Emission Frequency Modulation by Electronic Confinement Effect: Congo Red Incorporated within a Dendritic Structure

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Received: April 14, 2005; In Final Form: July 12, 2005

We have investigated the photophysical properties of an organic dye (Congo Red) incorporated within the internal cavities of a dendrimer (type polypropylenimine of fifth-generation modified with a dense shell of amino acids). In this paper we show that the luminescence properties of Congo Red encapsulated into the "dendritic box" can be modulated by the electronic confinement effect. The emission frequencies of this organic dye incorporated within the dendritic structure can be red shifted with respect to their emission in solution, and the magnitude of this shifting can be modulated under appropriate experimental conditions.

1. Introduction

Dendrimers are a new class of nanometric-size macromolecules characterized by a regular three-dimensional-like array of branch units[1] that currently are attracting the interest of a great number of scientists because of their unusual physical and chemical properties and potential uses. Thus, these macromolecular structures have enormous applications in catalysis,[2] material research,[3] and medical sciences.[4] Dendrimers are complex and structurally well-defined compounds that show a high degree of order and capability to contain specific chemical units in determined sites of their structure. Their structures are quite flexible and often lead to typical globular shapes.[1] Another important property of dendrimers is they can be designed to possess dynamic internal cavities of different size, where it is possible to encapsulate a variety of guest molecules.[5] This is the case of polypropylenimine dendrimers from the highest generations. Herein the guests can be really locked within the cavities of these macromolecular structures if an outer shell is constructed in the presence of the guest (forming up the so-called "dendritic box"). This hermetic shell is normally based on amino acids, which prevents the flux of matter through both sides of the dendrimer. In fact it has been reported that diffusion of guest molecules in these systems is unmeasurably slow because of the close packing of the shell.[5f]

Taking into account these premises, here we present the incorporation of an organic dye (Congo Red) within the internal cavities of a structurally modified fifth-generation polypropylenimine dendrimer and the photophysical characterization of the resulting adduct dye@dendrimer. We will show that the altered photophysic properties of the dye confined within the dendritic host can be interpreted as due to the spatial distortion of the electron density of the guest molecule induced by the repulsive interactions between the branches of the dendrimer and the guest.[6]

2. Experimental Section

Dendritic Structure Preparation and Encapsulation of Congo Red. The "dendritic box" was prepared according to the previously reported procedure.[5f] Fifty milligrams of Congo Red was added to a solution of 100 mg of the commercially available starburst fifth-generation dendrimer (with 64 amine end groups) in 12 mL of dichloromethane with 2 mL of triethylamine, and the solution was stirred for 24 h at room temperature. Then, 322 mg of N-*t*-BOC-L-phenylalanine N-hydroxy-succinimide ester was added, and the solution was stirred overnight. Dilution to 100 mL with dichloromethane was followed by washing with water and saturated $NaHCO_3$ solution. Then, the solution was dried over anhydrous $MgSO_4$, and the solvent was evaporated in vacuo to afford the dye-doped dendrimer. The product was purified by dialysis until no traces of free dye were detected by high-performance liquid chromatography.

Characterization. Steady-state fluorescence measurements were performed at 293 K on a FS900-CDT spectrometer from Edinburg Instruments. Fluorescence lifetime measurements were made using a pulsed hydrogen lamp FL900 as the excitation source (40 kHz), a Czerny-Turner monochromator (grating 1800/mm) to select the analyzing wavelength, and a Hammamatsu R955 photomultiplier tube as the detector. The fluorescence lifetimes were calculated by fitting the emission decays using the level 1 program implemented in the control software of the spectrometer.

