Substantial Intramolecular Charge Transfer Induces Long Emission Wavelengths and Mega Stokes Shifts in 6-Aminocoumarins

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ABSTRACT: Coumarins are deployed in numerous bioimaging and biosensing applications. Among various coumarin derivatives, 6-aminocoumarins attract increasing attention for their red-shifted emissions, mega Stokes shifts, and significant solvatochromism. These spectral characteristics together with weak emission intensities have historically been ascribed to the formation of the twisted intramolecular charge transfer (TICT) state in 6-aminocoumarins. In this work, we demonstrate that it is actually substantial intramolecular charge transfer (ICT) that is responsible for these fluorescent properties. Based on this new understanding, we reanalyzed the sensing mechanism of a 6-aminocoumarin based fluorescent probe and obtained close agreement with experimental data. Our results lead to a deeper understanding of the photophysics of 6-aminocoumarins and will inspire the rational development of novel fluorescent probes.

1. INTRODUCTION

Understanding the photophysics of fluorophores is essential to facilitate their widespread applications and enable the rational design of high-performance fluorescent probes. Among various chemical families of fluorophores, coumarin and its derivatives represent one of the most important classes of fluorophores in the blue and green region (Figure 1a).1 They are deployed in a broad range of bioimaging and biosensing applications,2,3 while their excellent optical properties also enable numerous uses in dye lasers,4 nonlinear optical materials,5 and dye-sensitized solar cells.6−11 The optical spectra of coumarins are often tuned by attaching substituents with varying electron-withdrawing power at position 3 or 4, as well as an electron-donating group at position 7 (Figure 1a).12,13 A judicious choice of these substituents permits effective control of the “push−pull” effect and intramolecular charge transfer (ICT) characteristics in coumarins, thereby shifting their absorption and emission spectra.13−17 This well understood mechanism has been applied in the design of many fluorescent sensors. In recent years, increasing attention has also been directed to 6-aminocoumarins, owing to their red-shifted emission wavelengths, considerably large Stoke shifts, and significant solvatochromism. These spectral properties endow 6-aminocoumarins with great potential in multicolor imaging and environmental sensing. Yet, the underlying photophysics of 6-aminocoumarins remains largely controversial.

In a pioneering study, Rettig and Klock proposed that the fluorescent characteristics of 6-aminocoumarins are due to the formation of the twisted intramolecular charge transfer (TICT) state upon photoexcitation.18 In the proposed TICT state, the amino substituent, which has a roughly planar alignment with respect to the coumarin moiety plane in the ground state (S0), rotates along the N−C6 axis by ~90°, resulting in a perpendicular alignment in the excited singlet state (S1). The TICT explanation has subsequently been accepted and referenced by many research groups.19−23 In recent years, 6-
aminocoumarins have turned out to be a useful platform for fluorescent sensors.24,29 Along with this rapid development, several research groups started to argue that no direct experimental evidence supports the TICT model in 6-aminocoumarins, using both fluorescence lifetime and pressure-dependent fluorescence intensity measurements as their justification.24,29 To discern the working mechanisms of 6-aminocoumarins and facilitate their rational design for high-performance probes, it is thus essential to resolve the controversy over their emission mechanism.

In this paper, we will reanalyze Rettig and Klock’s study by employing new (time-dependent) density functional theory (DFT/TD-DFT) calculations on a series of 6-aminocoumarins (Figure 1a). These reveal the limitations of the original quantum chemical calculations performed by Rettig and Klock, and provide an alternate view on the molecular origins of 6-aminocoumarins. We will demonstrate that substantial ICT from the electron-donating group at position 6 on the coumarin scaffold is actually responsible for the unique spectral properties of 6-aminocoumarins, and notably, no TICT occurs during this process. Based on this new understanding, we will re-examine the sensing mechanism of a 6-aminocoumarin derived sensor.

2. COMPUTATIONAL METHODS

DFT calculations were performed using Gaussian 09.30 Becke’s three-parameter and Lee–Yang–Parr hybrid functional (B3LYP)31–33 and a 6-31+G(d, p) basis set34 were used for all calculations, unless stated otherwise. The geometry optimizations of coumarins 1, 4–6, and 8 in both their ground and the first excited singlet states (S₀ and S₁) were performed in vacuo. Frequency checks were performed after each geometry optimization to ensure that minima on the potential energy surfaces (PES) were found. Following this, TD-DFT calculations were carried out on the optimized molecular structures to determine their peak absorption/emission wavelengths and molar extinction coefficients (oscillator strength).

