

Fluorescence Studies and Semiempirical Calculations on Alkali Ion Indicators¹

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Two newly synthesized cryptands act as sensitive Na⁺- and K⁺-selective indicators for cation concentrations above 20 μ M. The fluorescence properties change markedly upon cation binding. In addition, the free ligands exhibit a pronounced sensitivity to pH, which is considerably lower for the cation complexes. Time resolved fluorescence is characterized by a decay time of about 5 ns that is attributed to the diprotonated protolytic state of the uncomplexed ligands. Semiempirical calculations show the systematic influence of the nitrogen lone pairs or the N-H bond on the stability of the system. The cause of the strong fluorescence intensity increase observed upon protonation of the fluorescent cryptands may be attributed to an increase in the S₁-T_x energy gap as a consequence of bridgehead nitrogen protonation.

KEY WORDS: Cryptands; alkali ion indicators; pH sensitivity; semiempirical calculations; AM1.

INTRODUCTION

The alkali ions Na⁺ and K⁺ play an important role in many fundamental biological processes, e.g., in the transmission of signals in nerves. However, there is a lack of sensitive fluorescence indicators for the selective detection of Na⁺ and/or K⁺ in physiological systems. Therefore, two new fluorescent cryptands, the Na⁺-selective F221 and the K⁺-selective F222 (cf. Fig. 1) have been synthesized ([1]; details will be published elsewhere) according to the concepts of Lehn et al. [2]. A coumarin-like dye, the 4-trifluoromethylcoumarin residue,

is integrated in the macrocyclic structure. The new compounds can be used for the sensitive, fast, and selective fluorometric determination of alkali ions in aqueous solution.

SPECTROSCOPIC PROPERTIES OF F221 AND F222

The absorption spectra of F221 and F222 are very similar to that of the basic chromophore esculetin [3] and remain essentially unchanged upon binding of Na⁺ or K⁺. All fluorescence emission spectra are characterized by a broad band at λ_{max} 470 nm (excitation at 360 nm). Binding of Na⁺ to F221 and of K⁺ to F222 leads to large fluorescence intensity changes (Fig. 2a) and to a small shift of the emission maximum. Similarly, protonation of both bridgehead nitrogens leads to a large emission intensity increase. Spectrofluorometric titrations at different pH values were carried out to determine the apparent binding constants and cation selectivities.

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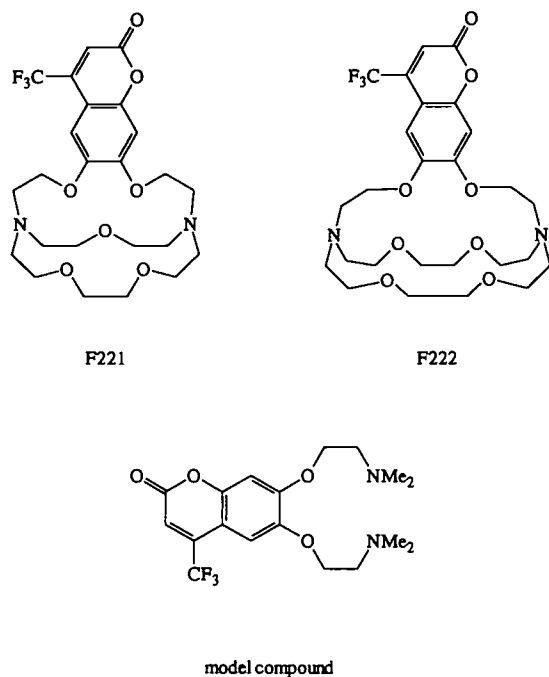


Fig. 1. Chemical structure of the fluorocryptands F221 and F222 and of a hypothetical model compound.

The dissociation constants of protolysis are similar to those of the parent compounds [2]. The stability constants of the F221–Na⁺ and of the F222–K⁺ complex are about $10^5 M^{-1}$; the K⁺/Na⁺ selectivity of F222 is 30 and the Na⁺/K⁺ selectivity of F221 is even as high as 250 [1].