FT-Raman spectra were recorded on a Bruker spectrometer, model RFS 100/s. The 1.064 nm line of a diode pumped Nd:YAG laser was used for excitation along with a high-sensitivity germanium diode detector, cooled to liquid nitrogen temperature. The laser Raman spectra were examined in the 180° scattering configuration using a sample holder specially designed for this study. Various laser powers were tried so that the optimum power (120 mW) was selected. The sample temperature was controlled by measuring the Stokes/anti-Stokes intensity ratio

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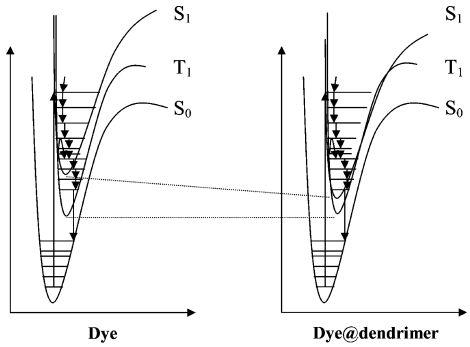
10.1021/jp051929e CCC: $30.25
Published on Web 08/12/2005

SCHEME 1. Proximity Effect Induced by the Dendritic Structure on S_0-S_1 Energy Gap of the Guest



of Raman scattered radiation. The spectral resolution and reproducibility were experimentally determined to be better than 4 cm^{-1}, and the number of scans varied from 400 to 1000 with recording times of 20 min to 1 h. The Raman spectra were corrected for instrumental response using a white light reference spectrum.

Combustion chemical analysis of the sample was carried out using a Fisons EA 1108-CHNS-O analyzer.

3. Results and Discussion

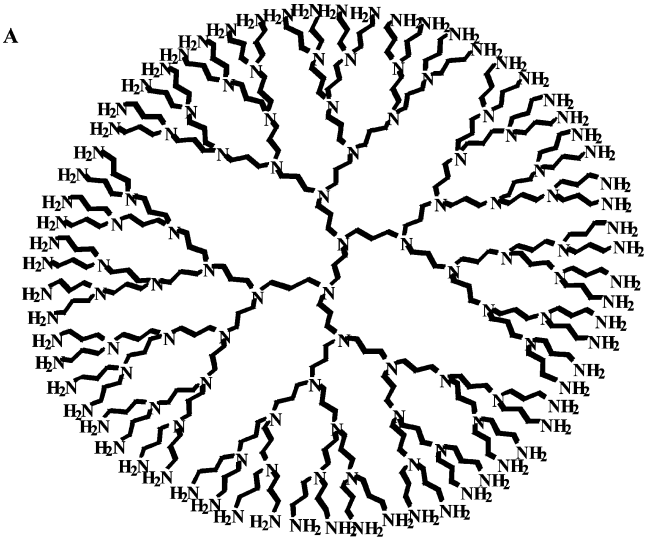
Dendritic structures interact with solvents through a combination of nonbonding interactions whose magnitude will depend on the hydrophilic and/or hydrophobic nature of both the solvent and the dendritic macromolecule. Depending on the nature of these interactions with the solvent, the guest molecule trapped into the dendritic box (in our case a dye molecule) can experience an alteration in the photophysical and photochemical properties in a specific way. In this sense, we envisaged that a chemical consequence of the encapsulation in a flexible core of a dendrimer is that solvation effects could lead to spreading or reducing of the original macromolecular dimensions by changing the solvent polarity, and this would leave no alternative but to temporarily deform the size of the dendritic cavities inside. As a consequence, guest molecules might interact differently with the dendritic walls influenced by the solvent environment.

As for other rigid or soft systems, when the cavity and the molecule dimensions are closer, the interaction with the guest could be sufficiently important as to experience a strong repulsion with the electronic orbitals of the host and the dye molecules themselves.[6] Such should be the case when solvents of opposite polarity to the dendritic system are employed. Indeed, the deformation experienced by the branches of the dendrimer (induced by the strong repulsion with solvent molecules) may cause an effective reduction in the size of the internal cavities, hence altering the highest occupied molecular orbital−lowest unoccupied molecular orbital (HOMO−LUMO) energy gap of the guest and resulting in appreciable modifications of the photophysical properties of the dye (see Scheme 1).

