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Tautomerization of lumichrome promoted by supramolecular complex formation with cucurbit[7]uril

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Dedicated to Professor Haruo Inoue on the occasion of his 60th birthday.

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ABSTRACT

Addition of cucurbit[7]uril to an aqueous solution of lumichrome led to the formation of a new red-shifted band in both the absorption and fluorescence spectra indicating that binding to this rigid macrocyclic host facilitated the partial transformation into isoalloxazine-type structure. Global analysis of the results of spectrophotometric and spectrofluorometric titrations provided $\log K = 3.92 \pm 0.06$ for the logarithm of the equilibrium constant of 1:1 association. The dual fluorescence of the complex was quenched by protons in a diffusion-controlled process. Cucurbit[7]uril was found to exert negligible effect on the deprotonation of lumichrome in basic solution.

1. Introduction

The mechanism and dynamics of photoinduced proton transfer of nitrogen-heterocyclic compounds have received widespread interest [1–5]. Bifunctional molecules, possessing both hydrogen bond donor and acceptor sites are often capable of tautomerization via proton transfer in the excited state [6–9]. It is known that lumichrome (Lc), a major product of the photodecomposition and biodegradation of riboflavin undergoes photoinitiated rearrangement from the alloxazine scaffold into isoalloxazine-type structure (Scheme 1) upon hydrogen bonding to pyridine derivatives or acetic acid in organic solvents [10–12]. Quantum chemical calculations showed that the driving force for this process is the considerable electron density redistribution upon light absorption, which leads to acidity enhancement of the N(1)–H hydrogen accompanied by the basicity rise at the N(10) position [13]. The calculated activation barrier of tautomerization proved to be much lower in the excited state than in the ground state [14].

Lc is unable to tautomerize neither in the excited nor in the ground states in water [15]. In the presence of β-cyclodextrin (βCD), 1:1 inclusion complex is produced in aqueous solution but no evidence was found for the transition into isoalloxazine-type structure [16]. Cucurbit[7]uril (CB7), a pumpkin-shaped macrocyclic compound comprised of 7 glycoluril units linked by pairs of methylene groups, has a similar cavity volume (279 Å^3) to that of βCD (262 Å^3) [17]. Despite the similar size, the binding properties of the two ligands are often entirely different owing primarily to the dissimilar characteristics of their portal regions. The hydroxyl groups lining the βCD cavity opening are not involved in a strong interaction with most embedded guests [18]. Because the hydrophobic environment in the cavity disfavors the incorporation of ionized guests, the acidity of dyes diminishes upon encapsulation in βCD. In contrast, the negative charge density of the carbonyl-laced portals of CB7 significantly contributes to the stability of the host–guest complexes and ensures the stronger binding of the positively charged species leading to marked rise in their pK_a upon confinement in CB7 [19–22].

We have previously shown that strong hydrogen bond acceptor anions induce tautomerization of Lc [23]. Complexation of two fluoride or acetate ions to Lc in acetonitrile and the interaction with 1:1 stoichiometry in 1–6 M water/acetonitrile mixtures brought about isoalloxazine-like absorption and fluorescence spectra because of the anion-promoted electron density redistribution in the heterocyclic rings [23]. As an extension of this work, we now reveal whether binding to CB7 is able to facilitate tautomerization of Lc and study the effect of pH on the fluorescence properties of the supramolecular complex.

2. Experimental methods

Lumichrome (Aldrich) were used without further purification. Cucurbit[7]uril (Aldrich) was dried in high vacuum for several days

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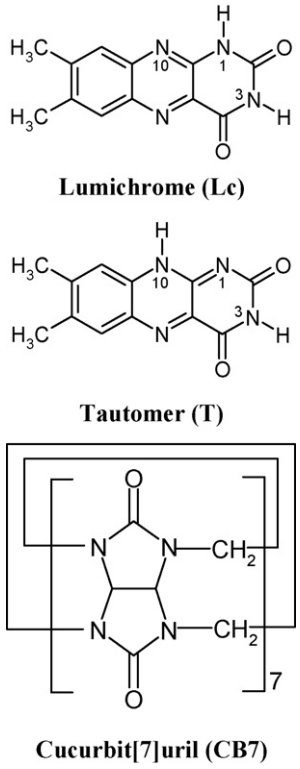

Scheme 1.

prior to use. The UV–visible absorption spectra were recorded on a Unicam UV 500 spectrophotometer. Corrected fluorescence spectra were obtained on a Jobin-Yvon Fluoromax-P photon-counting spectrofluorometer with excitation at 350 nm. Fluorescence decays were measured with the time-correlated single-photon-counting technique. A Picoquant diode laser (pulse duration ca. 70 ps, wavelength 372 nm) excited the samples, and the fluorescence decays were detected with a Hamamatsu R3809U-51 microchannel plate photomultiplier, which was connected to Picoquant Timeharp 100 electronics (36 ps/channel time resolution). Data were analyzed by a non-linear least-squares deconvolution method using Picoquant FluoFit software.

