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ENHANCEMENT OF INTRAMOLECULAR EXCIMER FORMATION OF 1,3-BICHROMOPHORIC PROPANES VIA APPLICATION OF HIGH PRESSURE AND VIA COMPLEXATION WITH CYCLODEXTRINS. PROTECTION FROM OXYGEN QUENCHING

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Abstract—The emission of several 1,3-bichromophoric (BC) systems has been investigated in aqueous solutions containing various cyclodextrins (CD's). In all cases where molecular models reveal that the correspondence of the size and shape of the eclipsed conformation of the BC system and the cavity CD is high, a stable CD (host)–BC (guest) complex is formed and in these cases, the ratio of excimer to monomer emission intensity (I_E/I_M) is much larger than that found in homogeneous solution. The effect of application of high pressure on the I_E/I_M ratio was investigated, as was the influence of O_2 quenching of excimer and monomer in homogeneous solutions, in aqueous micellar solutions, and in aqueous solutions of CD complexes. A strong protection from O_2 quenching is observed in the latter case.

INTRODUCTION

Inclusion complexes of aromatic compounds and cyclodextrins. Cyclodextrins form 1:1 complexes with a variety of organic molecules (Bender and Komiyama, 1978) in aqueous solution. These complexes have served as simple models for 'host–guest' systems that are held together by relatively strong non-covalent interactions. For example, cyclodextrins (CD's)‡ have been employed as models for the primary step of certain enzymes or of certain antigen–antibody reactions. The three commonly available cyclodextrins (α-CD, β-CD, and γ-CD) possess cavities with different inner diameters (~5, ~7 and ~9 Å, respectively). The felicitous matching of the 'host cavity' and the guest molecular volume appears to be a major factor in determining the equilibrium stability and conformational structure of cyclodextrin–molecule inclusion complexes. Depending on the dynamics of guest molecule exit from and entrance into the host cavity, complexation may serve to protect a hydrophobic guest from a hostile aqueous environment. In particular, an electronically excited guest molecule may be stabilized with respect to the unbound molecule in an aqueous or in a homogeneous hydrophobic environment. For example, if an electronically excited guest molecule is protected by inclusion from diffusional quenchers in the aqueous environment, an enhanced emission intensity and/or a longer emission lifetime may be observed.

If the correspondence of cavity size and host volume is a critical factor in determining the equilibrium stability of a complex, it is to be expected that stable complexes involving two (or possibly more) guest molecules are possible. As a variation of this possibility, a complex containing two guest groups linked by a flexible bond linkage should be possible.

Fluorescence probes have been employed to examine the molecule–cyclodextrin complexes involving inclusion of a guest molecule (or of guest molecules) in the hydrophobic cavity. For instance, in the presence of β-CD or γ-CD, sodium 8-anilianonaphthalene-1-sulfonate (ANS) exhibits a very strong fluorescence, whereas in aqueous solution or in the presence of α-CD, ANS shows only a weak fluorescence (Cramer *et al.*, 1967). These observations are interpreted in terms of the formation of 1:1 complexes of ANS and both β-CD and γ-CD, the latter hosts possessing an appropriate cavity size for an ANS guest. On the other hand, α-CD, whose cavity size is too small to comfortably include ANS, does not form comparably stable complexes with ANS.

Evidence for 2:1 guest–host complexes is available from the observation of enhanced excimer formation upon addition of γ-CD to aqueous solution of 1-naphthyl acetate (Ueno *et al.*, 1980). The corresponding effect is absent when α-CD or β-CD is added to aqueous solutions of 1-naphthyl acetate. Thus, the larger cavity of γ-CD, (as confirmed from molecular models) is evidently capable of including two molecules of 1-naphthyl acetate, whereas the

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‡*Abbreviations:* BC, bichromophoric; bichromophore; CD, cyclodextrin; I_E/I_M, the ratio of excimer to monomer emission intensities; ANS, sodium 8-anilinonaphthalene-1-sulfonate; DNP, 1,3-di-α-naphthylpropane; DNK, 1,3-di-α-naphthylpropan-2-one; DNL, 1,3-di-α-naphthylpropan-2-ol; PNK, 1-phenyl-2-α-naphthylpropan-2-one; PNA, 1-phenyl-2-α-naphthylpropan-2-ol.

smaller cavities of α-CD and β-CD can accommodate only one such guest molecule.

