Photophysical Properties of 3-[2-Cyano-4-(dimethylamino)phenyl]alanine: A Highly Fluorescent and Environment-sensitive Amino Acid with Small Molecular Size

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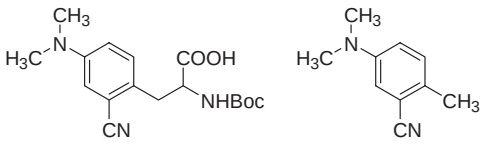
A new nonnatural amino acid 3-[2-cyano-4-(dimethylamino)phenyl]alanine (CDAPA) is virtually nonfluorescent in water but shows significant enhancement of fluorescence intensity and lifetime when the molecule partitions to nonaqueous environments, suggesting that this molecule is a good candidate as small fluorescent probe for proteins.

Fluorescent probes are powerful tools for studying the structure and dynamics of biomolecular systems such as proteins, membranes, and DNA. To achieve adequate detection of proteins, nonnatural fluorescent amino acids, whose emission spectra and quantum yields are sensitive to surrounding environments, have been developed.[1–5] In the course of studies on excited-state proton-transfer reactions of aniline and its derivatives,[6] we found that the fluorescence properties of *m*-cyanoaniline and its *N*-alkylated derivatives exhibit remarkable sensitivity to aqueous environment, e.g. the fluorescence quantum yield and lifetime of *m*-cyanoaniline in ethanol (0.19 and 6.2 ns, respectively) are reduced drastically in water (0.0019 and 45 ps, respectively).[7] Since the molecular size of the *m*-cyanoaniline and its *N*-alkylated derivatives is comparable to that of fluorescent natural amino acids (tryptophan, tyrosine, and phenylalanine), we expected that they can be used successfully in labeling proteins keeping the conformation intact.

We report here the synthesis of a fluorescent nonnatural amino acid, Boc-3-[2-cyano-4-(dimethylamino)phenyl]alanine (Boc-CDAPA; Boc = (*tert*-butoxycarbonyl) (Supporting Information),[10] and the photophysical properties of Boc-CDAPA and its chromophoric moiety, 3-cyano-4-methyl-*N*,*N*-dimethylaniline (CMDA) (Scheme 1), in aqueous and nonaqueous solutions. Fluorescence lifetimes (τ_f) in a picosecond range were obtained using a time-correlated single-photon counting fluorimeter using the third harmonic (266 nm) of a femtosecond mode-locked Ti:sapphire laser as an excitation source (Supporting Information).[10]

Figure 1 illustrates the absorption and fluorescence spectra of CMDA and Boc-CDAPA in cyclohexane (CH), acetonitrile (MeCN), ethanol (EtOH), and water (H_2O) at 293 K. The absorption and fluorescence spectra of both compounds are almost identical with each other except that a slight blue-shift is seen for the fluorescence spectrum of Boc-CDAPA in water. This implies



Boc-CDAPA **CMDA**

Scheme 1.

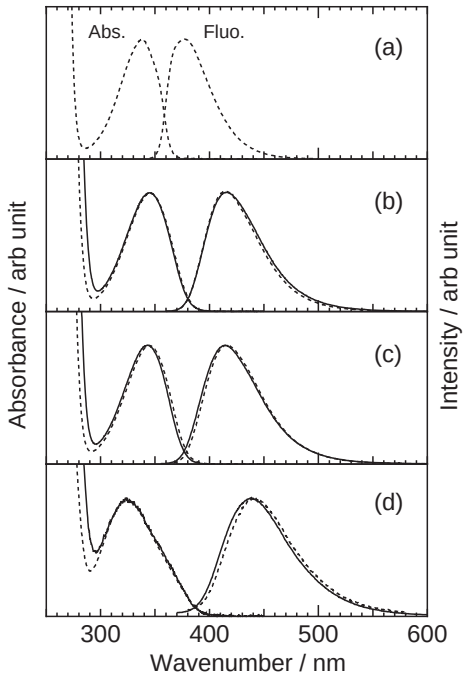


Figure 1. Absorption and fluorescence spectra of CMDA (broken line) and Boc-CDAPA (solid line) in (a) CH, (b) MeCN, (c) EtOH, and (d) H_2O at 293 K (The data for Boc-CDAPA in CH was not obtained because of the low solubility to CH).

