing as a prepulse in the plots of the experimental data (Figure 1). Before each measurement, the nitrogen-purged samples were thermally equilibrated for at least 15 min.

Chemicals and Sample Preparation. trans-Stilbene (Kodak) was scintillation grade and was used as received. Absolute ethanol was refluxed over Mg and then fractionally distilled under dry nitrogen and used immediately. Octanol and decanol (Gold Label, Aldrich) were fractonally distilled and were allowed to sit over

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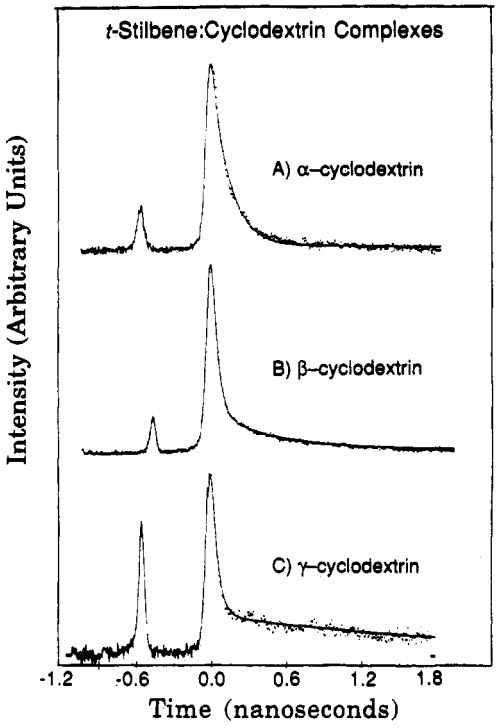

Figure 1. Fluorescence decay of *trans*-stilbene in the presence of (A) α-cyclodextrin, (B) β-cyclodextrin, and (C) γ-cyclodextrin, at room temperature.

cleaned, activated Type 4A molecular sieves, before use. The oligosaccharides α-, β-, and γ-cyclodextrin were purchased from Aldrich. The pureness of the samples (in aqueous solution) was verified by means of the UV absorption spectra (no absorption at wavelengths longer than 240 nm). After each experiment, absorption and emission spectra were recorded again as proof that no photoreactions (besides photoisomerization of *trans*-stilbene) had occurred upon laser irradiation.

Preparation of t-Stilbene/Cyclodextrin Complexes. The initial *trans*-stilbene concentration for each experiment was 10^{-4} M. In order to facilitate the complexation with cyclodextrins, a highly concentrated (5 mM) solution of *trans*-stilbene in methanol was prepared. Of this stock solution, 1 mL was then added to 49 mL of an aqueous cyclodextrin solution. Efficient complex formation between *trans*-stilbene and the cyclodextrins was achieved with high excess of the oligosaccharides (concentration ratios: 20:1 of α- and γ-cyclodextrin:*trans*-stilbene; 50:1 and 200:1, β-cyclodextrin:*trans*-stilbene). For comparison with the fluorescence decays in similar homogeneous solution, a dilute ($<10^{-6}$ M) solution of *trans*-stilbene in a mixture (1:4) of methanol and water was used. The solutions were stirred in the dark for at least 24 h and filtered (pore size of filter, 0.2 μm) to remove aggregates of *trans*-stilbene, which lead to a characteristic red shift of the fluorescence spectrum by about 10 nm. The complete removal of microaggregates was confirmed for each solution by means of the fluorescence spectrum.

Results and Discussion

In the presence of α-cyclodextrin, the fluorescence of *trans*-stilbene was found to decay as a single exponential. In contrast, in the presence of β- and γ-cyclodextrin, the excited singlet state decay of *trans*-stilbene was nonexponential but could be fitted to the sum of two exponentials (Figure 1). The short-lived component (of about 50 ps) is similar to the fluorescence lifetime of *trans*-stilbene in low-viscous alcohols (methanol and ethanol). The second, long-lived component (of several hundred picoseconds) had values typical for the decay times of stilbene fluorescence in highly viscous or rigid environments.[25-28] The contribution of the long component to the decay curve is also strongly dependent on temperature, as shown in Figure 2. Due to its low solubility, free *trans*-stilbene did not contribute to the observed fluorescence signal.

