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Rational Design of Biologically Important Chemosensors: A Novel Receptor for Selective Recognition of Acetylcholine over Ammonium Cations

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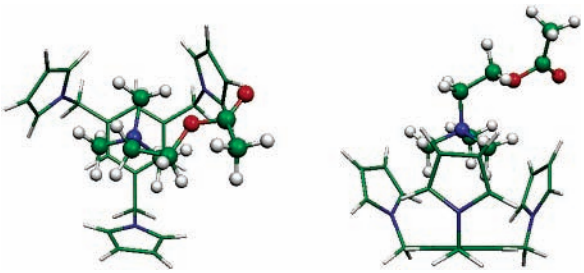
ABSTRACT



In consideration of competition between cation-π and hydrogen bond interaction forces, we designed a novel receptor, 1,3,5-tris(pyrrolyl)-benzene, which shows high selectivity for acetylcholine (ACh). The selectivity of the receptor for ACh over other ammonium cations is demonstrated by the ion-selective electrode (ISE) method in buffer solution. The binding free energy of the receptor with ACh in chloroform solution is measured to be 3.65 kcal/mol in the presence of chloride anion by nuclear magnetic resonance spectroscopy, and that in water is estimated to be much greater (~6 kcal/mol).

Recent advances in molecular recognition and supramolecular chemistry have made it possible to develop highly selective novel receptors for specific guests.[1] Among many factors, the cation-π interaction pioneered by Dougherty and co-workers[2] has been identified as a vital ingredient of the host−guest chemistry of biological systems[3] and the chemistry of nanostructures.[4]

Acetylcholine (ACh; $N^+(CH_3)_3CH_2CH_2OCOCH_3$), an important cationic neurotransmitter, tends to be bound to acetylcholinesterase via multiple cation-π interactions.[5] The ACh receptor has long served as a target molecule in designing potential therapeutic agents against various ail-

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ments, for example, myasthenia gravis, glaucoma, and possibly Alzheimer's desease.[6] Thus, the development of an appropriate receptor for ACh would aid the discoveries of novel drugs with understanding the binding mechanism. In addition, as ammonium-containing compounds are very important in chemical, biological, and physiological molecular systems, differential recognition of these species is extremely desirable.[7] Thus, we here report a systematically designed and synthesized novel receptor for ACh that displays a conspicuous preference for ACh over various ammonium cations.

To design an efficient receptor for ACh, we have employed a computer-aided receptor modeling approach.[8] In consideration that the optimal distance between the benzene centroid (or center of aromatic rings) and the nitrogen of NMe_4^+ is around 4.5 Å,[9] we have chosen the benzene-based tripodal system, which has attracted much interest recently (Figure 1).[10−12] In order for a receptor to be highly selective

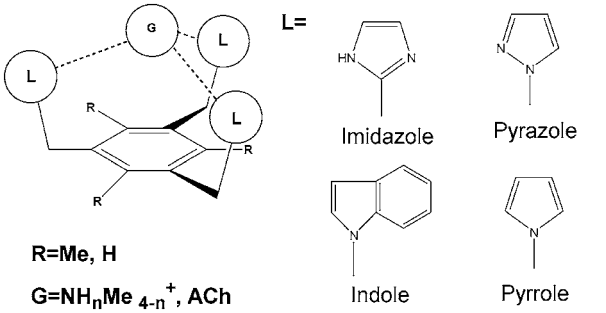

Figure 1. Schematics of tripodal receptors with subunits (**1**, L = imidazole; **2**, L = pyrazole; **3**, L = indole; **4**, L = pyrrole; **a**, R = Me; **,**: R = H).

for ACh or NMe_4^+ over NH_4^+, the dispersive-driven cation-π interaction must dominate the host−guest interaction,[9] while H-bond interaction with NH_4^+ should be minimized.

Since pyrazole[10] and imidazole[11] are known to have strong interactions with NH_4^+, and pyrrole and indole are predicted to provide very strong cation-π interactions,[13] we have carried out ab initio calculations[14] of tripodal receptors with subunits of imidazole, pyrazole, indole, and pyrrole (**1**−**4**), as shown in Figure 1.

From our theoretical investigations, the unmethylated benzene base (**1b**−**4b**) is found to be a better receptor for NMe_4^+, since methyl groups (**1a**−**4a**) tend to sterically hinder these subunits from facing the center of the receptor, hampering the formation of preorganized structure in favor of the cation-π interaction. Even in the unmethylated benzene base, only pyrrole subunits completely face the center and facilitate cation-π binding, while other subunits are skewed and as a consequence the cation-π interaction is diminished. In addition, ab initio calculations also indicate that the selectivity for NMe_4^+ over NH_4^+ is strongly enhanced for the receptors with pyrrole and indole subunits, while it is decreased for the receptor with the imidazole subunits. High level ab initio calculations predict that 1,3,5-tris(pyrrolyl)-benzene, **4b**, is an efficient receptor for NMe_4^+ over NH_4^+. This is further demonstrated from the free energy perturbation calculations employing molecular dynamics simulations.[15]

We confirm the selectivity and affinity of **4b** for ACh over various ammonium ions both theoretically and experimen-

