The Intramolecular Loss of Fluorescence by Lysine Derivatized with Naphthalenedialdehyde

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Derivatization of primary amines such as amino acids and peptides with naphthalenedialdehyde (NDA) in the presence of cyanide ion yields cyanobenzo[f]-isoindole (CBI) adducts that are highly fluorescent. However, the fluorescence is seriously quenched with amines that possess more than one primary amine site, as is the case with lysine. Although it was found that the adsorption of CBI_2-lysine on a solid substrate restored the fluorescence, the reason for the solution quenching, with respect to results for mono-derivatized amines, was investigated. The experiments to probe the quenching were based on the assumption that the mechanism responsible for quenching involved a charge-transfer (CT) excited state. Thus, it was found that the solvent properties of viscosity and polarity affected the lifetime and quantum yield of fluorescence in a manner consistent with the proposed mechanism.

Index Headings: Fluorescence; Analytical methods.

INTRODUCTION

Tagging of biological substances by derivatization to yield highly fluorescent products is an important strategy to achieve high-sensitivity liquid chromatographic analysis. For example, naphthalenedialdehyde (NDA) in the presence of cyanide ion is an excellent reagent for the derivatization of primary amines, such as amino acids and peptides, to give the fluorescent adduct, the cyanobenzo[f]isoindole (CBI).[1-4] These adducts have fluorescence quantum yields approaching unity and exhibit exceptional stability, making possible pre-column derivatization schemes for LC analysis. Thus, high-sensitivity detection in the attomole range has been reported for several different CBI-amines with laser-induced fluorescence detection in HPLC.[5-7]

One limitation of the NDA/CN reagent occurs when the primary amine possesses more than one derivatization site. The product of the derivatization, such as in the case of lysine, is CBI_2-lysine, which exhibits a substantially lower fluorescence quantum yield than those observed for the mono-derivatized CBI-amines.[8] However, the quantum yield increases substantially when CBI_2-lysine is adsorbed on a solid TLC substrate, becoming similar to that of the mono-derivatized amine.[9] For example, in Fig. 1 the fluorescence excitation and emission spectra of CBI_2-lysine is compared with those of CBI-alanine when they are both adsorbed on a TLC matrix. The fluorescence emission intensities are similar in magnitude in the adsorbed case; whereas in solution, a 20-fold difference exists, with CBI_2-lysine having a much lower quantum yield ($\phi_f = 0.02$).

The above observations stimulated the present investigation to find the reason for the loss of fluorescence for

a multi-derivatized amine such as lysine. A possible mechanism to explain the solution quenching of this CBI_2-lysine is the formation of a charge-transfer (CT) state, which competes favorably with the fluorescence de-excitation from the first, locally excited (LE), state. Such a CT state would require a molecular and conformational structure that is not possible with mono-derivatized CBI adducts. These CT or excimer states have been well documented in the literature. For example, naphthylalkyl-amines exhibit intramolecular exciplex formation, in which a decrease in the temperature of the solvent (higher viscosity) results in an increase in the fluorescence from the monomeric naphthalene and a corresponding decrease in the exciplex emission.[10] The viscosity dependence for excimer fluorescence is due to the fact that the electron donor and acceptor must attain a "sandwich" conformation during the lifetime of the excited state.[11-13] An increase in the viscosity serves to lower the rate at which this conformation can be attained.

Charge transfer complexes have been shown to be stabilized by increasing the polarity of the solvent. Amiri[14] demonstrated that the quantum efficiency and decay time of the intramolecular charge transfer complex of 4-di-cyanomethylene-1,2,3,4-tetrahydromethylquinoline increased as the polarity of the solvent was increased. p-Dimethylamino-benzonitrile (DMABN) exhibited dual fluorescence only in polar solvents with the absence of the CT fluorescence band in nonpolar solvents.[15] Eisenthal and co-workers[16,17] determined that the rate of CT formation was dependent on the polarity of the solvent, the rate increasing with more polar solvents. In addition, the CT fluorescence band of DMABN was found to be absent in very rigid matrices due to the inability of the molecule to undergo the necessary conformational change associated with CT formation.[18]

