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Molecular System Design for the Acetylcholine Fluorescent Chemosensor

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Abstract

We exploited a new molecular system - acetylcholine (neurotransmitter) detection system as a building block for the perfect molecular information system (sensing membrane of the chemical sensor) - using water soluble calix[n]arene-*p*-sulfonates which are useful even in *aqueous (water/methanol)* neutral solution. This achievement is due to several outstanding properties of these calix[n]arene derivatives such as low pK_a values, cation-interactions, and high water-solubility, etc.

1. Introduction

In the research and development of molecular sensing systems for information technologies, the miniaturization of devices down to the ultimate limits is one of the most important studies. With increasing demands for these miniaturized devices, the molecular design of recognition functional nanostructures playing a key role in the construction of artificial information molecular system consists of functional units such as molecules, supramolecules, and biologically active recognition centers related to the high information density, low power consumption, high flexibility, and high reproducibility, etc[1].

These well-designed nanostructure system could be useful for chemical and biochemical informatics. For instance, (bio)chemical sensing, electronically controlled drug release, molecular electronics, or bioelectronics. In this development of future information devices and their applications, especially in the field of (bio)chemical sensors, it is necessitated to understand molecular recognition functions between (bio)chemical species to be identified and well-designed sensing materials before the construction of nanostructural transducers.

From the above mentioned angles, we carefully examined the possibility of more simple as well as more functional recognition of acetylcholine (neurotransmitter) using water-soluble calix[x]arene-*p*-sulfonates (**1**) in aqueous neutral solution. This well known supramolecular receptor has been of interesting owing to their ease of preparation and distinguished molecular recognizable nature. Furthermore, they are now applied to the formation of information functional molecular system through molecular self assembly technology, molecular capsule formation and so on.

To the aim of this study we designed the cation-π interaction molecular systems which have been of much concern as a novel secondary force working in molecular recognition systems[2-5], enzyme active sites[6], and ion channels[7], etc. And also calix[x]arene derivatives can provide an

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ideal architecture for the evaluation of contributing cation-π interaction in the molecular sensor systems because they possess a π-basic cavity composed of four benzene rings and the various cavity shape can be easily created by immobilization of the conformational isomers[8-10].

2. Materials and Methods

Preparation of $\mathbf{1}_n$ (n = 4 and 6) has been described in well-known previous work and was carried out using the same process[11]. Compound **2** was synthesized as follows. 1-Pyrene carboxaldehyde (0.46 g, 2.0 mmol) dissolved in THF (20 mL) was added to the MeOH solution containing 1, 4-dimethylpyridinium iodide (0.47 g, 2.0 mmol) and piperidine (0.17 g, 2.0 mmol). After stirring this mixture at room temperature for 6 h, the product precipitation was filtrated, dissolved in MeOH, and ion-exchanged by addition of Ag_2O and HCl. The reaction mixture was filtrated and the filtrate was evaporated to dryness: yield 74%, mp (decomp.). 245 - 249 ℃; ^{1}H NMR (DMSO-d_6, 25℃) 4.30 (CH_3, s, 3H), 7.85 and 9.12 (CH=CH, d, 1H each), 8.12 - 8.98 (Pyrenyl-H, m, 13H). Anal. Calcd. for $C_{24}H_{18}NCl$: C, 81.00; H, 5.10; N, 3.94. Found: C, 80.90; H, 5.07; N, 3.96.

The apparatus used for the measurement of ^{1}H NMR spectra was a JEOL GX-400 (400 MHz) spectrometer. The methanolysis of acetylcholine and decomposition of **2** were confirmed by a conventional HPLC method (column Zorbax ODS, mobile phase MeOH) for the perfect molecular sensor system design.

3. Results and Discussion

Since the recognition events related to neurotransmitters proceed under physiological conditions, we here employed an 1:1 v/v water/MeOH medium at pH 8.0 (buffered with 0.1 M phosphate : corrected according to Bates' method)[12]. The use of MeOH was inevitable in order to suppress the aggregation of **2** in an aqueous system. It was confirmed that under these measurement conditions neither decomposition of **2** nor methanolysis of acetylcholine took place.