The fluorescence intensity difference at a given wavelength between the uncomplexed F221 and its alkali ion complex depends mainly on the intensity level of the free ligand, which increases much stronger with decreasing pH than the intensity of the complex (cf. Fig. 2b). The fluorescence intensity of the uncomplexed fluorocryptands at a given pH value thus depends on the degree of protonation of the macrocyclic nitrogens. A similar behavior upon protonation has also been reported for a different monocyclic system in mixed solvents [4]. Protonation of the fluorocryptands could also lead to a decrease in flexibility of the aliphatic ether bridges. The intramolecular redistribution of vibrational energy could be hindered in the protonated state leading to a rise of the fluorescence intensity.

Protonation of and metal ion binding to the cryptands can lead to topological structural changes involving the cation cage (Fig. 3). These different states could, for example, be characterized by different fluorescent properties. Therefore, time-resolved fluorescence studies were carried out on the uncomplexed F221 and F222 at

different pH values. In acidic media where both nitrogens are protonated, a monoexponential decay behavior was observed. The decay time of about 5 ns is comparatively large and may be attributed to inefficient vibrational quenching. Above pH 5 only the amplitude of the decay time decreases with increasing pH due to the population of the monoprotonated state. This result is consistent with the observation that the quantum yield of the monoprotonated form of these fluorocryptands is very low.

Since the fluorescence properties of the fluorophore seem to depend on the molecular conformation of the cryptands, which are influenced by the bridgehead nitrogen protonation, it is important to carry out theoretical calculations. Such calculations are especially of interest if they can also be based on sets of experimentally achieved structural coordinates. For this reason, the crystal structure analysis of F221 (Fig. 4) was performed. The central –OCH₂CH₂O– fragment of the larger ether bridge is disordered and possibly exists in two conformations, which can be considered as an expression of conformational flexibility; the N...N distance is 5.142 Å (details will be published elsewhere).

SEMIEMPIRICAL CALCULATIONS

An MO treatment should provide information concerning the mechanism of fluorescence deactivation and the significance of postulated conformations. Several conformation analyses based on the geometry obtained from the X-ray structure analysis were carried out using the random incremental pulse search (RIPS) method [5]. The most stable conformations were optimized semiempirically using the AM1-Hamiltonian [6]. The thermodynamic stability of the systems can be described using the standard heat of formation H_f .

The *unprotonated* cryptand is very flexible. No quantitative dependence of the thermodynamic stability to any set of atomic distances or bonding parameters can be found. Qualitatively, out–out conformations (cf. Fig. 3) appear to be more stable (2–3 kcal/mol) than in–in or in–out topologies. The influence of the nitrogen lone pair on the thermodynamic stability of the system is compensated by the flexibility of the cryptand moiety, so no selective interaction between lone pair and π -system can be detected.

The *protonation of one nitrogen* converts a lone pair into a σ -bond. This rehybridization leads to a more rigid structure. Characterizing the thermodynamic stability of the conformations according to the heat of for-

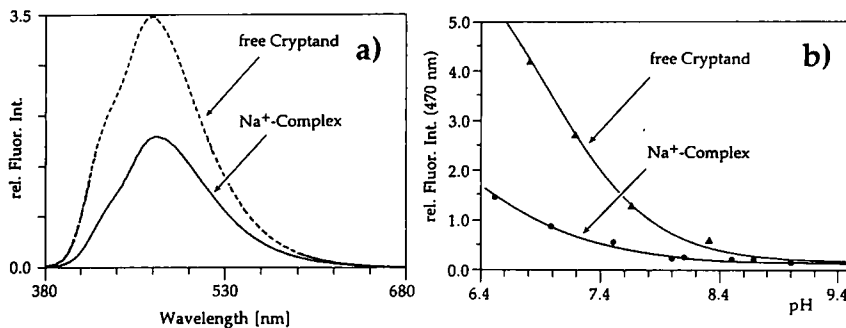


Fig. 2. Fluorescence properties of F221 and its Na^+ complex (25°C). (a) Emission spectrum of $6.25 \mu\text{M}$ F221 in 20 mM veronal/tetramethylammonium hydroxide, pH 8.05 (dashed line), and in the presence of 1.7 mM NaCl (solid line). Excitation at 360 nm. (b) Plot of relative fluorescence emission intensity at 470 nm versus pH for F221 (\blacktriangle) and its Na^+ complex (\bullet). Data of the Na^+ complex originates from individual titrations.