The fluorescence properties of CBI_2-lysine are compared to those of CBI-alanine in this study. Their spectral properties and fluorescence yields have been previously documented.[3] CBI-alanine possesses only one derivatization site, while CBI_2-lysine has two possible sites. The proposed energy diagram showing energy states for CBI_2-lysine is presented in Fig. 2. There are three manifolds for depopulating the LE state of CBI_2-lysine. Decay to the ground state can occur via fluorescence (k_{AF}) or through a nonradiative process at a rate designated as k_{AN}. In addition, depletion of population from the LE state can occur through formation of a proposed CT state (k_{AB}). The rate or efficiency of CT formation should be affected by the solvent viscosity, if formation of the CT state requires a conformational change during the lifetime of the excited state. Solvent polarity also affects the rate of CT formation, since an increase in the dipole
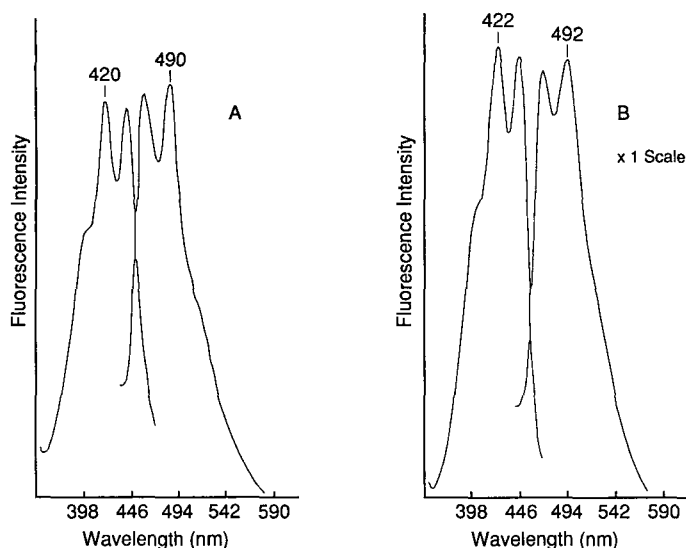
FIG. 1. The fluorescence excitation and emission spectra of 1×10^{-5} M of CBI-alanine and CBI_2-lysine deposited onto an aluminum oxide TLC surface. The emission spectra were obtained at an excitation wavelength of 420 nm.

FIG. 2. The proposed model for the intramolecular fluorescence loss of CBI_2-lysine. Energy level diagram of (1) CBI_2-lysine and (2) CBI-alanine.

moment of the CT state would be favored in more polar solvents. This rate can be indirectly evaluated as a function of solvent viscosity and polarity by monitoring how these solvent parameters affect the fluorescence quantum efficiency and lifetime of the LE state.

The energy diagram for CBI-alanine is also shown in Fig. 2. The formation of a CT state cannot occur in this derivative due to the lack of a second isoindole ring (electron acceptor or donor). Thus, only fluorescence and nonradiative processes serve to depopulate the excited state. The fluorescence quantum efficiency and lifetime of CBI-alanine, measured and compared with those obtained for the CBI_2-lysine system in solvents of various viscosities and polarities, are discussed herein.