3. Results and discussion

3.1. Absorption and fluorescence characteristics

Addition of CB7 to aqueous Lc solution leads to a slight bathochromic shift in the 270–350 nm domain, and a new, isoalloxazine-type band emerges above 430 nm (Fig. 1A). These spectral changes are evidence of complex formation. In the case of 1:1 binding and large excess of ligand, the equilibrium constant (K) is determined using the following relationship:

$$A_\lambda = A_\lambda^0 \left(\frac{1 + \varepsilon_C^\lambda / \varepsilon_0^\lambda K[\mathrm{CB7}]}{1 + K[\mathrm{CB7}]} \right) \quad (1)$$

where $(\varepsilon_C^\lambda / \varepsilon_0^\lambda)$ is the ratio of the molar absorption coefficients for the complexed and free Lc at a particular wavelength (λ), A_λ^0 and A_λ denote the absorbances in the absence and the presence of CB7, respectively. The results of spectrophotometric titration were evaluated by the global fit of Eq. (1) in the 300–500 nm range. The inset in Fig. 1A demonstrates that the calculated functions match the experimental data confirming the 1:1 binding stoichiometry of the complex, and $\log K = 3.86$ is obtained.

Binding to CB7 markedly alters the fluorescent behavior (Fig. 2). Gradual quenching of Lc emission is accompanied by the concomitant rise of an isoalloxazine-like band in the long-wavelength range with an isoemissive point at 493 nm. To gain insight into the details of the mechanism and the kinetics of the processes occurring in the excited state, time-resolved fluorescence measurements have been performed. In accordance with previously published results [15,24], an exponential decay with 2.7 ns fluorescence lifetime is obtained in water. Recording the fluorescence at 440 nm, a gradual decrease of the dominant fluorescence lifetime (τ_1) and the appearance of an emission with 5.1 ns lifetime (τ_2) and increasing amplitude are found when the CB7 concentration is raised. The latter fluorescence component is assigned to the tautomer–CB7 complex (T–CB7). Since practically complete binding is reached around 1 mM CB7 concentration, the shorter decay time ($\tau_1 = 1.7$ ns) obtained under this condition is attributed to the excited state of the complexed alloxazine form (Lc–CB7). Because of the insufficient difference in the fluorescence lifetimes of Lc–CB7 and free Lc, the decays of these two coexisting emissions cannot be resolved, and the alteration of their relative contribution leads to a slight change in τ_1 from 2.7 to 1.7 ns at 440 nm when the CB7 concentration is raised. The fluorescence decay parameters are invariant within the limits of experimental errors when the traces are detected at 590 nm. Because of the negligible contribution of Lc–CB7 emission at this wavelength, only a single-exponential decay of T–CB7