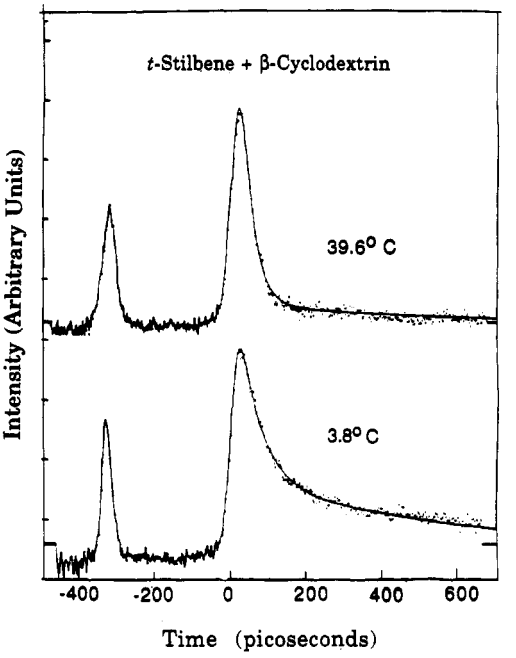

Figure 2. Fluorescence decay of *trans*-stilbene in the presence of β-cyclodextrin in aqueous solution, at two different temperatures: (a) at 39.6 °C and (b) at 3.8 °C. The pulse preceding the decay curve is used both as a time marker and for the deconvolution of the data.

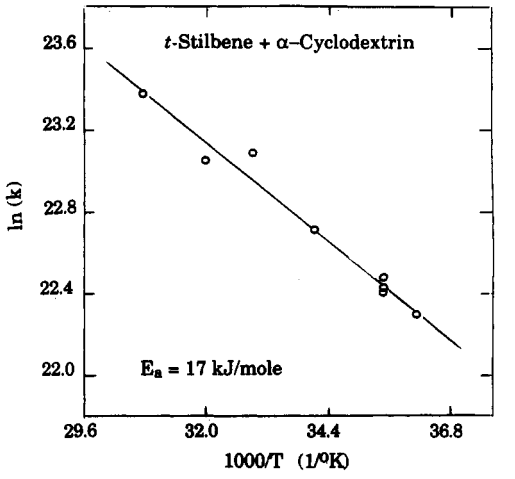

Figure 3. Temperature dependence on the photoisomerization rate of *trans*-stilbene in the presence of α-cyclodextrin in H_2O/CH_3OH (49:1).

For the stilbene and α- and β-cyclodextrin complexes, sufficient concentrations of complexed *trans*-stilbene were achieved, for a reliable data analysis. However, formation of a 1:1 complex between *trans*-stilbene and γ-cyclodextrin as so weak as to make the data analysis imprecise. Because the signal intensity obtained in the presence of γ-cyclodextrn was too weak for a quantitative data analysis, the photoisomerization of *trans*-stilbene interacting with γ-cyclodextrin will not be considered further, but the dynamics of excited-state *trans*-stilbene energy relaxation observed in the presence of α-and β-cyclodextrin will be discussed in detail.

α-Cyclodextrin. The excited-state-fluorescence decay rate of *trans*-stilbene, in the presence of α-cyclodextrin, was determined as a function of temperature (Figure 3). Over a temperature

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TABLE I: Arrhenius Rate Parameters for Photoisomerization of *trans*-Stilbene for the α-Cyclodextrin Complex Compared with Some Alcohol Solvent Systems

solvent system	$A/10^{13}$ s^{-1}	E_{act}/kJ/mol
α-cyclodextrin complex[a]	0.7 ± 0.4	17 ± 1
ethanol	1.0 ± 0.3	15.5 ± 0.4
octanol	1.0 ± 0.1	17.0 ± 0.5
decanol	2.4 ± 0.8	19.7 ± 0.8