EXPERIMENTAL

Reagents and Instrumentation. Alanine, lysine, and sodium cyanide were obtained from Sigma Co. (St. Louis, MO) and used without further purification. Naphthalenedialdehyde (NDA) was synthesized and purified according to published procedures.[1] All solvents were obtained from Aldrich Co. (Milwaukee, WI) and used as received. Fluorescence spectra were acquired on a Perkin-Elmer LS-5 fluorescence spectrophotometer (Oak Brook, IL). Fluorescence quantum efficiencies were calculated relative to CBI-alanine in 100% H_2O as a standard, since its quantum efficiency has been reported in the literature.[3] Fluorescence lifetimes were measured by the phase/modulation technique on an SLM Aminco 4800 spectrofluorometer (Urbana, IL) with modulation frequencies of 6, 18, and 30 MHz. This instrument allowed the measurement of lifetimes as short as 1 ns. Constant temperatures were maintained by circulating water/ethylene glycol from a refrigerated circulating bath (Neslab, Newington, NH) through a jacketed fluorescence cuvette (Uvonic Instrument Inc., Plainview, NY). All solutions were purged with N_2 prior to spectral acquisition. Molecular modeling was performed on a Digital Equipment
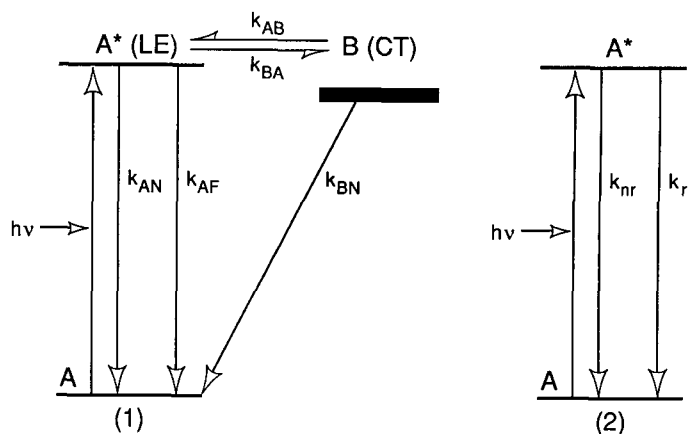
MicroVAX II computer (Maymard, MA) utilizing MacroModel version II software (Columbia University).

Synthesis of the CBI-amines. To a two-necked round-bottom flask, 25 mL of methanol, 147.2 mg of NDA (0.8 mmol), and 40 mg of NaCN (0.8 mmol) were added and mixed. One neck of the flask was stoppered with a septum, and a stream of argon was introduced through the other. The reaction flask was immersed into an acetone/dry-ice bath so that low temperatures could be maintained. The amine was dissolved in 1 mL of H_2O and introduced into the reaction flask via a syringe through the septum. The mixture was allowed to react for ~1 h with constant stirring. After the reaction was completed, the solvent was removed under reduced pressure with a residue remaining that was green (CBI-alanine) or yellow (CBI_2-lysine). The residue was dissolved in a small amount of methanol and then recrystallized with ether. The isolated crystals were filtered and dried under a stream of argon. The purity and identification of the derivatives were confirmed by proton and C-13 NMR and IR spectroscopy.

RESULTS

The electronic absorption spectra of CBI-alanine and CBI_2-lysine are very similar, with both showing absorption maxima at ~420 and 440 nm. The positions of these maxima were unaffected by changes in solvent polarity or viscosity. Measurement of the molar absorptivities for both adducts indicated a twofold increase for CBI_2-lysine, in comparison to CBI-alanine. The fluorescence emission spectra of these two derivatives displayed a maximum emission at ~485 nm, with the position of this maximum being insensitive to changes in the properties of the solvent. Careful inspection of the fluorescence emission spectra of CBI_2-lysine failed to show the presence of any band red-shifted from the fluorescence band at 485 nm. The only observed spectral difference between the fluorescence emission of CBI-alanine and CBI_2-lysine was the intensity of the emission, the former being more intense. The similarities in the wavelength maxima of the absorption and emission spectra of both adducts indicate that the ground and fluorescent electronic states of CBI_2-lysine are very similar to those of CBI-alanine.

Solvent	η (cp)	$E_t(30)$ (kcal/mole)[b]
Glycerol (25)	954	57.0
Water (20)	1.004	63.1
Butanol (20)	2.948	50.2
Butanol (40)	1.77	
Hexadecane (20)	3.34	30.8
Methanol (20)	0.597	55.5
Chloroform (20)	0.58	39.1
Ethanol (0)	1.77	51.9
Propanol (30)	1.77	50.7

[a] Temperatures are in degrees Celsius.
[b] Taken from Ref. 20.