Atomic contributions to the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) in selected compounds were calculated using Mulliken population analysis, based on the optimized molecular structures in the S₀ state.

The theoretical S₀ and S₁ PES of 4 and 5 in vacuo were constructed following the TICT model.35–37 In these calculations, the dihedral angles (θ), as defined by the average dihedral angle of C5–C6–N–C11 and C7–C6–N–C12 in the case of 4 and that of C6–C7–N–C11 and C8–C7–N–C12 in the case of 5, see Figure 1a) were fixed at various values, while other geometry parameters were freely optimized employing CAM-B3LYP/6-31+G(d,p) in the S₀ state.38 The CAM-B3LYP functional describes the overall PES profile accurately in systems involving varied amounts of charge transfer (i.e., when both the ICT and TICT states are of concern).39 CAM-B3LYP was thus used to construct the PES of 4 and 5.

3. RESULTS AND DISCUSSION

3.1. Re-Examining the TICT Model in 6-Amino-coumarins. Rettig and Klock have compared the optoelectronic properties of 6-aminocoumarins and 7-aminocoumarins from experiments, using 2 and 3 as examples (Figure 1a).18 Even though the emission of 2 is very weak with respect to that of 3, the Stokes shift of 2 is much larger (Δλ ~ 200 nm; Δν ~ 9500 cm⁻¹) relative to that of 3 (Δλ ~ 80 nm, Δν ~ 5200 cm⁻¹); the emission wavelength of 2 (∼580 nm) is significantly red-shifted with respect to that of 3 (∼430 nm) in alcoholic solvents. In addition, the fluorescence lifetime of 2 is quite long (9.4 ns in acetonitrile). These fluorescent characteristics of 2 are typical “signatures” of TICT emissions. To substantiate the TICT mechanism, Rettig and Klock performed “molecules-in-molecules” calculations, which showed large amounts of accumulated electron density in position 6 of the LUMO in the parent molecule, coumarin (Figure 1b). Attaching a donor group in position 6 consequently renders TICT energetically more favorable, as strong electrostatic interactions are then possible between the ionized donor group (positive) and the reduced coumarin moiety (negative, especially in position 6). Based on these observations, Rettig and Klock concluded that the emission of 2 should arise from TICT upon optical excitation.

More than three decades later, the rapid development of high-performance computing and the implementation of more accurate quantum chemical calculation methods (i.e., DFT/TD-DFT) allow us to re-evaluate Rettig and Klock’s conclusion. In contrast to their results, our calculations show that the electron density in the LUMO of 1 is actually higher in position 7 than in position 6 (Figure 1b). This suggests that the amino group in position 7 (and not in position 6) is prone to TICT, and Rettig and Klock’s quantum chemical calculations might be problematic. Indeed, further calculations of the potential energy surface (PES) along the rotational angle of the

Figure 1. (a) Molecular structures of 1−7 and atomic numbering schemes for 1, 4, and 5. (b) Individual atomic contributions to the electron density distributions in the LUMO and HOMO of 1; the sizes of the blue/red circles are proportional to the atomic contributions, and only contributions greater than 0.02 are shown; Rettig and Klock’s results were obtained by normalizing the squared HMO coefficients reported in ref 18. (c) S₀ and S₁ PESs of 4 (left) and 5 (right) in vacuo as a function of the rotational angle θ.

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dimethylamino group in 4 and 5 demonstrated that TICT is energetically unfavorable in position 6, but is potentially accessible in position 7 (Figure 1c). Compounds 4 and 5 are positional isomers that differ only with respect to the position of attachment for the donor substituent (4: position 6; 5: position 7). In the ground states of both 4 and 5, the torsional angle (θ) between the dimethylamino group and the plane of the coumarin scaffold is approximately 0°. As θ increases to 90°, which corresponds to the TICT state, the S1 PES of 4 reaches a maximum, concomitant with an energy increase of ~0.69 eV relative to that of the flat ICT state (θ = 0); TICT is thus energetically unfavorable in 4. In contrast to that, the TICT state of 5 corresponds to a local minimum in vacuo. This state often becomes accessible in polar solvents, as strong electrostatic interactions render it more stable than the ICT state.40

It should be pointed out here that, so far, emissions from the TICT states of coumarins have not been reported.35 Moreover, reducing the solvent temperature to 77 K in experiments (which essentially increases the solvent viscosity and potentially reduces molecular rotations) does not enhance the fluorescence of the 6-substituted aminocoumarin, 2 in ethanol.18 Conversely, coumarins substituted at position 7, which are susceptible to TICT, exhibit increased fluorescence intensity with increased solvent viscosity.37 Therefore, it can be concluded that substituents in position 7, but not position 6, facilitate TICT.