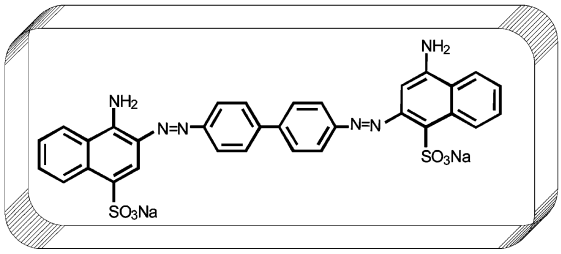
This phenomenon that had been anticipated by us for other rigid host systems such as zeolites was confirmed by experimental results, and theoretical calculations appeared in the recent literature.[6,7] Indeed, on the basis of the concept that the molecular orbitals (MO) of the adsorbates inside the zeolites are not extended over all the space (as they are in the gas phase) but instead are forced to limit within the zeolite walls (as in fact is occurring when a molecular guest is incorporated inside the cavities of a dendrimer), it has been proposed that such a "boxing effect" is stronger as the size of the confined guest

CHART 1. Molecular Structures of the Unmodified Fifth-Generation Polypropylenimine Dendrimer (A) and the Dye Congo Red (B)

A



B



approaches the zeolite cage dimensions, producing an energy increase of the MO, in particular those which are more diffused. Since the HOMO orbitals have been predicted to be more sensitive than the LUMO (on the basis of theoretical calculations), a strong reduction on the band gap of the frontier orbitals can be expected as compared to the gas phase. As a result, a bathochromic shift of the 0−0 transition should be the most direct consequence of this confinement effect and in fact is observed.[6]

Thus, to check the extent of this phenomenon to our dendritic system (testing the validity of our previous assumption), we studied the photophysics of the dye Congo Red incorporated into the dendritic cavities of a fifth-generation polypropyleneimine dendrimer whose internal voids were sealed with the L-phenylalanine amino acid (DAB-dendr-(NH-*t*-BOC-L-Phe)$_{64}$) by fluorescence and Raman spectroscopies. In this respect, it is important to notice that with dendrimers of lower generations this shell is not dense enough to capture the guests as they can be easily removed by extraction. Hence, only with the fifth-generation polypropylenimine dendrimer is it possible to build up the "dendritic box".[5f]

Chart 1 shows the molecular structures of the unmodified fifth-generation dendrimer (with 64 amine end groups) and the guest dye Congo Red. The amine groups will be subsequently modified with 64 (*t*-BOC)-protected amino acid molecules in the presence of the dye, hence forming a dense chiral shell that will trap the dye in the interior.

Once the dendritic system was built up according to the synthetic procedure detailed in the Experimental Section, the loading of Congo Red was calculated by elemental analysis. Hence, based on the sulfur content (which was 0.1%), the estimated loading level of the dye was ca. 0.36 dye molecules

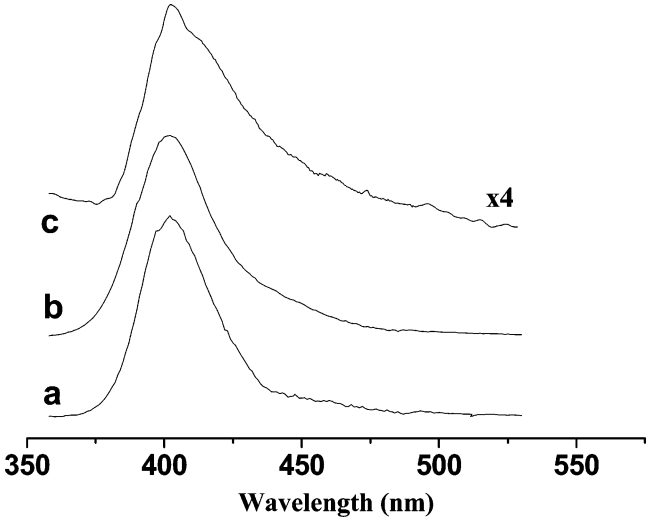


Figure 1. Emission spectra at room temperature of Congo Red after 350 nm excitation in neat solvents: (a) ethanol, (b) dichloromethane, (c) *n*-pentane.