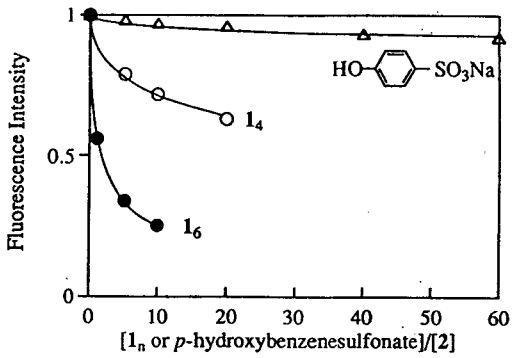

Fig. 1. Relative fluorescence intensity of **2** plotted against $\mathbf{1}_n$ or *p*-hydroxybenzensulfonate concentration: [2] = 1.00 × 10^{-4} M, 25 ℃, water/MeOH = 1:1 v/v, pH 8.0 with 0.1 M phosphate buffer, excitation (isobestic wavelength in the absorption spectra; 430 nm, emission; 580 nm.

Figure 1 shows the fluorescence intensity of **2** plotted against $\mathbf{1}_n$ or *p*-hydroxybenzenesulfonate (noncyclic reference compound) concentration. It is seen from Figure 1 that the relative fluorescence intensity (I/I_0) is efficiently quenched by $\mathbf{1}_4$ and $\mathbf{1}_6$ according to a typical saturation curve whereas *p*-hydroxybenzenesulfonate scarcely changes the fluorescence intensity[13]. Judging from the pK_a values of $\mathbf{1}_4$ and $\mathbf{1}_6$ (pK_{a1} = 3.3, pK_{a2} = 12.3, pK_{a3} = 12.9, pK_{a4} = 13.6 and pK_{a1} = 3.5, pK_{a2} = 5.0, p$K_{an,\ n\geq3}$ > 11.0 at 25 ℃, respectively[14-16]), one OH group is dissociated in $\mathbf{1}_4$ and two OH groups are dissociated in $\mathbf{1}_6$ at pH 8.0. These results show that these cavities containing one or two anionic phenolate units can include **2** owing to the cation-π interaction and quenched the singlet state of **2** in a pseudo-intramolecular manner[4a, 17]. From the analysis of the I/I_0 vs. $\mathbf{1}_n$ concentration plots by the Benesi-Hildebrand

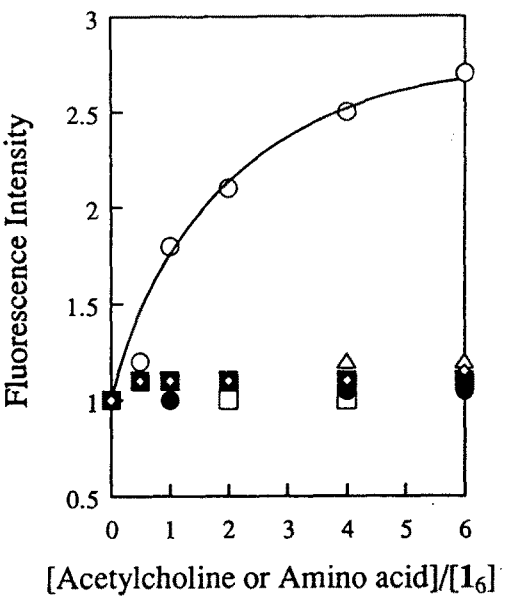

Fig. 2. Fluorescence regeneration: [2] = 1.00 × 10^{-4} M, [1_6] = 1.00 × 10^{-3} M, ○ acetylcholine, ● glycine, △ L-aspartic acid, □ L-proline, ■ L-phenylalanine ethyl ester hydrochloride, ◇ glycine methyl ester hydrochloride. Other conditions are the same as those in Figure 1.

equation, the association constants (K_{ass}) were estimated to be $10^{2.8}$ M^{-1} for 1_4 and $10^{3.8}$ M^{-1} for 1_6[18].