that only small perturbation due to the alanyl group to the electronic structure of the aromatic moiety is involved in Boc-CDAPA. The first absorption band of CMDA is red-shifted with changing the solvent from nonpolar CH to polar MeCN and EtOH. In contrast, it is noticed that in water the first absorption band is shifted to the blue. This can be attributed to hydrogen-bonding interactions between the amino lone-pair electrons in CMDA and water molecules. The molar extinction coefficient of CMDA in EtOH at the first absorption maximum (345 nm) was determined to be 2770 M^{-1} cm^{-1}. Although tryptophan possesses a larger extinction coefficient (5350 M^{-1} cm^{-1})[8] at its first absorption maximum (277 nm) in water (pH 7.3), the wavelength is much shorter as compared with Boc-CDAPA. The fluorescence of Boc-CDAPA appears in the blue region and the spectral position depends strongly on the solvent polarity, suggesting that Boc-CDAPA can be used as micropolarity probe. The absorption (λ_{max}^{abs}) and fluorescence (λ_{max}^{fluo}) maxima, and Stokes shifts ($\Delta\bar{\nu}$) of CMDA and Boc-CDAPA are listed in Table 1. The extremely large Stokes shift in H_2O suggests significant variation of solute–solvent interactions in water upon electronic excitation.

Figure 2 displays the fluorescence decay profiles of Boc-CDAPA in MeCN, EtOH, and H_2O (in phosphate buffer solu-

Table 1. Spectral properties and photophysical parameters of CMDA and Boc-CDAPA in solution at 293 K

Compound	Solvent	λ_{max}^{abs}/nm	λ_{max}^{fluo}/nm	$\Delta\bar{\nu}/10^3$ cm^{-1}	Φ_f	τ_f/ns	$k_f/10^7$ s^{-1}	$k_{nr}/10^8$ s^{-1}
CMDA	CH	338	377	3.06	0.15	5.80	2.7	1.5
	MeCN	346	415	4.81	0.48	29.4	1.6	0.18
	EtOH	345	416	4.95	0.42	23.3	1.8	0.25
	H_2O	324	442	8.24	0.0040	0.163	2.5	61
Boc-CDAPA	MeCN	345	415	4.89	0.54	26.9	2.0	0.17
	EtOH	343	414	5.00	0.48	22.5	2.1	0.23
	H_2O[a]	325	438	7.94	0.0070	0.274 (55%)	1.4	36
						0.536 (45%)	0.59	19

[a]Phosphate buffer solution, pH = 6.88.

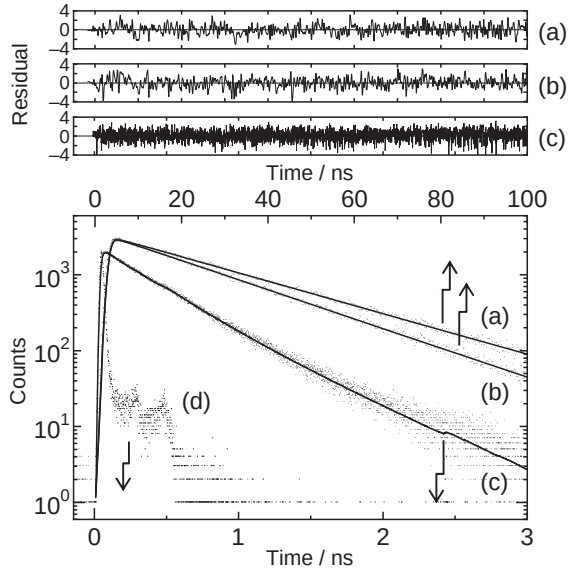


Figure 2. Fluorescence decay for Boc-CDAPA in (a) MeCN, (b) EtOH, and (c) H_2O (pH 6.88), excited at (a) 345, (b) 343, and (c) 266 nm and monitored at their fluorescence maximum wavelengths at 293 K, and (d) instrument response function (FWHM: ≈25 ps).