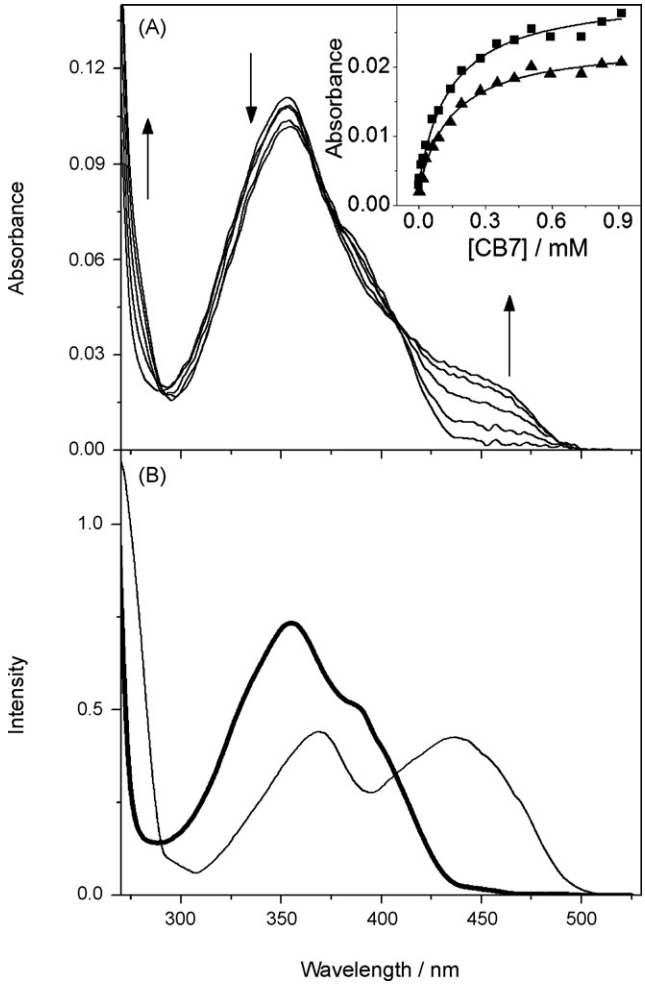

Fig. 1. (A) Absorption spectrum of 10.8 μM lumichrome aqueous solution in the presence of 0, 32, 143, 352 and 732 μM CB7. Inset presents the absorbance variation at 440 nm (■) and 460 nm (▲); the lines give the result of the global non-linear least-squares analysis. (B) Excitation spectra of the solution of 9.2 μM lumichrome and 684 μM CB7 in water ($\lambda_{emission}$ = 450 nm (thick line) and 600 nm (thin line)).

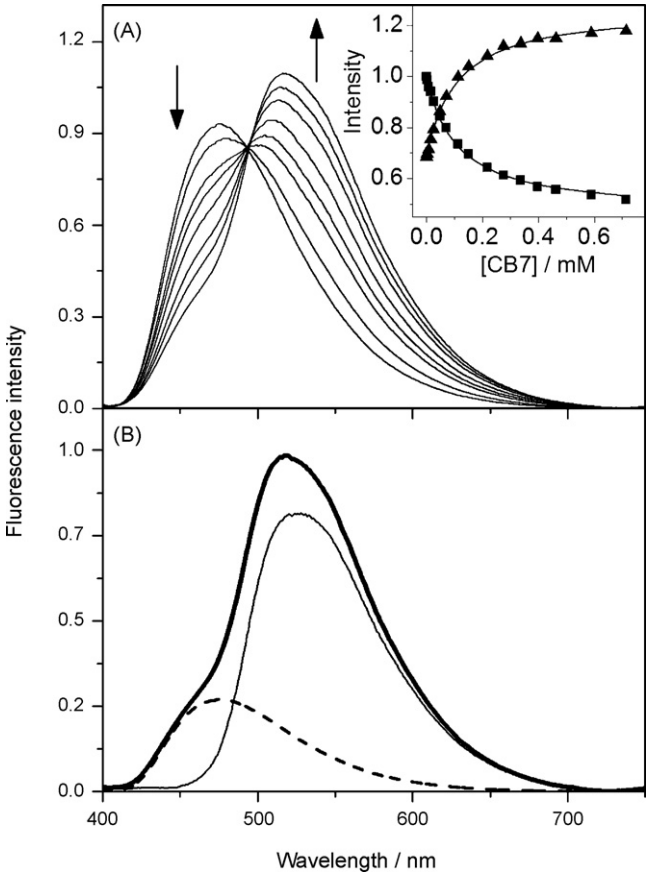

Fig. 2. (A) Change of the fluorescence spectrum of 10.8 μM lumichrome upon addition of 0, 15, 48, 70, 112, 217, 336 and 713 μM CB7 in water (excitation at 350 nm). Inset: fluorescence intensity variation at 474 nm (■) and 516 nm (▲); solid lines refer to the calculated functions. (B) Resolution of the dual fluorescence of lumichrome–CB7 complex (thick line); [Lc] = 10.8 μM, [CB7] = 1.01 mM, excitation at 350 nm; fluorescence spectrum of the complexed alloxazine form (dash line) and tautomer–CB7 (thin line).

fluorescence with 5.1 ± 0.2 ns lifetime is detected above 0.2 mM CB7 concentration. It is especially noteworthy that no grow-in appears. This shows that photoinduced tautomerization does not have significant contribution, and T–CB7 is already produced in the ground state. The lifetime of the singlet-excited state of this species is similar to that reported for lumiflavin (5.6 ± 0.2 ns) and riboflavin (5.15 ± 0.2 ns) in aqueous sodium phosphate buffer solution at pH 8 [25]. These closely related compounds have isoalloxazine structure.