[a] In H_2O/CH_3OH (49:1, v/v).

range of 5–55 °C, it was found that the excited *trans*-stilbene decay could be fit to a single exponential, with the decay rate being faster at higher temperatures. The excited-state decay time is considerably longer in the presence of α-cyclodextrin. As a typical example, it was found that when *trans*-stilbene was complexed to α-cyclodextrin, it had a lifetime of 137 ps, whereas in a homogeneous nonviscous medium it had a lifetime of only 34 ps (using H_2O/CH_3OH, 4:1 v/v, as solvent), at 20 °C. On the other hand, it is pointed out that the values in the excited-state lifetime of *t*-stilbene resulting from complexation to α-cyclodextrin are similar to those observed in *n*-alcohols of relatively high viscosity (*n*-octyl alcohol and *n*-decyl alcohol).

The observation of a single-exponential fluorescence decay is consistent with the existence of only one excited-state species of *trans*-stilbene, which is tightly bound to α-cyclodextrin. The remarkable increase of the fluorescence lifetime, compared to a similar homogeneous environment, is attributed to the strong hindrance of the rotation of one of the phenyl groups about the double bond as the molecule undergoes trans–cis isomerization. From the Arrhenius plot of the decay rate (Figure 3), an activation energy of 17 ± 1 kJ/mol and a preexponential factor of (7 ± 4) × 10^{12} s^{-1} are determined, which thus show that the rate is similar to that found in the relatively high-viscous octanol or decanol solvent systems, see Table I.

β-Cyclodextrin. The temperature dependence of the excited-state decay of *trans*-stilbene complexed to β-cyclodextrin was determined. A two-component decay was observed at all temperatures. The kinetics of the system could be expressed in terms of a double-exponential function, given by eq 1. As shown in

$$I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \quad (1)$$

Figure 4, a different temperature dependence is observed for the two decay times, τ_1 and τ_2. The value of k_1 ($1/\tau_1$) increases with increasing temperature, but the value of k_2 ($1/\tau_2$) is not affected significantly by a change in temperature (within experimental error). Furthermore, the ratios of the preexponential factors, A_1/A_2, are also found to vary with temperature, being an increasing function of temperature. Over the temperature range studied, it was found that the values of the short component, τ_1, are in good agreement with the fluorescence lifetimes of *trans*-stilbene in homogeneous solution (CH_3OH:H_2O = 1:4). For example, τ_1 = 35 ps at 20 °C but increases to τ_1 = 52 ps at 5 °C. The second decay component, τ_2, is of the order of 450 ps. The results of two series of measurements, with different concentration ratios of *trans*-stilbene and β-cyclodextrin (1:50 and 1:200), show only small differences in the values of the decay times (Figure 4), and the ratios of the preexponential factors, at a given temperature, are identical within experimental error (Table II).

The reason for the double-exponential fluorescence decay of *trans*-stilbene in the presence of β-cyclodextrin could be due to the presence of free (not complexed) stilbene molecules in addition to stilbene/β-cyclodextrin complexes present in solution. The free not complexed stilbene molecule would, presumably, have different decay kinetics when compared with one that would be complexed to the β-cyclodextrin. This idea can be ruled out because we found that free *trans*-stilbene (in the absence of cyclodextrin) did not contribute to the observed fluorescence signal, which is accounted for by its low solubility.