tion, pH = 6.88). The fluorescence decay curves of Boc-CDAPA in MeCN and EtOH follow single-exponential kinetics with relatively long lifetimes, 26.9 and 22.5 ns, respectively. It is noteworthy that the fluorescence decay rate in H_2O increases dramatically, and the decay profile no longer conforms to the single-exponential kinetics. The solid line represents the best fit of a double-exponential function ($F(t) = A_1e^{-t/\tau_1} + A_2e^{-t/\tau_2}$) superimposed on the experimental data points, using the parameters $A_1 = 0.31$, $\tau_1 = 274$ ps, $A_2 = 0.69$, $\tau_2 = 536$ ps and $\chi^2 = 1.079$. It can be seen from Table 1 that in MeCN and EtOH the fluorescence lifetime of Boc-CDAPA is almost identical with that of CMDA, and a substantial difference is seen in water: the fluorescence decay profile of CMDA in H_2O can be analyzed in terms of a single-exponential term with a lifetime of 163 ps ($\chi^2 = 1.017$). Although the origin of the two-exponential kinetics of Boc-CDAPA in water has not yet been clarified, one possible explanation is given in terms of different rotamers or conformers of the alanyl side chain of Boc-CDAPA, as has been reported for the similar behavior of tryptophan.[9]

The radiative (k_f) and nonradiative (k_{nr}) rate constants of CMDA and Boc-CDAPA in each solvent were calculated from τ_f and Φ_f. As shown in Table 1, the k_f value for Boc-CDAPA in MeCN and EtOH remains almost constant (≈2.0×10^7 s^{-1}) and is not different appreciably from those of CMDA, whereas in H_2O the k_f values for Boc-CDAPA are slightly smaller than that of CMDA. In contrast to the k_f values, the k_{nr} values of CMDA and Boc-CDAPA is influenced strongly by solvent properties. In water the k_{nr} values are much larger than those in nonaqueous solvents, indicating that fast radiationless processes are induced in water. The nanosecond laser photolysis experiments of Boc-CDAPA and CMDA using a XeCl excimer laser showed no transient absorption signal due to the triplet state. This suggests that the fast radiationless process of CMDA and Boc-CDAPA in water can be ascribed to internal conversion, as in the case of *m*-cyanoaniline.[7] The rapid $S_1 \rightarrow S_0$ internal conversion is a favorable deactivation mode for environment-sensitive fluorescent probes, because photochemical reactions that can occur from the S_1 and/or T_1 states are inhibited. In spite of the relatively small molecular size, Boc-CDAPA gives the first absorption band at much longer wavelength compared with tryptophan, and the strong blue fluorescence in nonaqueous environment is significantly quenched on exposure to water. These characteristic fluorescence properties of Boc-CDAPA demonstrate that Boc-CDAPA can be a candidate for novel fluorescent probe with small molecular size.

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References and Notes

1 B. E. Cohen, T. B. McAnaney, E. S. Park, Y. N. Jan, S. G. Boxer, L. Y. Jan, *Science* **2002**, *296*, 1700.
2 a) M. E. Vázquez, D. M. Rothman, B. Imperiali, *Org. Biomol. Chem.* **2004**, *2*, 1965. b) M. E. Vázquez, J. B. Blanco, B. Imperiali, *J. Am. Chem. Soc.* **2005**, *127*, 1300.
3 G. Sui, P. Kele, J. Orbulescu, Q. Huo, R. M. Leblanc, *Lett. Pept. Sci.* **2002**, *8*, 47.
4 A. Rzeska, J. Malicka, K. Guzow, M. Szabelski, W. Wiczk, *J. Photochem. Photobiol., A* **2001**, *146*, 9.
5 a) M. Taki, T. Hohsaka, H. Murakami, K. Taira, M. Sisido, *FEBS Lett.* **2001**, *507*, 35. b) H. Hamada, N. Kameshima, A. Szymańska, K. Wegner, L. Łankiewicz, H. Shinohara, M. Taki, M. Sisido, *Bioorg. Med. Chem.* **2005**, *13*, 3379.
6 a) S. Tajima, S. Shiobara, H. Shizuka, S. Tobita, *Phys. Chem. Chem. Phys.* **2002**, *4*, 3376. b) S. Shiobara, S. Tajima, S. Tobita, *Chem. Phys. Lett.* **2003**, *380*, 673.
7 J. Oshima, S. Shiobara, H. Naoumi, S. Kaneko, T. Yoshihara, A. K. Mishra, S. Tobita, *J. Phys. Chem. A* **2006**, *110*, 4629.
8 D. J. McLean, A. C. Giese, *J. Biol. Chem.* **1950**, *187*, 537.
9 H.-T. Yu, M. A. Vela, F. R. Fronczek, M. L. McLaughlin, M. D. Barkley, *J. Am. Chem. Soc.* **1995**, *117*, 348.
10 Supporting Information is available electronically on the CSJ-Journal web site.

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