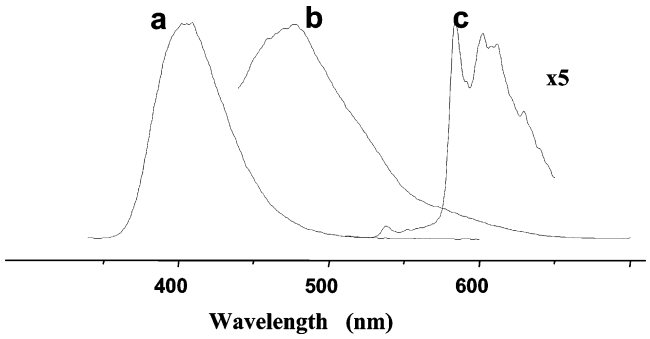


Figure 2. Emission spectra at room temperature of Congo Red included within the dendritic structure after 350 nm excitation: (a) ethanol, (b) dichloromethane, (c) *n*-pentane.

per dendrimer. Hence, a priori, this experimental data strongly points out the low probability that the dye might be forming dimeric associations that in principle could lead to incorrect interpretations about the photophysical behavior of site-isolated guest molecules within the cavities of a dendrimer.

Fluorescence Spectroscopy. The fluorescence emission (after $\lambda = 350$ nm excitation) of Congo Red in ethanol was recorded at room temperature. The emission spectrum consisted of a broad band with a maximum at ca. 402 nm. Similar results could be obtained when the spectrum was registered in the less polar solvents dichloromethane and *n*-pentane (see Figure 1).

Nevertheless, when the dye was encapsulated within the dendritic structure of DAB-dendr-(NH-*t*-BOC-L-Phe)$_{64}$, a progressive red shifting of the emission band could be measured as long as the medium polarity was reduced, from ca. $\lambda = 406$ nm in ethanol to $\lambda = 485$ nm in dichloromethane and to ca. $\lambda = 585$ nm in *n*-pentane. The fluorescence intensity in *n*-pentane was dramatically reduced, and the emission appeared as a structured band showing several peaks at ca. 485, 601, 612, and 632 nm (see Figure 2c). It is interesting to note that this progressive emission red shifting was reversible since the subsequent replacement (after evaporation) of *n*-pentane by solvents of higher polarity (on the same sample dye dendrimer) led to an ipsochromic shift of the fluorescence band.

Since the estimated loading of Congo Red was ca. 0.36 dye molecules per dendrimer, in principle it is not feasible that this extraordinary shifting might be attributed to a simple excimer or exciplex formation inside the dendritic structure. Moreover, the possibility that the observed luminescence properties were explained by an eventual solvation of the dye by solvent molecules was discarded from the very beginning by recording the fluorescence spectrum of the dye in neat solvents such as ethanol and *n*-pentane. In any case, the fluorescence spectra reproduced those spectra obtained with the dye-doped dendrimers. Herein, the explanation to these experimental results should be found in the increase of the spatial restrictions imposed by the dendritic host on the dye molecule, leading to an enhancement of the electronic confinement effect.[6] Effectively, the shift of the fluorescence band can be rationalized by taking into account that nonpolar solvents such as *n*-pentane can cause a dramatic reduction of the initial total volume of the macromolecule as well as a much closer self-association of dye-doped dendrimers leading to the formation of aggregates induced by the nonpolar character of the solvent as depicted in Figure 3.[8] A combination of both phenomena can lead to an effective reduction of the internal volume where the guest is located hence inducing changes in the band gap of the frontier orbitals of the dyes (a reduction of the HOMO−LUMO band gap) to an extent that will depend on the degree of interaction exerted by the branches of the macromolecule (see Scheme 1). This stronger confinement is reflected in the notable reduction of the excitation energy $S_0 \rightarrow S_1^*$ and, consequently, in the bathochromic shift of the fluorescence spectrum of the guests. Obviously, this effect is stronger when the internal cavities of the dendrimer became smaller (for the dendrimers used in the present study this requirement can be achieved when nonpolar solvents are used).