Another explanation for the double-exponential decay could be due to a mixture of 1:1 and 2:1 stilbene/β-cyclodextrin complexes, each giving different decay kinetics. This idea also can be ruled out by the following facts. First, we used a large excess

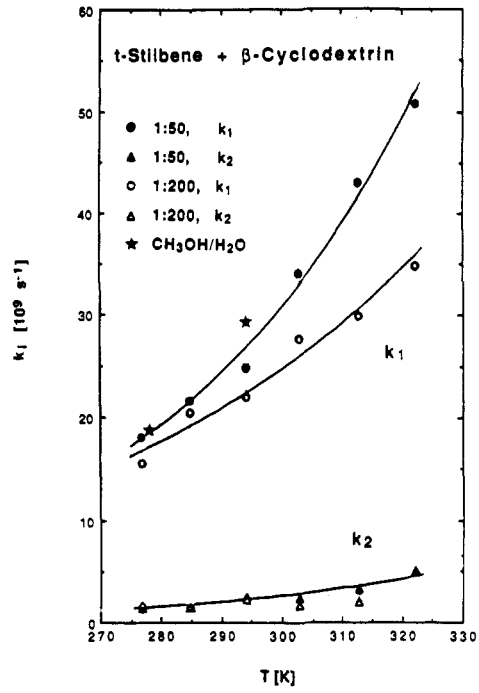


Figure 4. Fluorescence decay constants $k_1 = 1/\tau_1$ and $k_2 = 1/\tau_2$ of *trans*-stilbene in the presence of β-cyclodextrin in aqueous solution, as a function of temperature, for two different concentration ratios: [*trans*-stilbene]:[β-cyclodextrin] = 1:50 (full symbols) and 1:200 (open symbols). The stars (at 5 and 21 °C) indicate the decay constant obtained from the single-exponential decay of *trans*-stilbene in a homogeneous medium, CH_3OH:H_2O = 1:4.

TABLE II: Temperature Dependence on Decay Rates of the *trans*-Stilbene:β-Cyclodextrin Complexes[a]

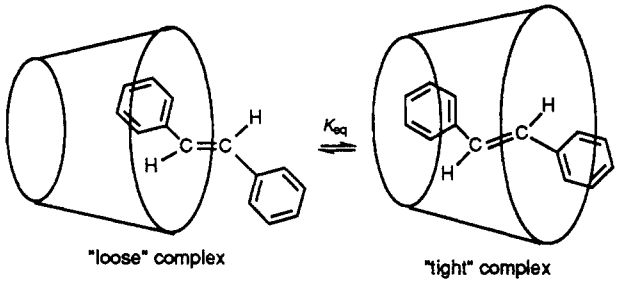
	1:50			1:200		
T/K	$k_1/10^9$ s^{-1}	$k_2/10^9$ s^{-1}	A_2/A_1	$k_1/10^9$ s^{-1}	$k_2/10^9$ s^{-1}	A_2/A_1
277	18.1	1.5	0.333	15.5	1.8	0.351
285	21.7	1.7	0.266	20.5	1.6	0.299
294	24.9	2.5	0.111	22.0	2.3	0.124
303	34.0	2.3	0.087	27.7	1.6	0.087
313	43.0	3.4	0.064	30.0	2.1	0.064
322	50.8	4.9	0.064	34.9	1.4	0.042

[a] Concentration ratios of [*trans*-stilbene]:[β-cyclodextrin] at 1:50 and 1:200 were taken in H_2O/CH_3OH (49:1). k_1, k_2, and the ratio A_2/A_1 are fitting parameters of a two-exponential fit (eq 1). Uncertainties are less than 10% of the values listed, based on averages from several data sets.

(up to 200:1) of the β-cyclodextrin, relative to *trans*-stilbene, to ensure 1:1 complexation. Second, we confirmed that the fluorescence spectrum of *trans*-stilbene in the samples containing β-cyclodextrin corresponded only to the monomer form, thus indicating that only 1:1 complexes were formed (dimer and higher aggregates of *trans*-stilbene have a red-shifted emission spectrum, relative to the monomer). Third, we found no evidence for a photodimer, which would be expected for a 2:1 complex, based on the UV spectrum of the sample taken after laser irradiation. In addition, with CPK models, it can be shown that the cavity space β-cyclodextrin is large enough for a single stilbene molecule (but not a pair of stilbene molecules) to fit into it.