The value of the radiative rate constant, k_r, of the LE state for CBI-alanine and CBI_2-lysine can be calculated from the integrated absorption and fluorescence spectra according to Eq. 1:[19]

$$k_r = 2.88 \times 10^9 \eta^2 \langle \nu_f^3 \rangle_{AV}^{-1} \int \epsilon \, d\ln \nu \qquad (1)$$

where η is the refractive index of the given solvent, $\langle \nu_f^{-3} \rangle_{AV}^{-1}$ is obtained experimentally from the integration of the fluorescence emission spectrum and the integration of the same spectrum multiplied by the cubed intensity value at each frequency. $\int \epsilon \, d\ln \nu$ is the integrated extinction coefficient, with k_r being the radiative rate constant (k_{AF} in Fig. 2, diagram 1). The value of k_r calculated from Eq. 1 for CBI-alanine in 90:10 MeOH:H_2O is 0.044 ns^{-1}. A similar value of 0.039 ns^{-1} is obtained for CBI_2-lysine. The radiative rate constant for CBI-alanine was also calculated through the use of Eqs. 2 and

TABLE II. The fluorescence lifetime (τ_f) and quantum efficiencies (ϕ_f) for CBI-alanine and CBI_2-lysine in butanol/hexadecane binary mixtures. The radiative (k_r) and nonradiative (k_{nr}) rate constants for CBI-alanine are also presented (calculated with Eqs. 2 and 3).[a]

CBI alanine

% Butanol	τ_f (ns)	ϕ_f	k_r (ns^{-1})	k_{nr} (ns^{-1})
90	13.3 (±0.7)	0.62 (±0.03)	0.047	0.027
80	13.0 (±0.6)	0.63 (±0.03)	0.045	0.027
70	14.3 (±0.7)	0.62 (±0.02)	0.043	0.027
60	14.1 (±0.7)	0.64 (±0.03)	0.046	0.026
50	13.6 (±0.6)	0.65 (±0.02)	0.048	0.026

CBI_2-lysine

% Butanol	τ_f (ns)	ϕ_f	ϕ_f/τ_f
90	3.1 (±0.3)	0.10 (±0.01)	0.032
80	3.9 (±0.3)	0.12 (±0.01)	0.031
70	5.7 (±0.2)	0.13 (±0.01)	0.023
60	7.4 (±0.3)	0.16 (±0.01)	0.022
50	9.0 (±0.4)	0.19 (±0.01)	0.021

[a] The standard deviations are given in parentheses.

TABLE III. The fluorescence quantum efficiencies (ϕ_f) and lifetimes (τ_f) of CBI-alanine and CBI_2-lysine in methanol/chloroform binary mixtures. The radiative (k_r) and nonradiative (k_{nr}) rate constants for CBI-alanine are also presented.[a]

CBI-alanine

% Methanol	τ_f (ns)	ϕ_f	k_r (ns^{-1})	k_{nr} (ns^{-1})
100	12.2 (±0.6)	0.70 (±0.03)	0.055	0.025
80	11.2 (±0.8)	0.68 (±0.03)	0.061	0.029
60	12.1 (±0.7)	0.67 (±0.02)	0.055	0.027
40	13.1 (±0.8)	0.71 (±0.04)	0.054	0.022
20	11.9 (±0.7)	0.66 (±0.03)	0.055	0.028

CBI_2-lysine

% Methanol	τ_f (ns)	ϕ_f	ϕ_f/τ_f
100	1.3 (±0.3)	0.030 (±0.005)	0.023
80	2.2 (±0.3)	0.048 (±0.005)	0.022
60	2.8 (±0.3)	0.059 (±0.007)	0.021
40	4.1 (±0.4)	0.069 (±0.006)	0.017
20	5.3 (±0.4)	0.078 (±0.007)	0.015

[a] The standard deviations are given in parentheses.

3, from the measured fluorescence quantum efficiency (ϕ_f) and lifetime (τ_f):

$$\phi_f = k_r/(k_r + k_{nr}) \qquad (2)$$
$$\tau_f = 1/(k_r + k_{nr}) \qquad (3)$$

where k_{nr} is the nonradiative rate constant. k_r calculated from these relationships resulted in values that agreed favorably with those from the use of Eq. 1 for the CBI-alanine system ($k_r = 0.050$ ns^{-1} in 90:10 MeOH:H_2O).

The influence of the solvent polarity upon the fluorescence emission properties of the CBI-derivatives was investigated in isoviscous solvents in order to minimize viscosity effects.[16,17] Binary mixtures of solvents whose room-temperature viscosities are equivalent but whose polarities differ dramatically were used. The selected solvents with the values of their viscosities and polarities, as measured by the empirical $E_t(30)$ scale,[20] are listed in Table I.