One may also consider the photophysical properties of the positional isomers 6 and 7, which likewise differ only with respect to the position of donor attachment on the coumarin scaffold (Figure 1a). In aqueous solution, their Stokes shifts were measured to be 189 nm (10 767 cm⁻¹) and 77 nm (5894 cm⁻¹), respectively, and their emissions peaked at 524 and 402 nm, respectively.19 Yet, the tremendously large Stokes shift and red-shifted emission of 6 cannot be attributed to TICT, as the donating amino substituent is constrained within a fused ring structure. Moreover, 6 possesses a lower quantum efficiency (QE) than 7.19 These results indicate that the large Stokes shift, long emission wavelength and low QE of 2 cannot arise exclusively from TICT.

3.2. Revealing the True Molecular Origins of 6-Amino-coumarin Photochemistry. To better understand the molecular origins of the different photophysical properties in 6- and 7-substituted coumarins, DFT and TD-DFT calculations were carried out on the ground (S₀), and first excited state (S₁) of 4 and 5. First, the results showed that the first absorption band in the UV-vis absorption spectrum of 4 should be located at longer wavelengths compared to that of 5 (Figure 2a). This effect can be rationalized by considering the electron densities in the HOMO and LUMO of 1 (Figure 1b). In position 6 of 1, the electron density is very high in the HOMO, but decreases distinctly in the LUMO. Therefore, attaching an electron-donating group in position 6 greatly destabilizes the HOMO and reduces the energy of the coumarin band gap.41 In contrast, the electron density in position 7 exhibits only minor changes for the HOMO–LUMO transition. Accordingly, an electron-donating group attached in position 7 induces similarly destabilizing effects in both the HOMO and LUMO, such that the resulting overall change of the energy band gap should be relatively small.

Second, the results showed that the large Stokes shifts in 6-substituted coumarins can be explained well by substantial amounts of ICT upon optical excitation. In 4, for example, the HOMO–LUMO transition is accompanied by a considerable decrease of electron density around the dimethylamino group in position 6, whereby the corresponding atomic contributions drop from 0.30 to almost 0 (Figure 2b). In contrast, the atomic contributions in 5 decrease less drastically, i.e., from 0.25 to 0.03, indicating smaller extents of ICT (Figure 2b). The amount of ICT in 4 and 5 can furthermore be quantified by the overlap index (Λ) of the HOMO and LUMO electron densities, since the S₁ states predominantly involve HOMO → LUMO transitions, whereby Λ carries a range of 0 to 1 and a larger Λ corresponds to increased ICT.42 The obtained DFT results showed a smaller Λ value for 4 (0.5840) compared to that of 5 (0.6635), reflecting a larger extent of ICT in 4 (Table 1). The extent of ICT is positively related to the degree of geometry relaxation in the excited state (since movement of the atomic nucleus follows that of electron flow), and eventually translates into the Stokes shifts of fluorophores. Indeed, the root-mean-square (RMS) of the cumulative atomic displacements of 4 upon optical excitation is substantially larger than that of 5, even though these two compounds are positional isomers (Table 1). In view of the large amounts of ICT and associated geometry relaxation upon excitation in 6-substituted coumarins, the observation of a large Stokes shift is hardly surprising (Figure 2a).

Intuitively, the larger ICT in 4 with respect to that in 5 upon optical excitation can be rationalized via resonance theory. In 5 (but not in 4), a full resonance pathway exists for electron delocalization between the amino donor and the ketone acceptor (highlighted in blue; Figure 2c). In other words, the donor group in position 7 participates more effectively in the delocalized π-system of coumarins. This is reflected in a more pronounced partial double-bond character of the N–C7 bond in 5 (1.377 Å), relative to the corresponding N–C6 bond in 4.
Excitations for 4 and 5 in vacuo seem to play a minor role (if any) in the activation of ∼
molecules. Under these conditions, a mechanism of 6-aminocoumarin derived aminocoumarins can be explained well by extensive ICT from oscillator strength. All units are in atomic units.

represents probably the best example (Figure 3a).

to the intrinsic fluorescence lifetime ($\tau_0$) via the Einstein transition probabilities of spontaneous emissions (eq 1). 