The observed kinetics can, however, be explained by a dynamic, temperature-dependent equilibrium (Scheme I) between *trans*-stilbene molecules that are tightly bound to cyclodextrin, with a relatively long fluorescence decay time (of some hundreds of picoseconds), and other stilbene molecules, which are located near the exit of the hydrophobic cavity at the moment of light excitation and emit with a relatively short lifetime (of about 50 ps). A rapid interconversion between these two forms in the excited state can be excluded, since that would yield a single-exponential decay, a result contrary to our observations. Consequently, the observed two decay times can be regarded as average fluorescence lifetimes

SCHEME I: Schematic Representation of the Dynamic Equilibrium between "Loose" and "Tight" *trans*-Stilbene/Cyclodextrin Complexes



of "loose" (loosely bound) and "tight" (tightly complexed) forms of *trans*-stilbene, which interconvert slowly on the time scale of the photoisomerization. On the basis of the excited-state dynamics alone, we cannot assign the two forms to distinct spatial arrangements of the molecules in the complex. We prefer to identify the different decay rates (τ_1 and τ_2), however, with average spatial arrangements that can be classified by the above two types of complexes. The differences between the values of the decay times with different concentration ratios (*trans*-stilbene:β-cyclodextrin) is probably due to a different distribution within the two groups of complex forms, although the effect is small.

The equilibrium constant for the interconversion of the two forms of the complex can be expressed as

$$K_{eq} = [\text{tight}]/[\text{loose}] \tag{2}$$

The equilibrium constant can be obtained experimentally by recognizing the fact that the relative population of the tight vs loose forms at zero time is given by the ratio of the preexponential factors (A_1 and A_2; see eq 1).[29] We can write the preexponential factor A_i (i = 1 and 2) as[30]

$$A_i = f_i(\lambda_{exc})\phi_{fi}(\lambda_{exc})\tau_i^{-1} = f_i(\lambda_{exc})k_{fi}^0 \tag{3}$$

where $f_i(\lambda_{exc}) = \epsilon_i[C_i]/\sum_i\epsilon_i[C_i]$ and $\phi_{fi} = k_{fi}^0\tau_i$. It follows that

$$K_{eq} = \frac{A_2 k_{f_1}^0}{k_{f_2}^0 A_1} = \frac{A_2\tau_2^0}{A_1\tau_1^0} \tag{4}$$

provided that $\epsilon_1 = \epsilon_2$ at 266 nm and that the emission spectra of *trans*-stilbene in the tight and loose complexes are identical. We finally point out the fact that the excited-state lifetimes are short (which makes competing nonradiative quenching processes less important) as well as the fact that the cyclodextrin structure does not have any special groups that would lead to enhanced quenching of the stilbene excited state. With these considerations in mind, we make the additional assumption that the relative radiative quantum yield for the two species are the same ($\phi_{f_1}/\phi_{f_2} \approx 1$), which leads to the relation

$$K_{eq} = A_2\tau_2/A_1\tau_1 \tag{5}$$

The fact that the ratio $A_2\tau_2/A_1\tau_1$ was found to be independent of stilbene concentration is consistent with the model that the equilibrium between the loose and tight forms does not depend on concentrations and confirms that the interconversion is a first-order process.

The free energy change, $\Delta G = -RT \ln K_{eq}$, is then given by

$$\Delta G = -RT \ln (A_2\tau_2/A_1\tau_1) \tag{6}$$

Plotting ΔG as a function of temperature (Figure 5) yields a straight line, with the slope identified as ΔS and the intercept as ΔH, as given by eq 7. The calculated values of $\Delta H = -32 \pm 5$