It is also possible for two different guest molecules to be included in a CD cavity, as evidence by the enhancement of fluorescence of aqueous solutions of γ-CD and 1-naphthyloxyacetic acid upon addition of cyclohexanol (Ueno *et al.*, 1981). This result is interpreted in terms of a 1:1:1 complex of naphthyloxyacetic acid–cyclohexanol–γ-CD, with the cyclohexanol serving as a molecular 'space regulator'.

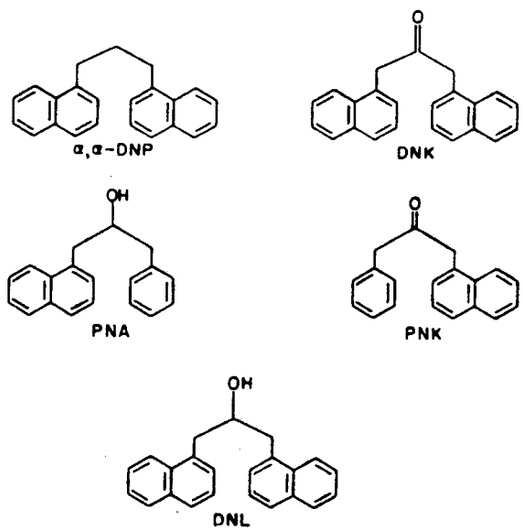
Photoluminescence probes. Photoluminescence of intrinsic or extrinsic probes is an important tool for the investigation of the structure and dynamics of macromolecules and especially of biomolecules (Fendler and Fendler, 1975). The bulk of published work at ambient temperature and pressure in aqueous solution involves the use of fluorescence methods by probes that have fast (< 20 ns) radiative rates. It is widely perceived that the phosphorescence and long lived fluorescence is generally too weak to be detected under the latter experimental conditions, because an inherently slow rate of emission allows radiationless quenching to dominate the deactivation of triplets and long-lived singlets. In particular, O_2 is considered as an important, effective and ubiquitous quencher of long-lived emission.

Luminescence probes with long (> 50 ns) lifetimes would provide information that extends and complements that which is available from the typical short-lived fluorescence probes: a longer lifetime provides (1) a greater opportunity for quenching and therefore is a more sensitive probe of accessibility and (2) a means of measurement of long rotational correlation times (characteristic of macromolecules). Furthermore phosphorescence and long-lived excimer fluorescence, provide useful spectral separation of the observed emission wavelengths and the exciting wavelength.

The phosphorescence of the tryptophan residues of proteins can be observed at room temperature in fluid solutions (Kai and Imakubo, 1979; Saviotti and Galley, 1974), a result which suggests that certain tryptophan residues 'buried' in the interior of the protein are remarkably protected from the typical bimolecular quenching processes which normally decrease the quantum yield of phosphorescence to immeasurably low values. Indeed, in the case of horse liver alcohol dehydrogenase, tryptophan residue phosphorescence is observed even in aerated solutions at ambient temperature in fluid (yet viscous) solution (Saviotti and Galley, 1974). This latter observation is most remarkable in view of the high diffusivity of the O_2 molecule and its general effectiveness at quenching long-lived emission (Geiger and Turro, 1975). It has been proposed that certain protein conformations strongly inhibit quenching of tryptophan triplets by dissolved oxygen, but that conformational fluctuations periodically 'expose' the 'buried' tryptophan triplets and thereby render them susceptible to O_2 quenching. In such a situation, certain dynamic aspects of protein structure may be probed by measuring phosphorescence quenching by O_2. Knowledge of the details of this protection from O_2 quenching could be of great utility in the design of mechanisms for protection of aromatic systems in proteins against oxidation and in the elucidation of respiration mechanisms. With this background in mind, we were curious to determine whether protection against O_2 quenching would occur when aromatic lumophores are included in the hydrophobic cavity of a CD molecule. Two other aspects of inclusion were of interest in our investigations: (1) how effective is inclusion in the induction of conformation changes of flexible bichromophoric systems?, and (2) how does the application of high pressure influence the equilibrium constants of inclusion and conformational structures?