Butanol and hexadecane have similar viscosities (at 20°C), but differ in their $E_t(30)$ values, butanol being the more polar solvent. Therefore, the polarity of the binary mixture can be altered without any significant viscosity change. The fluorescence quantum efficiencies and lifetimes of CBI-alanine and CBI_2-lysine, as measured in various butanol/hexadecane compositions, are shown in Table II. The values of k_r and k_{nr} for CBI-alanine, as calculated with Eqs. 2 and 3, also appear. The quantum efficiency and lifetime of CBI-alanine, as well as the k_r and k_{nr} values, are invariant to changes in the solvent polarity. However, for CBI_2-lysine, both ϕ_f and τ_f increase with a decrease in the solvent polarity.

Methanol and chloroform also have similar viscosities, but differ in their $E_t(30)$ values (see Table I). This binary solvent mixture was also investigated for its effect on the
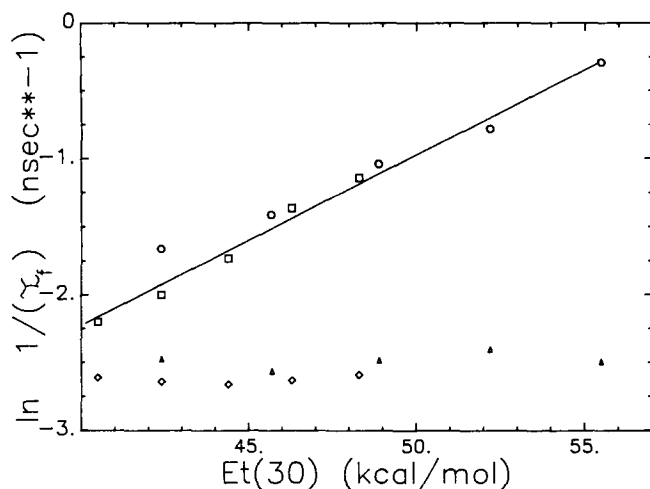
FIG. 3. Values of ln(1/τ_f) versus solvent E_t(30) are plotted for CBI$_2$-lysine and CBI-alanine. (□) CBI$_2$-lysine and (◇) CBI-alanine in butanol/hexadecane binary mixtures. (O) CBI$_2$-lysine and (△) CBI-alanine in methanol/chloroform binary solvent mixtures.

FIG. 4. Plot of ln (1/τ_f versus 1/T for CBI$_2$-lysine in the alcohol series ethanol, propanol, and butanol.

fluorescence properties of CBI-alanine and CBI$_2$-lysine. The results are tabulated in Table III. Again, the quantum efficiency and lifetime, as well as k_r and k_{nr}, for CBI-alanine, remain constant with respect to changes in the solvent polarity. For CBI$_2$-lysine, both ϕ_f and τ_f exhibited an increase as the solvent polarity decreased. In Fig. 3, the values of ln(1/τ_f) vs. E_t(30) for CBI-alanine and CBI$_2$-lysine in the binary mixtures of butanol/hexadecane and methanol/chloroform are plotted. A linear dependence of ln(1/τ_f) on the solvent polarity is seen for CBI$_2$-lysine (r = 0.994) indicating a dramatic change in the dipole moment for the excited state of CBI$_2$-lysine. In the case of CBI-alanine no such dependency on solvent polarity is observed.

The second method to achieve an isoviscous condition in order to investigate the polarity effects upon the fluorescence properties of CBI$_2$-lysine is to incorporate neat solvents whose E_t(30) values differ but whose viscosities may be adjusted to a given value by changing the temperature. The solvents with the temperatures necessary to maintain a viscosity of 1.77 cp are listed in Table I. The results are presented in Table IV for CBI$_2$-lysine. In the alcohol series of ethanol, propanol, and butanol, an increase in the E_t(30) value of the solvent resulted in a corresponding decrease in the fluorescence quantum efficiency and lifetime, irrespective of the lower temperature necessary to maintain the isoviscous condition in the more polar solvents. These results are in accordance with the polarity dependence previously obtained. The

rate of CT state formation increases in more polar solvents.