The room-temperature fluorescence lifetimes for Congo Red incorporated into the dendritic structure were adjusted to single-exponential functions. Figure 4 shows the fluorescence decays obtained for Congo Red@dendrimer in solvents with different polarity. These constants, which were lower than those measured for the pure dye in polar solvents ($\tau = 2.3$ ns, in ethanol), decreased substantially from ethanol (1.2 ns) to dichloromethane (0.6 ns) and *n*-pentane (<200 ps). This kinetic behavior resulting

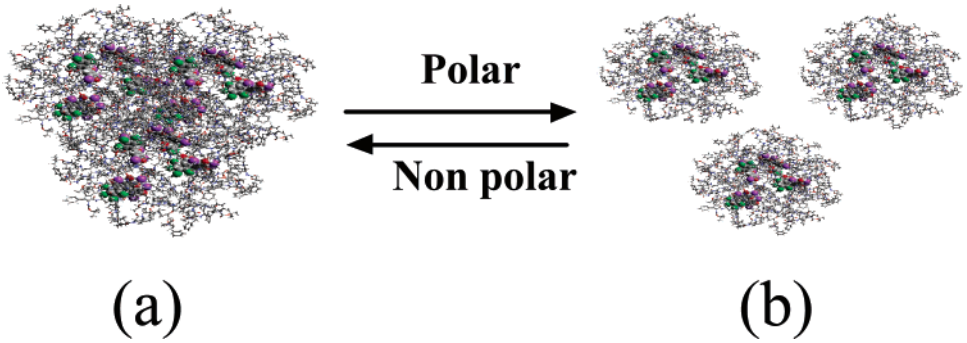


Figure 3. Theoretical simulation of the possible equilibrium experienced by three dendritic structures with molecules of dye incorporated in nonpolar (a) and polar (b) solvent.

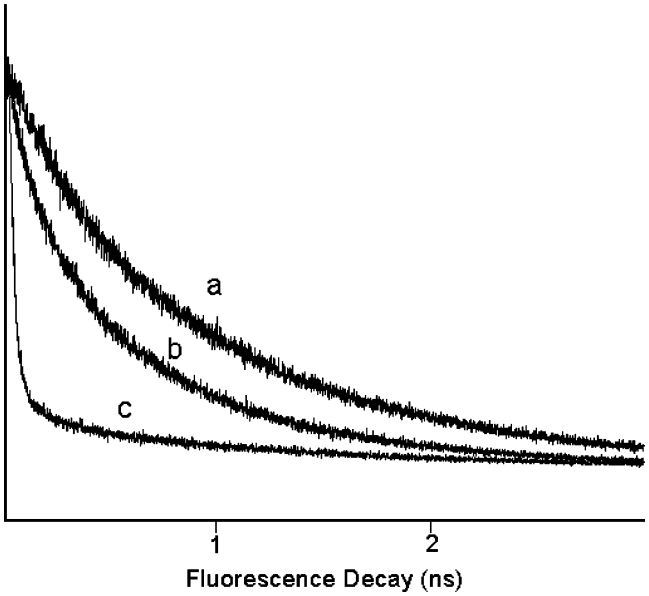


Figure 4. Normalized fluorescence decays of Congo Red in ethanol (a) and Congo Red incorporated within the dendritic structure in dichloromethane (b) and *n*-pentane (c).

from the orbital-confinement effect enhances the nonradiative deactivation pathway due to the closer proximity between the lowest excited singlet states.[6]