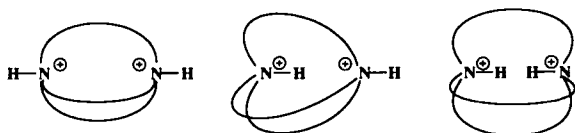


Fig. 3. Different principal conformations of a cryptand in the diprotonated state.

mation and the longest absorption band, the following conclusions can be made.

- The localization of the N–H bond or N–lone pair within the cavity stabilizes the aromatic system.
- The degree of stabilization of the N–H group effect (7–10 kcal/mol) appears to be two to three times stronger than the influence of the N–lone pair.

If the N–H bond is located within the cavity, a partial charge transfer of this bond to the π -system is accompanied by an increase of the bond length and a decrease in the corresponding bond order can be calculated. The electron density of the N–H bond or the nitrogen lone pair inside the cavity stabilizes the aromatic system.

With regard to representative *diprotonated* conformations, the same effects as described above can be calculated. Furthermore, the effect of two N–H bonds is stronger than that of a single one which is consistent with the experimental evidence mentioned above. From a qualitative energetic point of view, protonated cryptands should prefer in–in conformations.

The topology of the cryptands has a marked influence on the thermodynamic stability of the fluorophores. The physical reason for this effect has to be investigated. Since protonation changes the intensity of the fluorescence but not the location or shape of the bands, the energy of the involved orbitals may not be affected. The

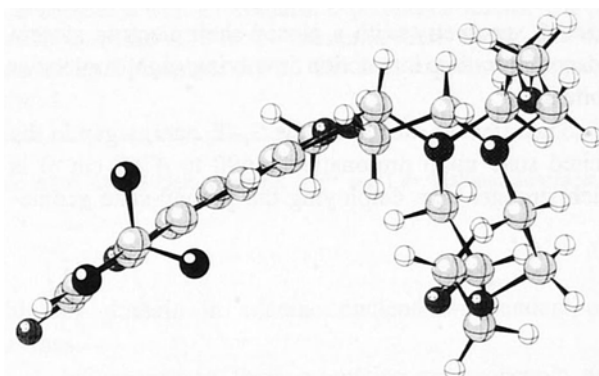


Fig. 4. X-ray structure analysis of F221 crystallized from methanol ($\text{C}_{24}\text{H}_{31}\text{F}_3\text{N}_2\text{O}_7 \cdot 0.5 \text{CH}_3\text{OH}$; monoclinic crystals of space group $C2/c$).

population of the excited states changes when nonradiative processes such as internal conversion (IC) or intersystem crossing (ISC) can occur. To investigate these possibilities quantum mechanically, the geometries of the ground and excited states of F221 and F222 have to be minimized. To save computational effort, a hypothetical model compound (assumed to exhibit the same properties as the original fluorophore including the involvement of the N–lone pairs) was chosen (Fig. 1).

Comparing the data characterizing the AM1-optimized energy minima of the unprotonated and protonated model compound in the ground state, the following statements can be made:

- The enthalpy of protonation of the model compound is about 12 kcal/mol smaller than that of $\text{NMe}_3 \rightarrow \text{HNMe}_3^+$ ($\Delta H_f = 162.1$ kcal/mol).
- The molecular orbitals in the region of the HOMO/LUMO are stabilized by ~ 3 eV, the N–

lone pair is no longer part of the active space upon σ -bond conversion as a consequence of protonation.

To describe the ISC-transition probability, the energetic difference between the first excited singlet state S_1 and the nearest triplet state T_x can be used. This energy difference increases from 1600 to 2700 cm^{-1} upon protonation, by applying the ground-state geometry. In the protonated form the rate constant k_{ISC} is smaller than for the unprotonated state, which leads to an increase in fluorescence intensity.

Since fluorescence emission starts from a vibronically relaxed excited state, the same calculations were carried out using AM1-optimized S_1 geometries. The electronic transitions were calculated using the optimized S_1 geometry with a closed-shell electron system and configuration interaction involving eight molecular orbitals.

The effect of increasing the S_1 - T_x energy gap in the excited state upon protonation (1690 to 4760 cm^{-1}) is much stronger than employing the ground-state geome-

tries. Both considerations may be the explanation of the strong fluorescence enhancement.

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