MATERIALS AND METHODS

Materials. Bichromophores (BC) used in this study (see Scheme 1 for structures) are 1,3-di-α-naphthylpropane (DNP), 1,3-di-α-naphthylpropan-2-one (DNK), 1,3-di-α-naphthylpropan-2-ol (DNL), 1-phenyl-2-naphthylpropan-2-one (PNK) and 1-phenyl-2-naphthylpropan-2-ol (PNA). The preparation and the purification of DNP, DNK and PNK were accomplished by literature methods (Chandross and Dempster, 1970; Antonius *et al.*, 1979; Weed, 1981).



Scheme 1. Monomer/excimer guest probes.

DNL and PNA were prepared by quantitative reduction of the corresponding ketones with sodium borohydrode and crystallization of products with hexanes. α-, β- and γ-Cyclodextrins (Aldrich) were used as received.

Absorption- and fluorescence-spectra and lifetime measurements. UV absorption spectra were taken on a Cary 118 Spectrophotometer, emission spectra were recorded on a SPEX Fluorlog fluorimeter and a Hitachi–Perkin–Elmer spectrophotometer. Lifetimes were measured using the photon-counting technique as described earlier (Turro and Aikawa, 1980). The details of the high pressure cell were described in a previous paper (Turro and Okubo, 1981). Aqueous solutions of the CD's were made up and stirred with ca 100 μ*M* BC for at least 2 days. The concentration of the probe was determined from the absorption of filtered solutions.

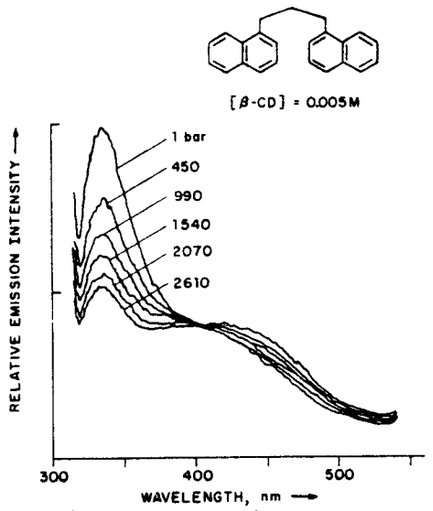


Figure 1. Fluorescence spectra of aqueous solution of saturated DNP in the presence of β-CD ($5_m M$) under high pressure at 25°C. [DNP] = $\sim 2\,\mu M$. Pressure values in bars are given in the figure.

RESULTS

Evidence for DNP complexes with β-CD and γ-CD

Saturated aqueous solutions ($\sim 0.3\,\mu M$) of DNP show a very weak emission consisting mainly of monomer ($I_E/I_M \sim 0.01$). Based on the emission intensity and solubility characteristics, no complexation occurs when α-CD is added to aqueous solutions of DNP. By the same criterion, however, complexes are formed between β-CD and γ-CD (Figs. 1 and 2). For the β-CD:DNP complex ([β-CD] = 5 mM), the ratio of excimer to monomer emission increases ($I_E/I_M \sim 0.3$) substantially over the value in pure water ($I_E/I_M \sim 0.01$) and, remarkably, the emission of the γ-CD:DNP complex ([γ-CD] = 5 mM) is due almost entirely to the excimer ($I_E/I_M > 10$). The quantum yield of excimer fluorescence is 0.074 for [γ-CD] = 10 mM and [DNP] = 10 μM. When the concentration of γ-CD is increased, the monomer and excimer emissions are enhanced in strength with the latter emission being more strongly enhanced. This result is suggestive of an association equilibrium between DNP and γ-CD.