An *effective* activation energy, E_a, between the LE and CT states of CBI$_2$-lysine can be determined from an Arrhenius-type relationship:[16,17]

$$\ln 1/\tau_f = \ln Bf(\eta) - E_a/RT \qquad (4)$$

where B is the pre-exponential frequency factor and $f(\eta)$ is the viscosity function (which is constant for the isoviscous solvents). A plot of ln (1/τ_f) versus 1/T is shown in Fig. 4. The plot is linear, yielding a negative activation energy, E_a, of −0.90 kcal/mole.

The effect of solvent viscosity on the fluorescence of CBI-alanine and CBI$_2$-lysine was evaluated with glycerol/water binary mixtures. Glycerol and water have nearly the same E_t(30) values, thereby minimizing polarity effects upon the fluorescence properties. However, their room-temperature viscosities are dramatically different (see Table I). The results are summarized in Table V. The fluorescence quantum efficiency and lifetime of CBI-alanine as well as its radiative and nonradiative rate constants were unaffected by changes in the solvent viscosity. For CBI$_2$-lysine, the fluorescence quantum efficiency and lifetime were observed to increase with an increase in the solvent viscosity.

DISCUSSION

The similarity in the shape and wavelength maxima of the visible absorption spectra of CBI-alanine and CBI$_2$-lysine indicates that significant electronic interaction between the isoindole rings in the ground-state conformation of CBI$_2$-lysine is absent. This factor, coupled with the molar absorptivity of CBI$_2$-lysine being twice that of the CBI-alanine system, with the maximum in the fluorescence emission occurring at approximately the same wavelength and with the position of this maximum being insensitive to the properties of the solvent, suggests that the observed fluorescence of CBI$_2$-lysine occurs from an excited electronic state nearly identical to that of CBI-alanine. We label this state the locally excited (LE) state.

The polarity of the solvent does not affect the energetics of the LE state. However, it does affect the lifetime by influencing the rate of formation of a state which

TABLE IV. The fluorescence quantum efficiencies (ϕ_f) and lifetimes (τ_f) for CBI$_2$-lysine in neat alcohols at their isoviscous temperatures.[a]

Alcohol	τ_f (ns)	ϕ_f
Ethanol	2.4 (±0.2)	0.046 (±0.006)
Propanol	2.8 (±0.4)	0.060 (±0.007)
Butanol	3.0 (±0.3)	0.070 (±0.008)

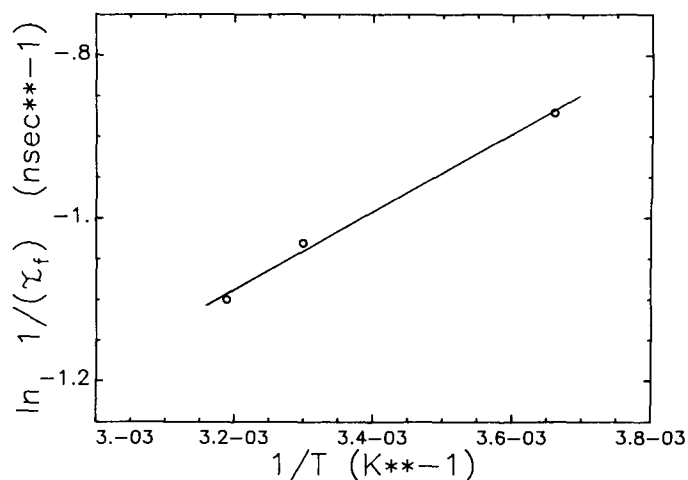[a] The standard deviations are given in parentheses.

competes favorably to diminish the fluorescence quantum yield. The potential energy of such a CT state is expected to be lowered with respect to the LE state as the solvent polarity is increased. This lowering reflects the solvent interaction with the charge-separated CT state. The fluorescence quantum yield and the lifetime of the LE excited state thus correspondingly decrease as the solvent polarity is increased, in accordance with the proposed mechanism.