The modulation of the fluorescence lifetime of Congo Red by the dendrimer host in different solvents can be connected with the bathochromic shift observed in the fluorescence emission. This effect is more pronounced in the adduct Congo Red@dendrimer in nonpolar solvents. In these cases, the guest molecule matches the internal cavities of dendritic structure, and the S_0-S_1 energy gap in Congo Red confined is smaller compared with that of Congo Red pure. As a result, and due to this proximity effect, the S_1 and S_0 states can become partially degenerated due to the vibrational−electronic (vibronic) coupling between both electronic states, and this effect clearly could be responsible for the enhaced nonradiative deactivation pathways. In the same manner, if k_{nr} (nonradiative) constants are increased by the proximity effect, then the fluorescence lifetime is clearly shorter as can be deduced from the following relation

$$\tau = \frac{1}{k_{nr} + k_r}$$

Considering the dramatic decrease of the fluorescence lifetime associated to this proximity effect, the magnitude of the radiationless decay rate induced by electronic confinement effect is truly remarkable. This effect has been described in many aromatic compounds and heterocycles, and we think that this is a reasonable mechanism whereby fluorescence lifetime can greatly be affected by confinement within a dendritic structure.

FT-Raman Spectroscopy. The near-IR−FT-Raman spectra of Congo Red incorporated into the dendritic structure are shown in Figure 5. As can be seen there, the most characteristic peaks appear in the range from 1590 to 1450 cm^{-1}, where C═C stretching vibrations of naphthalene and biphenyl moieties are expected. The most intense peak observed at ca. 1150 cm^{-1} corresponds to the N−C stretching. These vibrations experience a shift in going from ethanol to dichloromethane and *n*-pentane. This shift is remarkably higher in *n*-pentane and apparently depends on the interactions established between the branches forming dendritic cavities and the dye molecules. Interestingly, the shift of these vibration bands goes in the same direction as

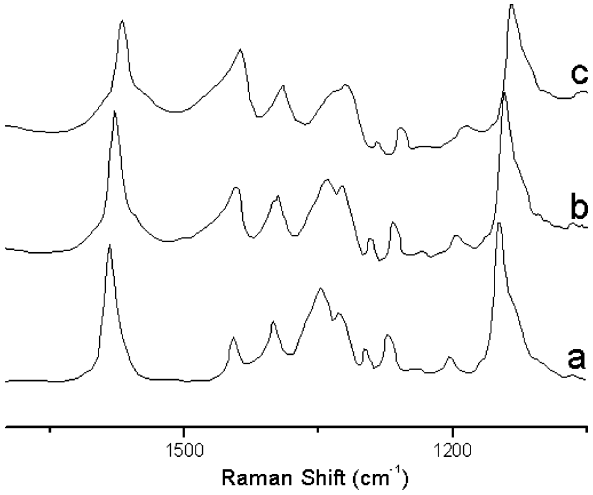


Figure 5. FT-Raman spectra of Congo Red@dendrimer in ethanol (a), dichloromethane (b), and *n*-pentane (c).

expected from the fluorescence results and can be rationalized as due to the electronic confinement.[6] The confinement effect involves a general weakening of bonds that can be reflected in a decrease of the vibrational frequencies.[9]

Other confined spaces (i.e., zeolites) affect the guest−host relationships in a similar way.[6] In fact, it has been reported that the photophysical properties of several probe molecules such as naphthalene, anthracene, or 2,3-diazabicyclo[2.2.1]hept-2-ene within pure silica zeolites are strongly affected by the zeolite host.[6] As stated, one of these effects is the bathochromic shift of the 0−0 transition that the molecule undergoes by inclusion in the rigid matrix of a zeolite. The magnitude of this shifting is more pronounced when the cavity and molecule dimensions are closer, and it appears in principle to be lower than the one attained in this case for our dye-doped dendrimer.

In summary, the ability of the dendritic shell to create a confined environment in determined experimental conditions can be taken as an easy way to modify the emission spectra of dye molecules trapped in the cavities of dendrimers. The spectral shift in the emission band can be interpreted as a direct consequence of the orbital confinement effect, whose magnitude depends exclusively on the solvent polarity.

These results may have important applications from a technological point of view. For instance, dye-doped dendrimers can be used to enlarge the emission frequency range of laser dyes simply by choosing a solvent or a mixture of solvents of adequate polarity.[7]

Acknowledgment. Generous financial support by the Comisión Interministerial de Ciencia y Tecnología (CICYT) of Spain (Project MAT2002-04421-C02-01) and from NASA (Grant NAG10-335) is gratefully acknowledged.

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