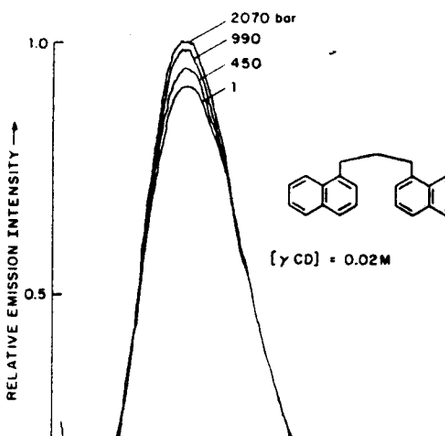
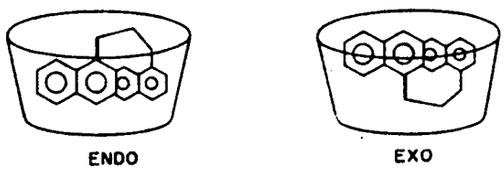


Figure 2. Fluorescence spectra of aqueous solution of saturated DNP in the presence of γ-CD (0.02 M) under high pressure at 25°C. [DNP] = $\sim 10\,\mu M$. Intensity scale is larger than that of Fig. 2 by a factor of 25.



Scheme 2. Possible conformations of DNP in γ-cyclodextrin.

From solubility data the equilibrium constants, K_{eq}, for complexation of DNP with α-CD, β-CD and γ-CD were found to be ~ 0, $840 \pm 150\,M^{-1}$, and $5800 \pm 2000\,M^{-1}$, respectively.

Molecular models reveal that two structurally different complexes which favor excimer formation are plausible (Scheme 2). In one structure (endo) the naphthalene rings are eclipsed and buried in the cavity of the CD; in the other structure (exo) the naphthalene rings are eclipsed, but exposed to the aqueous environment. On the basis of protection from O_2 quenching (*vide infra*) we suggest that the endo structure is favored.

The emission behavior of CD complexes with other bichromophoric systems

Relative intensities of excimer and monomer emission, I_E/I_M, are listed in Table 1 for some other BC probes. Emission features of DNK in the presence of three kinds of CD's were similar to those of DNP. However, the alcoholic derivative, DNL, showed relatively weak excimer emission with γ-CD. This may be due to strong interactions of the OH group of the probe with γ-CD via hydrogen bonding, which could retard the inclusion. PNK was found to show strong excimer formation in the presence of β-CD as well as γ-CD. The smaller size of PNK compared with DNP or DNK may be the principal factor involved in determining the observed I_E/I_M values. For PNA, a significant excimer peak appeared in the presence of γ-CD only. From these results, it may be concluded that the size, shape of guest or host molecule, and the specific interactions between them are important for determining the relative magnitude of monomer and excimer emissions.

Oxygen quenching

The data in Table 2 provides a summary of the effectiveness of O_2 quenching of DNP monomer and

Table 1. Relative intensity of excimer and monomer emission of some 1,3-bichromophoric propane probes

Probe*	H_2O	Hexanes	α-CD	β-CD	γ-CD
DNK†	~0.1	7	0.2	0.7	4
DNL†	~0.2	1	0.2	0.4	0.5
PNK‡	~0.1	2	0.1	4	4
PNA‡	~0.04	0.08	0.04	0.07	0.5

*[Probe] = 0.1 mM; [CD] ~ 5 mM.
†$\lambda^{M}_{max} \cong 340$ nm; $\lambda^{E}_{max} \cong 420$ nm.
‡$\lambda^{M}_{max} \cong 340$ nm; $\lambda^{E}_{max} \cong 395$ nm.

excimer emission under various conditions. The pertinent parameter is the emission lifetime, under nitrogen purging, air purging and oxygen purging. Although the bulk concentration of O_2 is not known for these systems, a rough measure of the effectiveness of protection can be estimated by a comparison of the lifetimes under 1 atm of N_2 and under 1 atm of O_2. By this method it is seen that γ-CD provides substantial protection of both excimer and monomer emission, whereas in homogenous solution nearly all of the monomer and excimer emission can be quenched by O_2.

Emission of DNP in cyclodextrins as a function of applied pressure

The effect of applied pressure on the excimer/monomer intensity ratio for DNP in β-CD and in γ-CD is summarized in Figs. 1 and 2, respectively. It is seen that for the β-CD:DNP system, the I_E/I_M ratio increases sharply as the pressure increases, but for the γ-CD:DNP system, the I_E/I_M ratio is relatively invariant upon application of pressure.