The foregoing results establish that 1_n can satisfy the first requirement (i.e., fluorescence quenching by included 2). The second requirement is related to the fluorescence regeneration which must be induced by selective substitution of 2 with acetylcholine. We tested this requirement using 1_6 which gave the larger K_{ass} for 2. In Figure 2, acetylcholine (Cl^- salt) was added to a 1:1 v/v water/MeOH solution containing 1_6 (1.00 × 10^{-3} M) and 2 (1.00 × 10^{-4} M). The fluorescence intensity of 2 was increased with increasing acetylcholine concentration. This indicating that acetylcholine could substitute 2 bound to the 1_6 cavity. From the analysis of the I/I_0 vs. acetylcholine concentration (Figure 2) by the substitution method, the association constant (K_{ass}) was calculated to be $10^{3.5}$ M^{-1}. In contrast, the increase in the fluorescence intensity was scarcely induced by the addition of amino acids (such as glycine, L-aspartic acid, L-proline, L-phenylalanine ethyl ester hydrochloride, glycine methyl ester hydrochloride, etc.). The results establish that amino acids bearing a primary or secondary ammonium group cannot compete with 2 for the 1_6 cavity. It is now clear that 1_6 can also satisfy the second requirement that the fluorescence regeneration occurs selectively only with acetylcholine[19].

To obtain further evidence for inclusion and substitution of 2 we measured the ^1H NMR spectra in 1:1 v/v D_2O/CD_3OD (a mixed solvent prepared from D_2O buffered to pD = 7.4 with 0.2 M phosphate and CD_3OD). Since 2 was not so soluble as to obtain the well-resolved NMR spectra, we used *N*-methyl-4-picolinium iodide (3) instead of 2. All chemical shifts of 3 (5 mM: δ_{N^+Me} 4.33 ppm, $\delta_{2\text{-}H}$ 8.61 ppm, $\delta_{3\text{-}H}$ 7.90 ppm, and $\delta_{4\text{-}CH3}$ 2.67 ppm) moved to higher magnetic field in the presence of 1_6 (5 mM: $\Delta\delta_{N^+Me}$ -0.78 ppm, $\Delta\delta_{2\text{-}H}$ -1.12 ppm, $\Delta\delta_{3\text{-}H}$ -0.80 ppm, and $\Delta\delta_{4\text{-}CH3}$ -0.52 ppm), indicating that this guest was included in the π-basic cavity 1_6. When acetylcholine (Cl^- salt: 5 mM) was added, all chemical shifts attributable to 3 moved to lower magnetic field ($\Delta\delta_{N^+Me}$ 0.30 ppm, $\Delta_{2\text{-}H}$ 0.39 ppm, $\Delta\delta_{3\text{-}H}$ 0.17 ppm, and $\Delta\delta_{4\text{-}CH3}$ 0.10 ppm from those in the presence of 1_6 and 3) whereas the N^+Me protons in acetylcholine shifted to higher magnetic field (from 3.22 ppm to 2.31 ppm)[20].

The results are consistent with the conclusion derived from the fluorescence spectroscopic studies. Based on the foregoing findings, one can now propose Figure 3 which unequivocally substantiates an artificial-signaling detection (molecular information functional) system for acetylcholine in an aqueous (water/methanol) system.

