Nondestructive Detection of Acetylcholine in Protic Media: Artificial-Signaling Acetylcholine Receptors

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Because of their potential for nondestructive detection and high sensitivity, there is great interest in the development of molecular sensors that change optical properties in response to the presence of biologically important chemical species.[1,2] With this goal in mind, we recently reported a new class of spiropyran derivatives possessing a molecular recognition site, of which isomerization to the open-colored merocyanine forms was induced by recognition of specific guest molecules.[3] We now present artificial-signaling acetylcholine receptors, in which the presence of acetylcholine (not other neurotransmitters) induces a large fluorescence enhancement in protic media (H_2O, MeOH, EtOH, etc.). The biological significance of these receptors lies in the fact that acetylcholine is one of the most abundant neurotransmitters in nerve cells, and that no reliable methods for the chemical transformation of acetylcholine to its fluorescent derivatives in the presence of other neurotransmitters are currently available.[4]

Resorcinol/acetaldehyde tetramer **1** is well-known to form tetraphenolate $\mathbf{1}^{4-}$ in alkaline media. The tetraphenolate adopts a bowl-shaped conformation with four intramolecular OH···$^-$O hydrogen bonds and can incorporate not only acetylcholine (**3a**) but also other polyalkylammonium cations into the cavity by the electrostatic and/or cation–π interactions.[5] We found that pyrene-modified *N*-alkylpyridinium cations (**2**) were also bound to the receptor (the association constants were similar to that obtained for the acetylcholine analogue:[6] 23 (**2a**), 5.3 (**2b**), and 7.6 (**3b**) × 10^4 M^{-1} in 0.01 M KOH/MeOH) and that orange fluorescence of the pyridinium dyes was strongly quenched by the complexation (Scheme 1). This type of quenching is

