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Relative fluorescence yields of dansyl amino acids: a sensitive probe for structures in solution

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Abstract

While dansyl amino acids and their anions show no evidence of fluorescence quenching by intramolecular electron transfer, small but reproducible variations in fluorescence yield among a series of 16 closely related derivatives allow spatial and electronic aspects of structure to be probed by these measurements. Values in aqueous solution relative to that for dansylamide ($DnsNH_2$, 1.0) range from 2.8 ($DnsNH^-$) to 0.69 ($DnsProO^-$) and may be rationalized on the basis of inductive, H-bonding, hydrophobic and steric effects.

Keywords: Dansyl amino acids; Fluorescence quenching; Relative quantum yield; Fluorescent probe. Comparative fluorimetry

1. Introduction

The quenching of fluorescence is frequently used as a probe for photoinduced electron transfer (see for example [1]) and, in this context, may form the basis for an efficient chemical sensor [2]. We have shown by detailed product analyses that the photolysis of simple peptides entails intramolecular electron transfer from terminal carboxylate and, where appropriate, side-chain chromophores to the peptide bond [3]. In order to examine these processes in more complex peptides, we are evaluating fluorescence quenching in readily available or synthesized derivatives of amino acids. We report here results obtained with a series of *N*-5-dimethylaminonaphthalene-1-sulphonyl (dansyl) amino acids and their anions (**1**) which, despite their frequent and widespread use in fluorometric and UV-spectrometric assay of amino acids and peptides [4], do not appear to have been studied in detail hitherto. Variations in fluorescence yield do not correlate with expected modes of intramolecular electron transfer, however. They are best explained in terms of steric and electronic features of molecular structure and thus illustrate thereby the potential value of comparative fluorimetry as a probe for structural information in solution.

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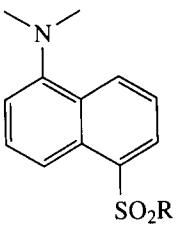
2. Experimental details

2.1. Materials

Dansylamino acids and their cyclohexylamine or dicyclohexylamine salts were supplied by Sigma, designated 99% pure. HPLC-grade acetonitrile and doubly deionized water were used as solvents. Sodium hydroxide solution (10^{-1} mol dm^{-3}) was BDH Convol analytical grade.

2.2. Instrumentation and procedure

Fluorescence emission spectra of the dansyl compounds (approximately 3×10^{-5} mol dm^{-3} in 98:2 water: acetonitrile or in 98:2 10^{-2} mol dm^{-3} aqueous sodium hydroxide:acetonitrile) were recorded in triplicate over the range 400–650 nm ($\lambda_{ex} = 325$ nm; sampling period, 5 s), using an Applied Photophysics spectrofluorometer equipped with a 250 W xenon lamp, an EMI 9813 photomultiplier and an Ortec 9315 photon counter and interfaced to a microcomputer for data acquisition and control. Stock solutions were made up using 50% water–50% acetonitrile and diluted with water, because of the low solubility of some dansyl compounds in water. The fluorometer was calibrated immediately before and after each set of measurements, by recording in triplicate the fluorescence emission from 470 to 490 nm ($\lambda_{ex} = 325$ nm; sampling period, 200 ms) of a standard com-



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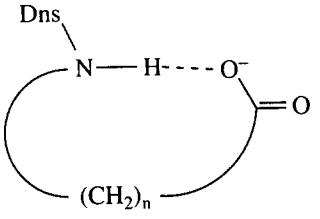

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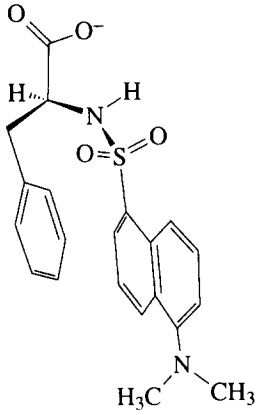

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Scheme 1.

prising ovalene in a Perspex block, thus enabling correction to be made for instrument fluctuations and the measurement of relative quantum yields.

The fluorescence intensities were corrected for inner filter effects using [5]

$$f_0 = f_{obs}\frac{2.3A(d_2-d_1)}{10^{-Ad_1}-10^{-Ad_2}} \quad (1)$$

where f_0 is the corrected fluorescence intensity, f_{obs} is the measured value, A is the absorbance at the excitation wavelength, and d_1 and d_2 are the path lengths corresponding to the extremities that define the width of the emission beam.