$$\Delta G = \Delta H - T\Delta S \tag{7}$$

kJ/mol and $\Delta S = -103 \pm 23$ J mol^{-1} K^{-1} are in agreement with

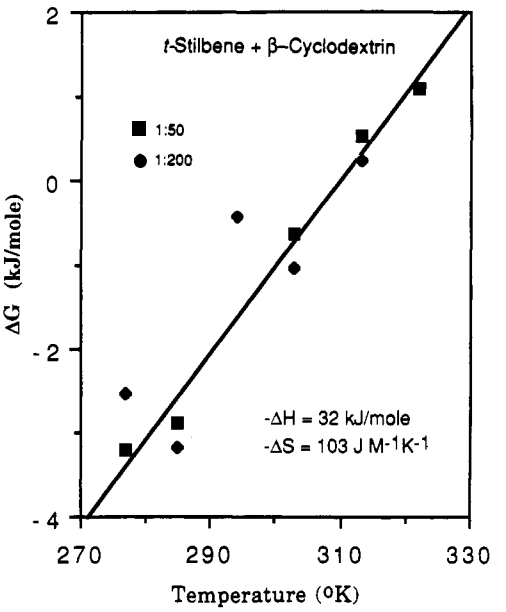


Figure 5. Free energy difference, $\Delta G = -RT \ln (A_2\tau_2/A_1\tau_1) = \Delta H - T\Delta S$, between the two types of *trans*-stilbene/β-cyclodextrin complexes (see text), as a function of temperature.

typical data of these thermodynamic parameters for similar processes of weakly bound complex formation (for example ΔH = −40 kJ/mol, ΔS = −100 J mol^{-1} K^{-1} for intermolecular excimer formation with pyrene.[31]

The appearance of positive values of ΔG in the diagram (Figure 4) is readily explained by the difference in binding strengths for the two complexes. At low temperatures, the tight complex is favored. Indeed, near the freezing point of the liquid (4 °C), the relative population of the tight complex is roughly 35% that of the loose complex. At elevated temperatures, only the loose form dominates, giving rise to the observed fast decay kinetics. The nonpolar ground-state stilbene molecule prefers the nonpolar cavity of the cyclodextrin, relative to the polar solvent, and thus will be thermodynamically more stable in a configuration that can optimize this. The tight complex affords such a nonpolar environment for the stilbene molecule, whereas the loose form does not. Although the tight form is thermodynamically more stable, we can easily see why the photoisomerization proceeds more slowly compared to the loose form. In the tight form, there will be greater restriction for molecular motion (a degree of freedom that is necessary for the trans–cis isomerization) and thus the rate of excited-state decay will be slower. For the loose form, which is preferred at elevated temperatures, the friction experienced by the stilbene molecule will be less and thus the rate of excited-state decay is fast. The results observed for the γ-cyclodextrin complex (which shows that there are two forms of the complex) also support this overall view. Finally, the α-cyclodextrin complex, owing to the fact that its small cavity can accommodate only one phenyl group of the stilbene molecule, yields only a single average form for the complex, and thus, the kinetics of photoisomerization is described by a single process. The reaction rate of photoisomerization of *trans*-stilbene complexed to α-cyclodextrin is to be compared to the tight form of the β-cyclodextrin complex. At room temperature, the α-cyclodextrin complex yields a decay time of 137 ps, but for the tight β-cyclodextrin complex, it is 450 ps. The slower reaction time for the tight form presumably reflects the fact that the stilbene molecule is imbedded more deeply into the cavity, which thus immobilizes the molecule more than in the case of the α-cyclodextrin complex, for which only a portion of the stilbene molecule can be encapsulated. A recent investigation of complexation of a stable nitroxide spin probe has been interpreted in terms of two slowly interconverting (on the EPR time

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scale) forms the probe–cyclodextrin complex.[32]