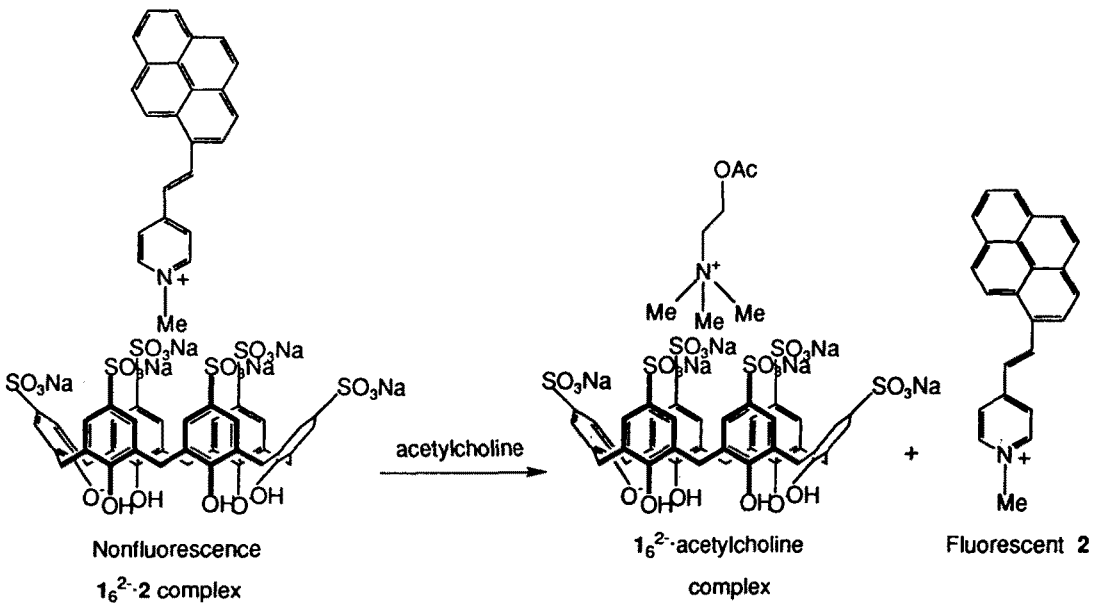

Fig. 3. Schematic representation of acetylcholine-signaling molecular-sensing system.

4. Conclusion

The present study offers an improved fluorescent-sensing molecular-system which is useful for the formation of information-functional nanostructured -interfaces. The success in this molecular system design is due to several outstanding characteristics inherent in $\mathbf{1}_n$ that is, (i) the pK_a values of the OH group (first dissociation for $\mathbf{1}_4$ and first and second dissociation for $\mathbf{1}_6$) are very low, (ii) the phenolate anion thus formed makes the cavity π-basic enough to include 2 or acetylcholine owing to the cation π-interaction[4a, 17], and (iii) the phenolate anion can act as a quencher for included 2. We believe that the $\mathbf{1}_n$ · 2 combined molecular system has enabled us to apply a convenient fluorescence method for the selective and nondestructive histochemical analysis of acetylcholine against amino acids in a biological information-system studies. And also this molecular sensor system has several unique potentials for the functional nanosystems of molecular informatics.

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[19] We evaluated whether other onium guests could regenerate the fluorescence intensity of 2. At [onium guest]/[1_6] = 6.0 we obtained the following I (in the presence of onium guest) / I_0 (in the absence of onium guest) values: $Et_2N^+Me_2OTs^-$ (1.25), Et_3N^+HCl (1.60), $Me_3S^+ClO_4^-$ (1.69), $Et_3N^+MeClO_4^-$ (1.70) and $Et_4N^+ClO_4^-$ (1.70). The results indicate that these onium guests can partially compete with 2 for the π-basic cavity but the I/I_0 values are all smaller than that for acetylcholine (2.70). Important from a practical viewpoint is the finding that as shown in Figure 2, this system is insensitive to biologically-ubiquitous ammonium groups in α-amino acids.

[20] In the $1_6 \cdot 3$ complex the protons in 3 distinctly shifted as described in the text whereas those in 1_6 scarcely moved. The chemical shift changes ($\Delta\delta$) assuming 100 % complexation were -0.83 ppm for N^+Me, -0.83 ppm for 3-H, and -0.54 ppm for 4-CH_3 (2-H was folded by Ar-H of 1_6). The N^+Me protons in acetylcholine also shifted to higher magnetic field ($\Delta\delta$ -1.04 ppm) in the presence of excess 1_6 (10 fold) where 100 % complexation can be assumed.

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고 광 락

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