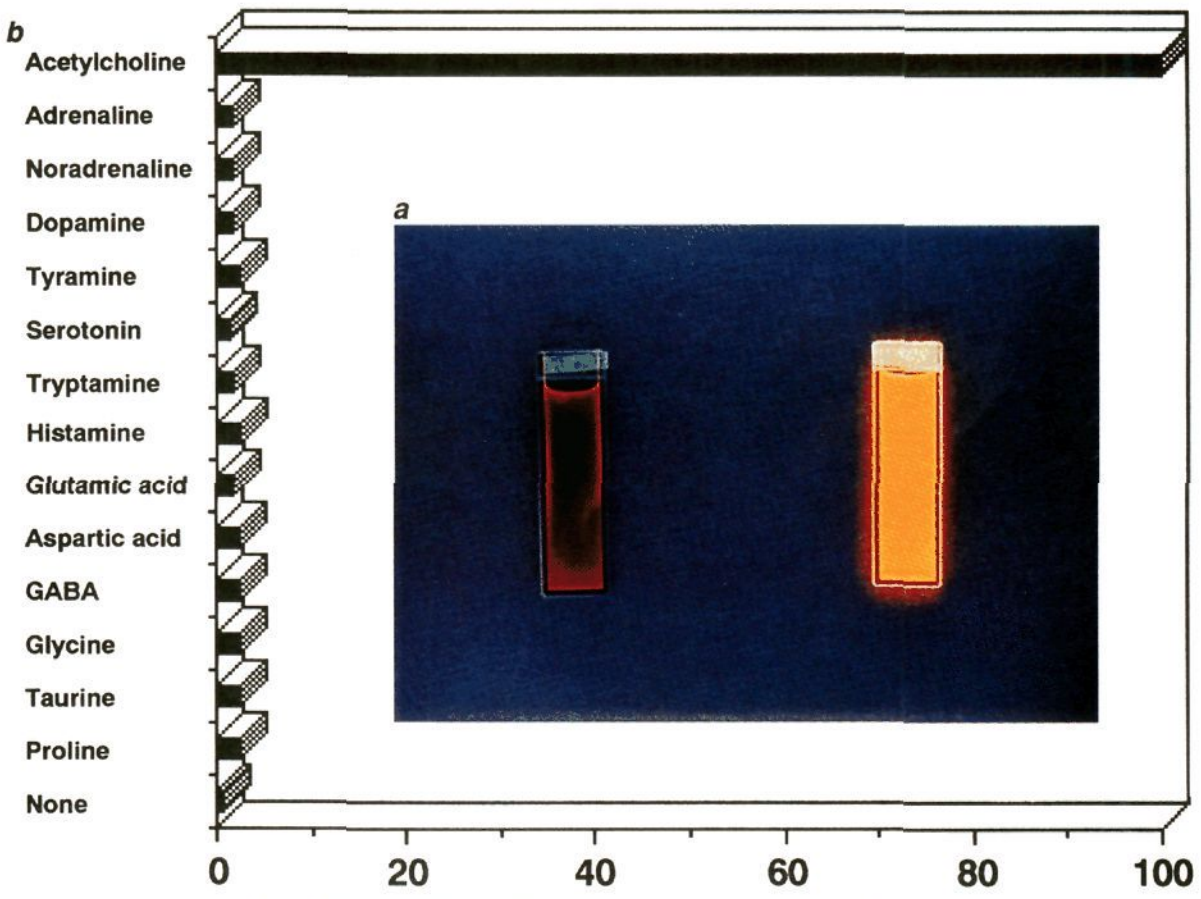
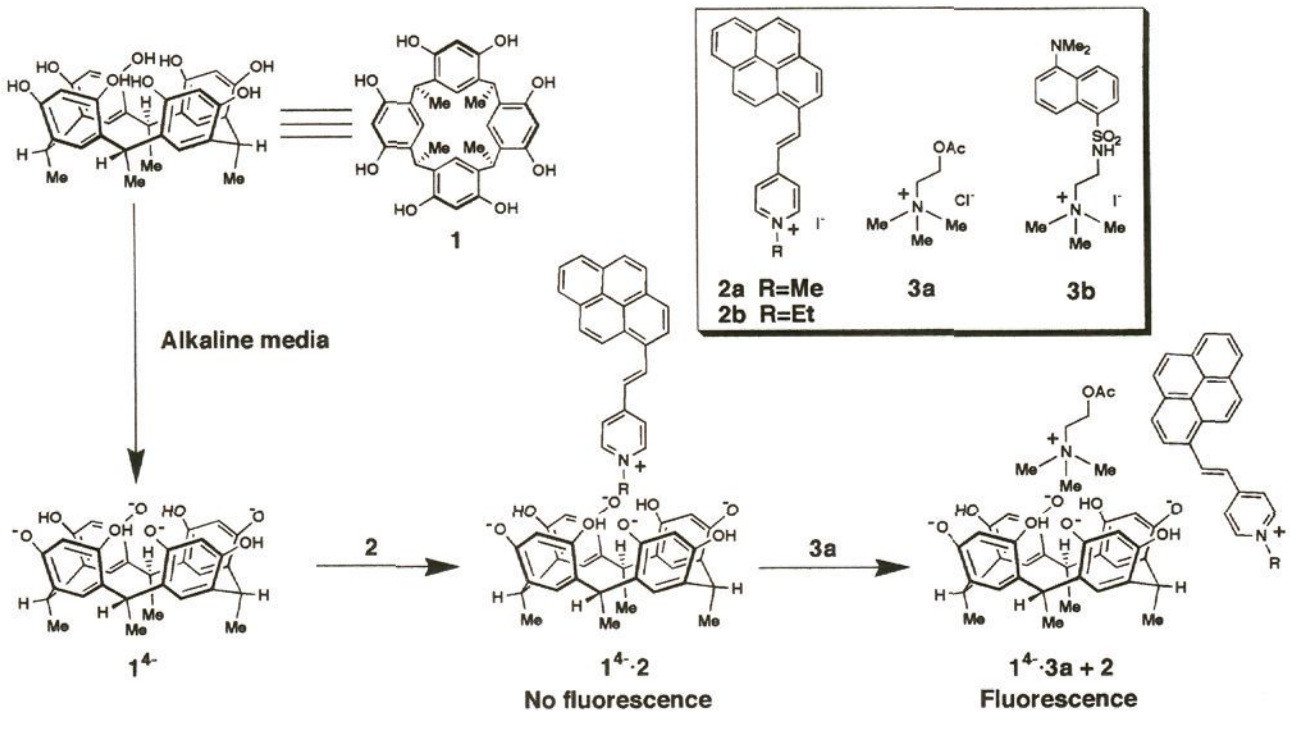


Figure 1. Fluorescence regeneration: (a) (left) before addition of acetylcholine and (right) after the addition; (b) relative fluorescence emission intensities at 567 nm of **2a** (1.0×10^{-4} M) in the presence of **1** (2.0×10^{-4} M) and various neurotransmitters (2.0×10^{-4} M) in 0.01 M KOH/MeOH. The excitation wavelength was 430 nm.