The coexistence of the two different forms of the complexed lumichrome (Lc–CB7 and T–CB7) in the ground state is also supported by the excitation spectra recorded in solution containing 684 μM CB7, where Lc is almost completely complexed. As shown in Fig. 1B, the excitation spectrum resembles the absorption of unbound Lc when the monitoring wavelength is 450 nm indicating the very similar absorption characteristics of Lc and its Lc–CB7 complex. Detection at 600 nm gives an entirely different excitation spectrum, which matches the well-known absorption spectrum of lumiflavin [15,26] indicating that Lc binding to CB7 induces partial tautomerization into isoalloxazine-type form (Scheme 1).

As the variation of the spectra presented in Fig. 2 originates from a process in the ground state, the results of fluorescence titration can be analyzed using a relationship analogous to Eq. (1). Global fit of the fluorescence intensities measured as a function of CB7 concentration in the 405–725 nm range gives $\log K = 3.97$, which is in fair agreement with the corresponding value derived from absorption spectra (*vide supra*). Since spectrofluorometric titrations provided $\log K = 2.98$ for the logarithm of the association constant between Lc and β-cyclodextrin [16], we can conclude that a significantly stronger complex is produced with CB7. As a representative example, the inset of Fig. 2A demonstrates the good correlation between the calculated and experimental data at two wavelengths. The lower panel (Fig. 2B) exhibits the resolution of the fluorescence spectrum of the complexed Lc. The shape of the longer wavelength band closely resembles that reported for lumiflavine aqueous solution [15], confirming again the CB7-assisted tautomerization of Lc.

Unfortunately, the low solubility of the free and complexed Lc in water thwarted a determination of the structure of the complex by NMR spectroscopy, but the solubility enhancement upon addition of CB7 may imply inclusion complex formation. There is enough space in the CB7 cavity to accommodate Lc effectively. The hydrophobic dimethylbenzene moiety can be embedded in the macrocycle, whereas the high electron density of the carbonyl oxygens at the portal provides good hydrogen bond acceptor sites for interaction with the N(1)–H group. The hydrogen bonding of N(1)–H probably increases the charge density of the heterocyclic rings, thereby inducing the transition into an isoalloxazine-like form. There is a fraction of complexed Lc in which N(1)–H moiety does not form hydrogen bond with the guest. This type of complex has alloxazine-like properties.

3.2. Effect of pH variation

As seen in Fig. 3A and B, the fluorescence spectra of the free and complexed Lc are completely different in acidic solutions. In the latter case, dual fluorescence remains irrespective of pH. In the absence of CB7, our results agreed with those reported by Lasser and Feitelson [24], who established that the fluorescence intensity diminution with gradually increasing acid concentration originates from the dynamic quenching of the excited Lc by protons with a rate constant of $2.5 \times 10^{10}\ M^{-1}\ s^{-1}$. The protonation of Lc in the ground state takes place only in highly acidic solutions since the pK_a of

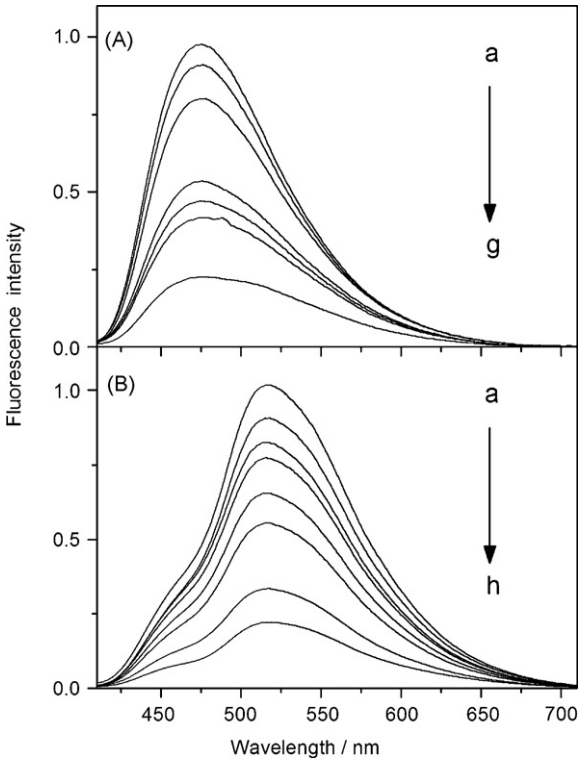

Fig. 3. (A) Fluorescence spectrum of Lc at pH (a) 6.51, (b) 2.79, (c) 2.42, (d) 2.11, (e) 1.99, (f) 1.74, (g) 1.46 and (B) that of Lc–CB7 complex at pH (a) 6.60, (b) 3.89, (c) 3.13, (d) 2.80, (e) 2.48, (f) 2.30, (g) 1.91, (h) 1.63; [Lc] = 10 μM, [CB7] = 570 μM, excitation at 350 nm.