The correction factors, which were not sensitive to the precise values of d_1 and d_2, ranged from 1.14 to 1.22. The fluorescence intensities were then normalized to constant excitation intensity using the emission intensities measured for the ovalene standard. As all the compounds measured are closely related and, with only two exceptions (see below), give fluorescence spectra with maxima at 515 ± 3 nm, with similar bandwidths, the peak intensities have been taken to be proportional to the integrated areas under the fluorescence spectra. Dividing these by the absorbance values then gives the relative quantum yields [5]. Because similar solvent mixtures were used throughout, no correction was made for refractive index or polarization effects.

Analysis of the values from seven independent measurements of the fluorescence yield for dansylglycine made with freshly prepared solutions over a period of several months gave a standard error of 1.0%, well within that calculated from errors estimated for each measured parameter (2.9%) and confirming the long-term stability of the ovalene standard.

UV absorption spectra of the dansyl compounds were measured over the range 250–450 nm on a Kontron Uvikon 860 spectrophotometer.

3. Results and discussion

Absorption and emission spectra for dansylglycine (DnsGlyOH) are shown in Fig. 1. λ_{max} values for absorption throughout the series lie at 326 ± 3 nm except in the case of $DnsNH^-$ (313 nm) and $DnsGlyO^-$ (317 nm), and λ_{max} values for emission are at 515 ± 3 nm except for $DnsNH^-$ (507 nm) and $Dns(CH_2)_2\overset{+}{N}(CH_3)_3$ (521 nm).

Fluorescence yields are presented in Table 1. Error analysis (see Section 2) suggests that variations exceeding ± 0.04 are significant.

Photoinduced electron transfer can occur as either oxidation or reduction of the chromophore, the potential for both being enhanced considerably by excitation [6]. Neither intramolecular electron transfer to nor intramolecular electron transfer from the dansyl moiety, however, is a sustainable hypothesis for explaining the variations observed in Table 1. Rather, comparisons within groups of compounds suggest that the influence of steric and electrostatic effects is being demonstrated. Thus a substantial increase in fluorescence of the amide anion is anticipated by the well-known enhancement of electron-donating substituents [7], and similar through-bond electrostatic effects, on a smaller scale, are apparent in the comparisons: **d**, **c** and **e**, **j**. Progressive effects of both electronic and steric aspects of structure are seen in data for the series **a**, **d**, **e**, **f**, **g** and **h**, **i**, **k** (Fig. 2). Replacing one H by $CH_2CO_2^-$ or $CH(CH_3)CO_2^-$ in $DnsNH_2$ leaves the fluorescence yield unchanged, an inductive enhancement by carboxylate apparently being counterbalanced by a reduction caused by substituting CH_2 (or $CH(CH_3)$) for H (cf. **a** and **c**). Lengthening the chain by one carbon atom (**e**, **i**) reduces the enhancement, but it is more than restored by further chain extension in **f**, **k** and **g**. The ability to support a nearly linear intramolecular H bond (**2**) may account for this trend, the electronic effect of which imparts some of the character seen fully developed in **b**.

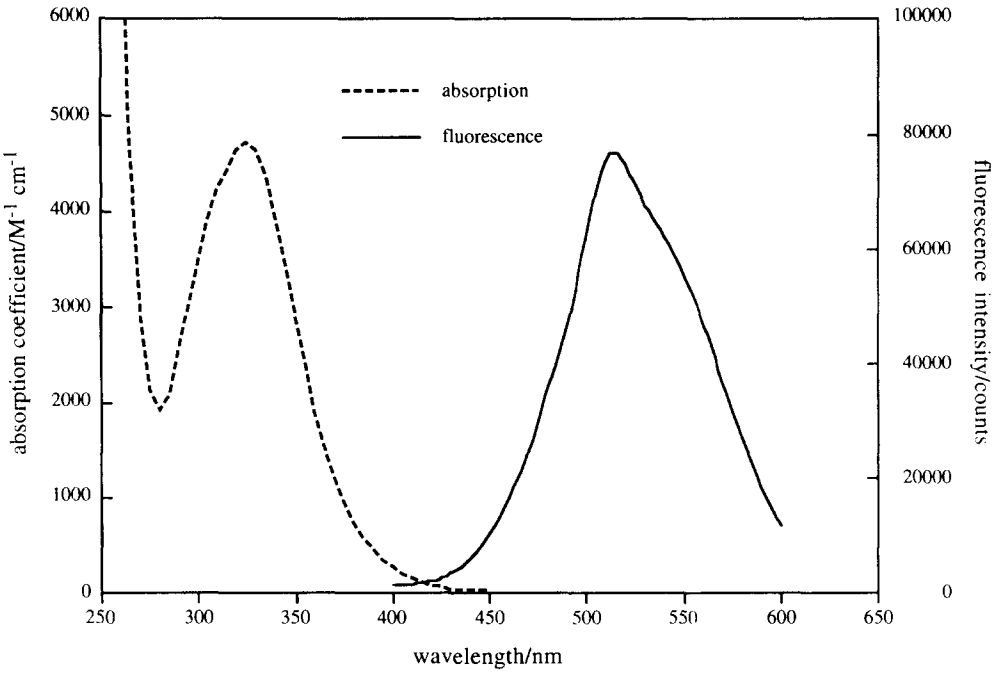

Fig. 1. Absorption and fluorescence emission spectra of dansylglycine (DnsGlyOH) in 2% acetonitrile in water. The fluorescence emission is uncorrected for the instrumental response function.