The radiative rate of the fluorescence lifetime is a function of the five rate constants, as proposed for the levels in Fig. 2. If it is assumed that the values of k_{AF} and k_{AN} are similar to the radiative and nonradiative rate constants for CBI-alanine and that k_{BN} is independent of the solvent properties, then only k_{AB} and k_{BA} are affected by changes in the polarity of the solvent. In more polar solvents, the equilibrium ($K_{eq} = k_{AB}/k_{BA}$) will favor the CT state of CBI_2-lysine with respect to the LE state. The results given in Fig. 3 are consistent with these conclusions. The slope of the plot in Fig. 3 (0.125) reflects the sensitivity of the LE/CT equilibrium towards solvent polarity. The equilibrium, as already stated, shifts in favor of the CT state as the solvent polarity is increased. The present results do not allow us to assign values to k_{AB} and k_{BA}. However, it may be that the fluorescence decay is dominated by k_{AB}. If so, the expression:[16,17]

$$k = k' \exp[A(E_t(30) - 30)]/RT \qquad (5)$$

is appropriate, where k' is the polarity-corrected rate constant and A is the sensitivity of the barrier height to solvent polarity (the slope of Fig. 3). It should be noted, however, that the rate constant k_{BA} may not be negligible when compared to k_{AB} in the solvent systems investigated. If it were, the ratio ϕ_f/τ_f should be independent of any changes in the solvent polarity. Inspection of the ratio ϕ_f/τ_f for CBI_2-lysine (see Tables II and III) indicates that this ratio is not constant, but actually increases as the polarity of the solvent is increased. Furthermore, the establishment of a rapid pre-equilibrium between the LE and CT states cannot be assumed. If equilibrium populations of these states were established, it can be shown that the ratio ϕ_f/τ_f would be equal to the quantity $k_r/(K_{eq} + 1)$. As the solvent polarity is increased, K_{eq} is expected to increase, and ϕ_f/τ_f would decrease, contrary to our results (Tables II and III).

The apparent negative activation energy seen from the data of Table IV and shown in the plot of Fig. 4 is similar to the results obtained for the DMABN system reported by Eisenthal and co-workers.[16,17] The barrier height between the LE and CT states is effectively decreased at lower temperatures (negative activation energy) because of the increased polarity of the solvents at lower temperatures. The slope of a plot of ln k' (the polarity-corrected rate constant obtained from Eq. 5) versus $1/T$ should yield the activation energy between the LE and CT states of CBI_2-lysine. Such a plot for the data of Fig. 4 yields a polarity-corrected activation energy of 3.2 kcal/mole, consistent with C-C single-bond rotational energies. However, the assumption underlying the validity of Eq. 5 is that $k_{AB} \gg k_{BA}$. As discussed earlier, k_{BA} may not be negligible and, therefore, the calculation of the activation energy between the LE and CT states through the use of Eq. 5 should be regarded as provisional. In

TABLE V. The fluorescence quantum efficiencies (ϕ_f) and lifetimes (τ_f) for CBI-alanine and CBI_2-lysine in glycerol/water binary solvent mixtures. The radiative (k_r) and nonradiative (k_{nr}) for CBI-alanine are also presented.[a]

CBI-alanine

% Glycerol[a]	τ_f (ns)	ϕ_f	k_r (ns^{-1})	k_{nr} (ns)
88 [78]	19.5 ($\pm$0.9)	0.67 ($\pm$0.03)	0.034	0.017
92 [156]	19.3 ($\pm$0.9)	0.69 ($\pm$0.04)	0.036	0.016
94 [221]	19.8 ($\pm$0.7)	0.66 ($\pm$0.04)	0.033	0.017
96 [359]	19.8 ($\pm$0.7)	0.65 ($\pm$0.05)	0.032	0.018
98 [546]	19.8 ($\pm$0.9)	0.64 ($\pm$0.04)	0.032	0.018

CBI_2-lysine

% Glycerol	τ_f (ns)	ϕ_f
88 [78]	12.9 ($\pm$0.6)	0.12 ($\pm$0.02)
92 [156]	13.3 ($\pm$0.6)	0.17 ($\pm$0.02)
94 [221]	15.4 ($\pm$0.5)	0.19 ($\pm$0.02)
96 [359]	16.1 ($\pm$0.5)	0.20 ($\pm$0.03)
98 [546]	16.6 ($\pm$0.7)	0.23 ($\pm$0.03)

[a] The numbers given in brackets are the viscosities of the binary solvent mixture.

[b] The numbers in parentheses represent the standard deviations.

order to clarify these issues, a direct measurement of k_{AB} is needed.