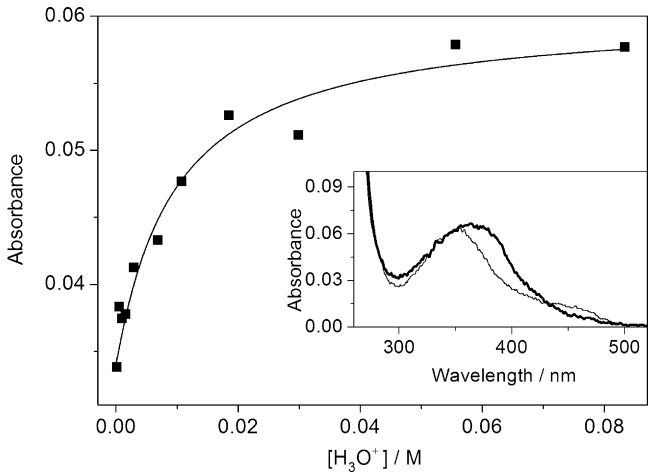

Fig. 4. Absorbance change at 385 nm with increasing acidity in the solution of 6.9 μM Lc and 700 μM CB7. Line represents the best fit. Inset displays the absorption spectra at pH 3.71 (thin line) and 1.08 (thick line).

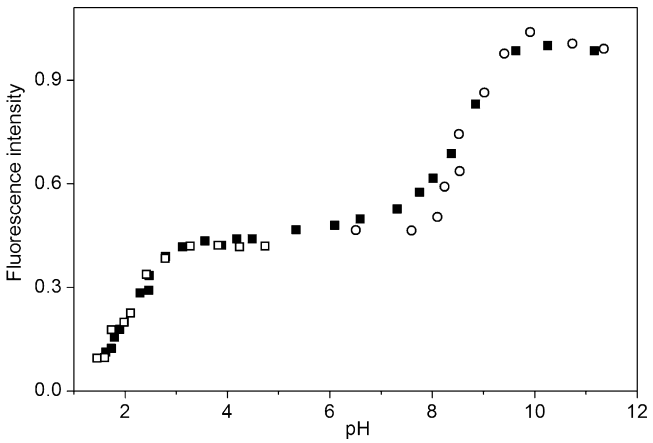

Fig. 5. Effect of pH on the fluorescence intensity of Lc measured at 450 nm (□), 550 nm (○) and for the Lc–CB7 complex at 450 nm (■).

this process is −2.4. The protonated Lc is nonfluorescent and its absorption spectrum has a maximum at 390 nm [24]. When the pH was decreased from 4.5 to 0.8 in the presence of 0.7 mM CB7, we observed a slight growth and a concomitant diminution of the absorbance in the 360–425 nm range and above 425 nm, respectively, indicating a partial protonation of the complex in the ground state (inset to Fig. 4). These spectral alterations show that the complex remains stable even in strongly acidic solution. If competitive binding of a proton to CB7 expelled Lc from the complex, no Lc protonation would take place under our experimental conditions. The dual fluorescence in the 4.5–0.8 pH domain also confirms that the complex does not disintegrate. Non-linear least-squares fit of the H_3O^+ concentration dependence of the absorbance at 385 nm gave $pK_a \approx 2$ for the protonated complex (Fig. 4). This is close to the pK_a 2.2 value reported for the conjugated acid of CB7 [27] suggesting that the binding of a proton to the Lc–CB7 complex at the carbonyl oxygen of the ligand causes the slight change in the absorption spectrum.