Table 1
Relative fluorescence yields of dansylamino acids and related compounds in 98:2 water:acetonitrile

Dansyl derivative	R	Relative fluorescence yield
a amide	NH_2	1.00
b amide anion[a]	NH^-	2.82
c glycine	$NHCH_2CO_2H$	0.92
d glycine in base[a]	$NHCH_2CO_2^-$	0.99
e β-alanine CHN[b]	$NH(CH_2)_2CO_2^-$	0.94
f γ-aminobutyrate CHN	$NH(CH_2)_3CO_2^-$	1.07
g ϵ-aminocaproate DCHN[c]	$NH(CH_2)_5CO_2^-$	1.16
h alanine CHN	$NHCH(CH_3)CO_2^-$	0.99
i aspartate D(CHN)[d]	$NHCH(CH_2CO_2^-)CO_2^-$	0.91
j (2-aminoethyl)trimethylammonium perchlorate	$NH(CH_2)_2\overset{+}{N}(CH_3)_3$	0.72
k glutamate D(CHN)[d]	$NHCH(CH_2CH_2CO_2^-)CO_2^-$	0.99
l proline	$N[(CH_2)_3]CHCO_2H$	0.71
m proline in base[a]	$N[(CH_2)_3]CHCO_2^-$	0.69
n phenylalanine	$NHCH(CH_2Ph)CO_2H$	1.10
p phenylalanine CHN	$NHCH(CH_2Ph)CO_2^-$	1.06
q methionine CHN	$NHCH[(CH_2)_2SCH_3]CO_2^-$	1.24

[a]98% aqueous solution with 2% acetonitrile and 10^{-2} mol dm^{-3} NaOH.
[b]Cyclohexylamine salt.
[c]Dicyclohexylamine salt.
[d]Di(cyclohexylamine) salt.

Fluorescence yields are often enhanced by molecular rigidity [7] and the reduced conformational mobility introduced by hydrophobic stabilization is consistent with the trend of the series **h**, **p**, **q**. The side chains in phenylalanine and methionine derivatives can each reach close proximity with the dansyl group and are strongly encouraged to do so in an aqueous environment (e.g. **3**).

The reason for the low value for proline remains unclear. The comparison **a** and **c** suggests that alkylation of the amide reduces the fluorescence yield; so a greater effect in the same direction may be expected on dialkylation, but a theoretical basis for this is not obvious. Alternatively, the reduction arises as it does with analogous 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD) derivatives and other amino acid fluorophors which is attributed to a trisubstituted nitrogen atom acting as an H-bond acceptor [8]. A third possibility is that the conformations of the sulphonamide group in this derivative are necessarily restricted by the prolyl moiety, and it is possible

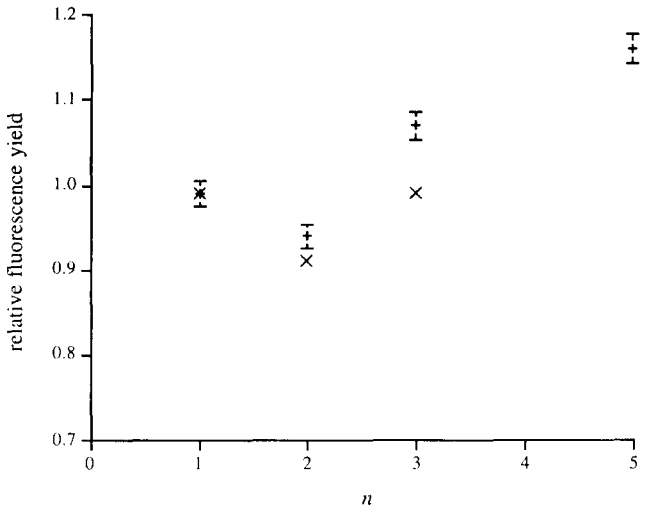

Fig. 2. Relative fluorescence yields in the series $DnsNH(CH_2)_nCO_2^-$ ($\vdash + \dashv$) and $DnsNHCHR(CH_2)_{n-1}CO_2^-$ ($\times$) in which n represents the number of carbon atoms between N and CO_2^-.

that they do not include those that are optimal for emission and which are more readily accessible in open-chain derivatives.

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