Table 2. Fluorescence lifetimes of DNP under various conditions at 25°C

System	Emission*	τ, ns N_2	Air	O_2	%Q†
γ-CD	E	90	91	87	3
	M	43	43	38	11
β-CD	E	42	35	33	21
	M	23	21	17	26
HDTBr	E	28	26	21	25
	M	9	9	8	11
HDTCl	E	47	42	23	51
	M	27	25	19	30
SDS	E	38	32	23	39
	M	18	17	13	28
CH_3CN	E	50	10	<3	
	M	28	6	3	89
C_2H_5OH	E	29	13	5	83
	M	9	6	3	67
n-Hexane	E	32	7	2	94
	M	8	4	2	75

*E = Excimer emission; λ_{max} 420 nm; M = Monomer emission; λ_{max} 350 nm.
†Fractional quenching = $(\tau_{N_2} - \tau_{O_2})/\tau_{N_2}$; values approximate only.

Table 3. Influence of pressure on the excimer to monomer intensity ratio for naphthalene in aqueous solutions of cyclodextrins at 25°C

Pressure (bar)	I_E/I_M α†	β‡	γ§
1	0.01	0.22	0.08
1540	—	0.40	0.10
2610	—	0.41	0.10

*Cyclodextrin concentration fixed at 5 mM.
†α-Cyclodextrin. Naphthalene concentration ~0.1 mM.
‡β-Cyclodextrin. Naphthalene concentration ~0.2 mM.
§γ-Cyclodextrin. Naphthalene concentration ~0.1 mM.

Emission of naphthalene in cyclodextrins as a function of applied pressure

As evidenced from the enhancement of fluorescence, naphthalene forms complexes with β-CD and γ-CD, but not with α-CD. Excimer emission is much stronger, relative to monomer emission, upon complexation with γ-CD ($I_E/I_M \sim 0.22$) than upon complexation with β-CD ($I_E/I_M \sim 0.08$ at 1 bar). For the β-CD:naphthalene complex, application of high pressure results in a significant increase in the relative excimer emission ($I_E/I_M \sim 0.41$ at 2600 bar); a similar, but smaller, increase results when high pressure is applied to the γ-CD:naphthalene complex ($I_E/I_M \sim 0.1$ at 2100 bar; Table 3 and Fig. 3).

DISCUSSION

Chemical reactions generally involve molecular collisions which provide activation and which bring

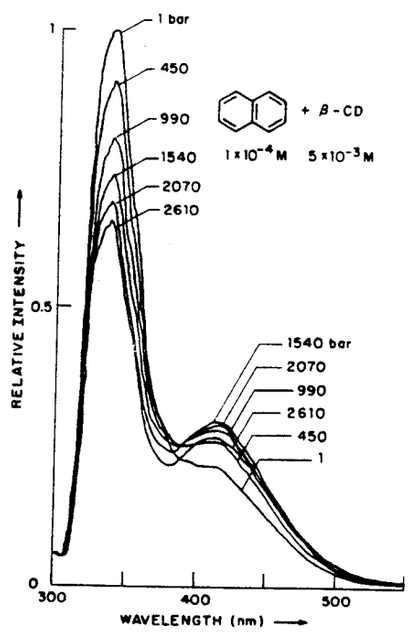


Figure 3. Fluorescence spectra of naphthalene in the presence of β-CD in aqueous media. Pressure influence. [Naphthalene] = 0.1 mM. [β-CD] = 5 mM.

reactants into reactive configurations. Complexation of reactants can provide a means of special rate acceleration or retardation depending on the configuration of the reactants in the complex. The cavities of the CDs provide a fascinating medium of restricted space which can bind individual aromatic groups, intermolecular pairs of aromatic groups or intramolecular paris of aromatic groups. In the latter case the CDs can induce or select conformations in the reactant(s).

Our results demonstrate, in agreement with other recent investigations (Emert *et al.*, 1981), that a 'proper fit' of the equilibrium concentration of the eclipsed conformations of 1,3-bichromophoric propanes can be greatly enhanced by inclusion in a CD cavity. The simple and convenient ratio of excimer to monomer emission serves as a probe of such conformation effects.

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