The effect of solvent viscosity on the fluorescence property of CBI_2-lysine indicates that a molecular conformational change is associated with the formation of the CT state. The orientation of the isoindole rings in the ground state of CBI_2-lysine, when adsorbed on a solid substrate, does not result in efficient CT state formation, because the fluorescence emission of CBI_2-lysine is very close to that of CBI-alanine. Similarly, a high-viscosity solution also impedes CT state formation. A possible reason is that the "sandwich" conformation, in which the isoindole rings are essentially parallel to each other in close proximity, is unlikely to be attained during the lifetime of the LE excited state. This assumes that a molecular conformational change is required during the excited state for CT formation.

Large overall conformational changes necessary to achieve the structure required for CT state formation may seem unlikely during the excited-state lifetime because of entropic effects and the relatively large size of the isoindole rings. Nevertheless, the reduced quantum yields for CBI_2-lysine in low-viscosity solvents are consistent with an efficient CT state formation.

A computer graphical search was conducted to assess whether any particular ground-state conformation was preferred for CBI_2-lysine. All C-C and C-N single bonds were allowed to freely rotate, and the total energy associated with each conformation was the sum of the energy due to torsional strain, nonbonded interactions, bond stretching, bond bending, electrostatics, and van der
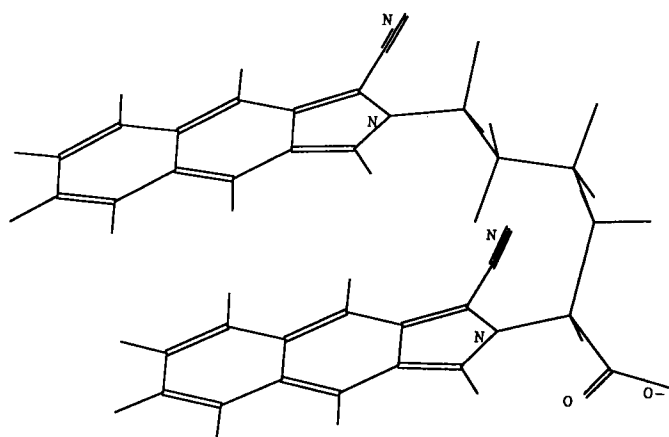
FIG. 5. A low-energy molecular conformation of CBI_2-lysine obtained from the computer ground-state conformational search.

Waals interactions. No solvent or π-π interactions were considered (they could be repulsive in nature). Several low-energy conformers were found, one of which is shown in Fig. 5. As is evident from this conformation, the isoindole rings may be in a stacked, sandwich-type configuration that places the rings in close proximity to one another. Although no solvent interactions were included in the computational search, a stacked-type geometry would be favored in polar solvents because of the minimization of any hydrophobic interactions between the solvent and the isoindole rings. The distance between the isoindole rings in the conformer is $\sim$4 Å, thereby precluding solvent from being between the two isoindole rings. The number of C-C or C-N bond rotations required to attain such a sandwich-type conformation in this case would be minimal in comparison to a conformation in which the isoindole rings are far apart and unstacked. The energy associated with this latter type of conformer results in the predicted energy being considerably higher in comparison to the conformer shown in Fig. 5. Qualitatively, the ground-state conformation favored by the computational modeling is consistent with the observed fluorescence properties of CBI_2-lysine. The effects of viscosity on the observed ϕ_f and τ_f of CBI_2-lysine indicate that the required conformation for the CT state is not identical to that of the ground state. Further investigations are required in order to determine the degree of reorientation required for CT formation.

The absence of a fluorescence band red-shifted from the fluorescence band of the LE state indicates that the fluorescence quantum efficiency of the CT state is extremely small in the solvent systems investigated. Although the CT state is stabilized by more polar solvents, the nonradiative processes for depopulation of the CT state overwhelm the radiative process and thus result in reduced fluorescence yields. This observation has been seen in a number of different systems.[21-24] The apparent lack of fluorescence from the CT state of CBI_2-lysine precludes fluorescence methods as a means to obtain direct kinetic measurements on this state. An alternative method would be the use of time-resolved absorption spectroscopy,[25-29] which could provide information on the electronic structure and the decay dynamics of that excited state.

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