Stern-Volmer plot of the fluorescence intensity of the complex at 520 nm showed a good linear correlation as a function of H_3O^+ concentration with a slope of $k_q\tau_0 = 130\ M^{-1}$. Taking the fluorescence lifetime $\tau_0 = 5.1$ ns determined at this wavelength in neutral solution, $k_q = 2.5 \times 10^{10}\ M^{-1}\ s^{-1}$ is derived for the rate constant of T–CB7 fluorescence quenching. An identical k_q value is obtained from the slope of the H_3O^+ concentration dependence of the reciprocal fluorescence lifetime monitored at 520 nm. The fluorescence decay time of excited Lc–CB7 measured at 450 nm, which is 1.7 ns in neutral solution, is also shortened by the addition of acid, but the small changes and the low intensity of the signal do not allow precise determination of the quenching rate constant.

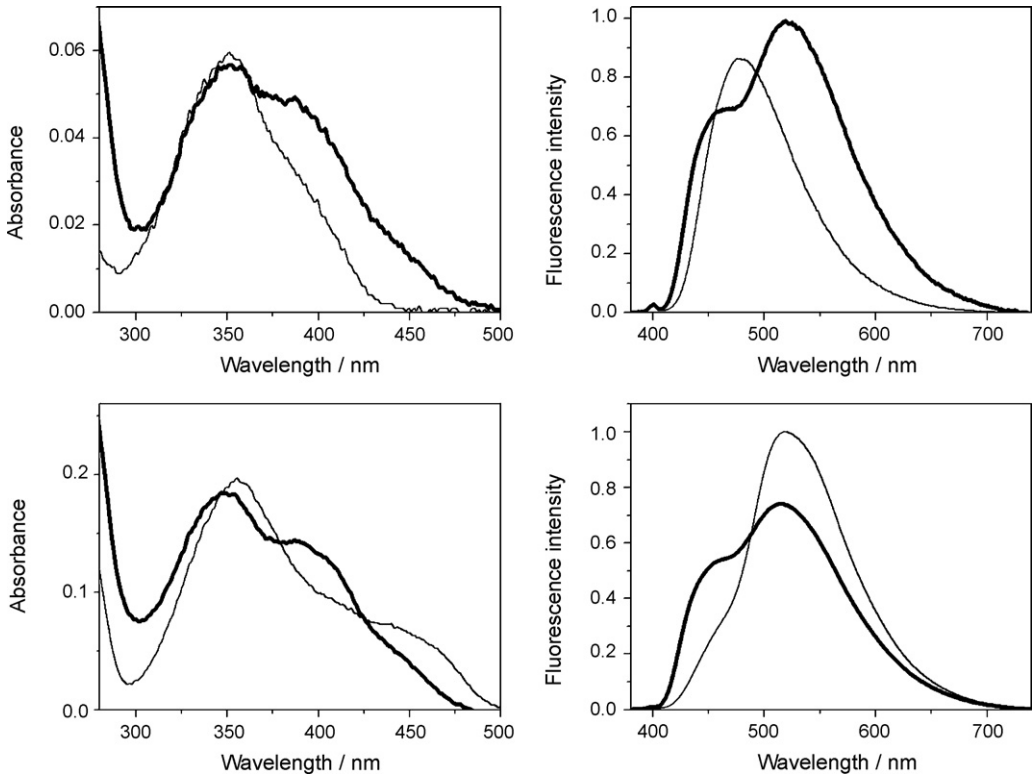

Fig. 6. Absorption and fluorescence spectra of the free (upper graphs) and complexed lumichrome (lower graphs) at pH 6.8 (thin lines) and 10.8 (thick lines).

The filled symbols in Fig. 5 display the fluorescence intensity of the Lc–CB7 complex at 450 nm as a function of pH. For the sake of comparison, the normalized fluorescence intensity of aqueous Lc solution at 450 and 550 nm is also presented (open symbols). It is seen that an analogous pH dependence is observed for the complexed and free Lc in acidic medium implying that dynamic quenching by H_3O^+ plays the dominant role in both cases.

As the pH was increased in basic medium, parallel fluorescence enhancement was found in the presence of CB7 at 450 nm and in the aqueous Lc solution at 550 nm (Fig. 5) indicating that Lc is not protected against deprotonation by OH^- in the CB7 complex. The alteration of the absorption and fluorescence spectra on going from pH 6.8 to 10.74 is compared in the absence and presence of CB7 in Fig. 6. It is apparent that the spectra of Lc become very similar in water and CB7 solution at pH 10.74 indicating that CB7 exerts a negligible effect on the deprotonation of Lc.

In conclusion, we have demonstrated that the binding to CB7 fosters partial transformation of lumichrome into a structure of an isoalloxazine-type electron density distribution even in the ground state. To the best of our knowledge, this is the first example for a cucurbituril-induced tautomerization.

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