Owing to its low $f$ and low transition energy ($E$) values, the $\tau_0$ of 4 is higher than that of 5. This increased intrinsic emission lifetime allows the competing nonradiative decays to substantially reduce fluorescence, resulting in lower quantum efficiencies in 6-substituted coumarins. Indeed, Krzyżkowiak et al. have shown that hydrogen bond interactions significantly reduce the quantum yield of 6-aminocoumarin.

$$\tau_0 = \frac{c^3}{2Ec^2f}$$  

where $\tau_0$ is the intrinsic fluorescence lifetime, $c$ is the speed of light, $E$ is the transition energy of fluorescence, and $f$ is the oscillator strength. All units are in atomic units.

In summary, the spectral properties and quantum yields of 6-aminocoumarins can be explained well by extensive ICT from the amino group in position 6 to the coumarin scaffold.

3.3. Reanalyzing the Sensing Mechanisms of 6-Aminocoumarin Derived Fluorescent Sensors. Understanding the true molecular origins of ICT allows us to resolve the ostensible “mysteries” that have surrounded the sensing mechanism of 6-aminocoumarin derived fluorescent sensors. To this end, a fluorescent probe for monoamine oxidases (8) represents probably the best example (Figure 3a).

Sensor 8 was developed by the Sames group in a seminal work. The design of 8 had been motivated by the limiting TICT rotation of the amino group in position 6 (highlighted in blue) upon forming reactant 6 (Figure 3a). Surprisingly, although the fluorescence brightness of 6 is ~40—50-fold stronger than that of 8, an ~8—9-fold greater emission manifests in 8 which originates from an enhanced light absorption efficiency; and the quantum efficiency of 6 is only ~5 times as high as that of 8. In fact, Sames and co-workers have correctly pointed out that the limiting TICT rotation seems to play a minor role (if any) in the fluorescence activation of 8.

These facts are not surprising at all, in light of our demonstration herein that 6-aminocoumarins (including 8) do not energetically favor the TICT state (Figure 1c).

Furthermore, our DFT/TD-DFT analysis shows that the first absorption band of 6 is substantially stronger than that in 8, in good agreement with experimental data (Figure 3b). This strong absorption in 6 is mainly attributed to a significant $S_0 \rightarrow S_1$ transition, which is dominated by a HOMO-1 → LUMO transition (Figure 3c). The HOMO-1 → LUMO transition in 6 experiences a small amount of ICT, as indicated by their large spatial overlap ($\Lambda = 0.7430$). This large overlap in turn leads to a great molar extinction coefficient in 6.

Finally, the amino group in position 6 of 8 experiences a significant geometry change upon photoexcitation. This group becomes more planar in the excited state ($S_1$) than in the ground state ($S_0$), as highlighted in red circles.

In summary, the fluorescence intensification in 6 with respect to 8 is not due to blocking TICT. Instead, its molecular origins lie in both the ICT tuning (which enhances absorbance) and...
structural rigidification (which increases the quantum yield) of 6. In fact, the extent of ICT from the substituent in position 6 to coumarin scaffold plays a vital role in determining the wavelength and the fluorescence intensity of the electronic output. Modulating the electron-donating strength of this substituent thus allows the development of a broad range of fluorescent sensors.

4. CONCLUSIONS

Our DFT/TD-DFT calculations show that substantial amounts of ICT afford weak emissions and significantly large Stokes shifts and long emission wavelengths in 6-aminocoumarins; these spectral properties are not caused by the formation of the TICT state as previously thought. Our analysis is reinforced by these spectral properties are not caused by the formation of the ICT a...old plays a vital role in determining the...features. In fact, the extent of ICT from the substituent in position 6 to coumarin scaffold plays a vital role in determining the wavelength and the fluorescence intensity of the electronic output. Modulating the electron-donating strength of this substituent thus allows the development of a broad range of fluorescent sensors.

Notes

The authors declare no competing financial interest.

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