Scheme 1



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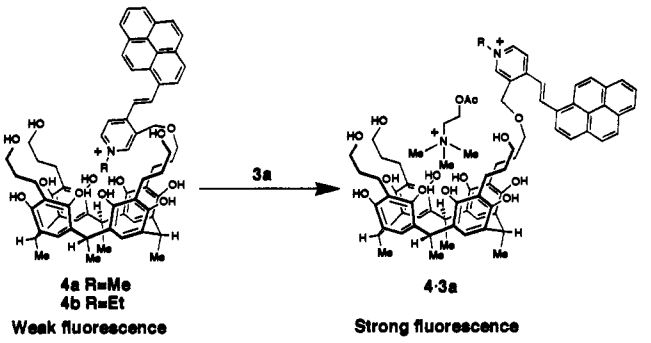
satisfactorily explained by the electron transfer from 1^{4-} to the excited **2** (PET mechanism).[7] The fluorescence regeneration was observed by the addition of acetylcholine to the nonfluorescent solution of $1^{4-}\cdot 2$ (Figure 1a). On the other hand, the fluorescence spectra were not affected by the addition of any of the other low molecular weight neurotransmitters (Figure 1b). The same results were obtained in water. The regenerated fluorescence emission was attributed to the increasing proportion of the free-pyridinium dye to that of the complexed one by the addition of acetylcholine. Indeed, this interpretation was verified by the following NMR studies. To a CD_3OD solution (0.7 mL) of **2b** (2.0 μmol) and NaOD (15 μmol) was added **1** (2.0 μmol), and the ^1H NMR spectra were measured. The pyridinium-Et protons were largely shifted upfield by 1.94 (CH_3) and 3.19 (CH_2) ppm, reflecting the formation of $1^{4-}\cdot 2b$. Subsequent addition of acetylcholine (2.0 μmol) to the solution resulted in a fair relaxation of the upfield shifts, while the acetylcholine signals were shifted upfield when compared to those of acetylcholine alone. These results clearly indicated that a considerable amount of the complexed **2b** was displaced by acetylcholine under these conditions as depicted in Scheme 1. The high selectivity observed here is due to the strong interaction between **1** and the quarternary ammonium moiety of acetylcholine: there are no such structures in other neurotransmitters.

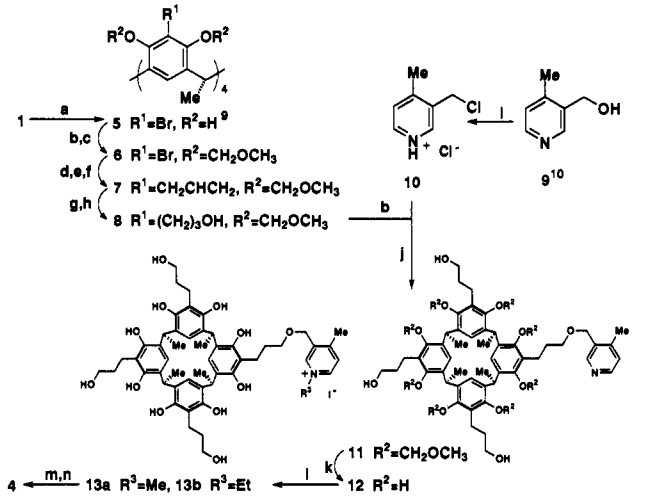
It is preferable, however, that such detection be carried out in neutral media. Unfortunately, in neutral protic solvents, the interaction of **1** with **2** was too weak to obtain substantial changes in their fluorescence spectra.[8] We thought that this difficulty could be overcome by attaching the pyridinium dyes to the wall of **1**: intramolecular interaction and the resulting intramolecular quenching of the fluorescence would be more efficient (Scheme 2). Thus, we synthesized fluorescence probe modified artificial acetylcholine receptors **4**, which were identified on the basis of ^1H NMR (500 MHz), FAB-MS (glycerin matrix), and elemental analyses (Scheme 3). The solubility of **4** is poor in neutral water, so that EtOH was used for the measurements.

The receptors **4** thus prepared showed weak fluorescence in neutral EtOH, indicating that the intramolecular quenching occurred efficiently. The addition of acetylcholine (3.0×10^{-2} M) to the solution (**4a**: 3.0×10^{-4} M) produced a strong

Scheme 2



Scheme 3[a]



[a] (a) NBS, 2-butanone; (b) NaH, DMF; (c) $ClCH_2OCH_3$; (d) *n*-BuLi, TMEDA, THF; (e) CuI; (f) allyl bromide; (g) 9-BBN, THF; (h) H_2O_2, NaOH, H_2O; (i) $SOCl_2$, CH_2Cl_2; (j) **10**; (k) HCl, H_2O, MeOH; (l) MeI or EtI, acetone; (m) 1-pyrenecarboxaldehyde, MeOH; (n) piperidine.

fluorescence emission (ca. twice). This spectral behavior illustrated that acetylcholine was accommodated into the cavity, so that the pyrene-modified pyridinium moiety was released from the cavity into the bulk MeOH, which resulted in the fluorescence enhancement. The conformational change in the receptors was confirmed from ^1H NMR spectra, consisting of data obtained for the intermolecular processes previously described. No other neurotransmitters induced such enhancement.

In summary, we have developed artificial-signaling acetylcholine receptors. In the cases of the intermolecular (the easily prepared pyridinium dyes **2** and the receptor **1**) and intramolecular (the fluorescence probe modified receptors **4**) systems, selective off/on signaling in alkaline media and in neutral protic media were observed, respectively. The present results suggest that our methods may be applied to the histochemistry of acetylcholine. The design of more efficient and selective receptors is now under way.

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