Conclusions

Using picosecond dynamic measurements of the photoisomerization reaction of *trans*-stilbene, we have been able to show the effect of restricted environments caused by complexation to cyclodextrins on reaction rates. We also determined that the nature of the complex depends on the cavity size. A single complex is formed between *trans*-stilbene and α-cyclodextrin molecules, resulting in a relatively slow single-exponential decay, compared to nonviscous homogeneous media. In the presence of β-cyclodextrin, on the other hand, two distinct complexes are formed that result in a double-exponential fluorescence decay of *trans*-stilbene. The nature of the two complexes for the large cavity cyclodextrins can be viewed as a tightly bound form (inside the cavity), leading to a relatively long decay time (comparable to the fluorescence lifetime in rigid environments), and a loose association, displaying a comparably fast decay of the stilbene fluorescence like in nonviscous media. In the case of complexation with β-cyclodextrin, values of $\Delta H = -32 \pm 5$ kJ/mol and $\Delta S = -103 \pm 23$ J mol^{-1} K^{-1} were determined for the differences of enthalpy and entropy between the two forms of complexes between *trans*-stilbene and β-cyclodextrin. It is emphasized that which type of complex is formed is determined by the size of the cyclodextrin cavity. With α-cyclodextrin, providing a fit for only part of the stilbene molecule, only a tightly bound complex is formed, whereas with β-cyclodextrin both types of complexes are formed. In summary, picosecond time-resolved methods permitted for the first time the identification of two distinctly different complexes that yield vastly different reaction dynamics for the photoisomerization reaction of *trans*-stilbene. The results provide a model for reactions in restricted environments.

Acknowledgment. We thank the Air Force Office of Scientific Research and the National Science Foundation for their generous support of this research. G.L.D. thanks DAAD (Deutscher Akademischer Austauschdienst) for a NATO fellowship during 1987/88.

Registry No. *trans*-Stilbene–α-cyclodextrin complex, 122383-06-6.

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Kinetic Study of Monomer and Excimer Fluorescence of Pyrene-Substituted Phosphatidylcholine in Phosphatidylcholine Bilayers

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We have shown by time-dependent fluorescence methods that pyrene fluorescence of 1-palmitoyl-2-pyrenedecanoyl-*sn*-glycero-3-phosphatidylcholine in the gel phase differs from that in the fluid phase. The mechanism can be explained by a rather simple scheme, which involves two separate paths for excimer formation produced by two intraphase configurations of pyrene moieties in the membrane. The first path is a diffusion-controlled excimer formation process with a rate constant of about 10^8 M^{-1} s^{-1}. The second path is a static process, where the pyrene moieties are aggregated in such a way that only a small rotational motion is required for them to attain the excimer configuration. The ratio of pyrene moieties in this state able to form excimers by a diffusional process to those able to form them by a static process can be calculated. The relative weighting factor between the states increases with concentration. The proposed scheme and calculated rate parameters are in good agreement with stationary measurements. Excimer formation in the gel phase is probably not controlled by lateral diffusion but by rotational processes, as the rate parameter for the excimer formation is about the same order of magnitude as in the fluid phase. Inconsistencies between the monomer and excimer decay parameters in the gel phase indicate that there are more than three excited species present.

Introduction

If information on the molecular dynamics and structural organization of biological membranes is to be obtained it is important to study both static and dynamic molecular interactions. In the past 5 years the use of fluorescent probes in biomembrane research has greatly increased. This is mainly due to the sensitivity and simplicity of the techniques used, coupled with the development of the necessary instrumentation in modern physicochemical and biophysical laboratories.

Two types of fluorescent probes have been employed. The first type is a fluorescent molecule, which does not have to be related to the component membrane molecule, but which strongly partitions into the hydrophobic interior of the bilayer. This kind of molecule is commonly used to monitor the microviscosity of its surroundings. The second type of probe is more or less closely related to the membrane molecule; it is usually its analogue with a covalently coupled fluorescence moiety in its hydrocarbon chain. This type of probe mimics the properties of the unlabeled lipid component of the membrane bilayer. If a fluorescent labeled lipid probe in the membrane system is used, it is possible to monitor the isomerization of lipid alkyl chains, rotational and wobbling diffusion, vertical fluctuation, and the lateral diffusion of fluorophores and unlabeled components. The deviation of the labeled lipid from the ideal behavior of the unlabeled lipid is a measure of the degree of its mimicry.

Molecular pyrene as well as pyrene-labeled lipids have been employed as fluorescent probes to study several phenomen involving membranes such as interbilayer lipid transfer,[1-5] lateral

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