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(14) (a) We have carried out high level of ab initio calculations (MP2/6-31G*//B3LYP/6-31G*) despite the large size of the molecular system. Compared with the case of **2b**, the relative binding energy of the NMe_4^+ complex with respect to the NH_4^+ complex is enhanced for **4b**, **3b**, and **1b** by 17, 13, and −13 kcal/mol, respectively. In the cases of **1b** and **2b**, the binding with NH_4^+ is much favored over that with NMe_4^+ because of the N···HN hydrogen bonding between the N atom and the cation. We have considered the solvent effect by nanosolvation by three chloroform/water molecules, since the coordination number of the pyrrole is three. In consideration of the interaction between NMe_4^+/NH_4^+ with three $CHCl_3$ molecules in the chloroform solution, **4b** shows an energy gain of 11 kcal/mol in favor of NH_4^+ over NMe_4^+. On the other hand, in consideration of the interaction between NMe_4^+/NH_4^+ with three H_2O molecules in the aqueous solution, **4b** shows the energy gain of 13 kcal/mol in favor of NMe_4^+ over NH_4^+. Indeed, the binding energy of **4b** with NMe_4^+ in the dielectric medium of chloroform is calculated to be 13 kcal/mol using the self-consistent reaction field (SCRF) method. The effective binding free energy at room temperature (∼40% of the binding energy in the cases of hydrated cations)[11] is roughly estimated to be ∼5 kcal/mol, in reasonable agreement with the NMR experimental value in the presence of Cl^- ions (3.65 kcal/mol) that is considered to be 1−2 kcal/mol smaller than that in the absence of Cl^- ions. (b) Calculations were carried out using Frisch, M.; Frisch. M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zarkzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*; Gaussian Inc.: Pittsburgh, PA, 1998.

tally. The calculated binding energy of **4b** with ACh is almost the same as that with NMe_4^+, as expected. The ab initio predicted structure of ACh bound to **4b** is shown in Figure 2.

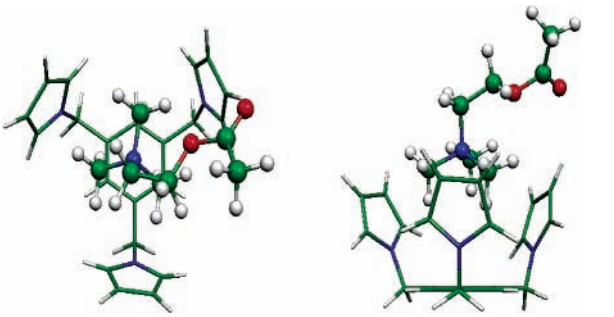

Figure 2. Top and side views of the calculated structures of **4b**·ACh.

To verify our prediction, we synthesized **4b**, which was achieved in one pot from readily available pyrrole, *t*-BuOK, and 1,3,5-tris(bromomethyl)benzene with 36% yield in THF.[16] Then, we performed an ion-selective electrode (ISE) study,[10,17,18] which confirms the highly selective binding of **4b** to ACh over NH_4^+ by 24 times (log $K_{ACh/NH_4^+} = -1.38$) in buffer solution (Figure 3).[19] The selectivity of **4b** to ACh over NH_4^+ at pH 7.4 is almost the same as that of pH 8.0. The selectivities of other ammonium cations decrease in the order log $K_{ACh/NH_4^+} = -1.38$; log $K_{Me_4N^+/NH_4^+} = -1.26$; log $K_{Me_3NH^+/NH_4^+} = -1.11$, log $K_{Me_2NH_2^+/NH_4^+} = -0.97$, log $K_{MeNH_3^+/NH_4^+} = -0.91$.

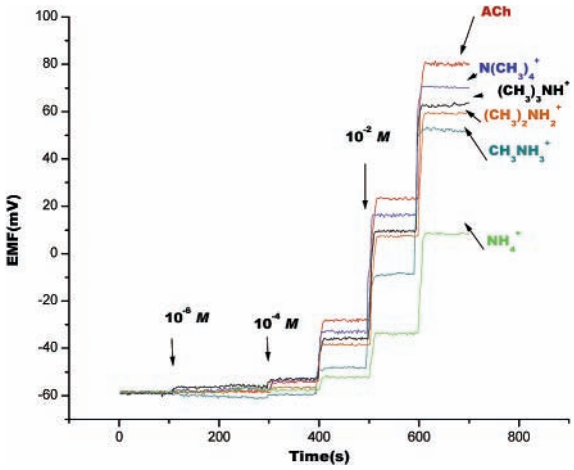

Figure 3. Electropotential graph showing ISE responses of **4b** to ACh and NH_4^+ in pH 8.0 buffer solution at 298 K.

To investigate binding affinity of **4b** for ACh, we performed nuclear magnetic resonance (NMR) titration.[20]

The binding free energy and association constant in chloroform solution in the presence of Cl^- ions are 3.65 kcal/mol and 472 M^{-1}, respectively.[21] We note that this binding energy seems to be not large. However, recently, Roelens and co-workers[22] have reported that the free energy significantly depends on the counterions. In particular, in the presence of Cl^- ions, the binding free energy for ACh is much smaller than other cases as a result of the ion pair formation. It is thus expected that the binding free energy of **4b** for ACh in the absence of Cl^- ions (the measurement was not feasible because of the poor solubility) would much increase (possibly close to our predicted value ~5 kcal/mol).[14] Although the binding strength between ACh and **4b** in the chloroform solution may not be strong because of the

(15) The free energy perturbation calculation of **4b** in 128 water molecules (for 1 ns simulation) using our previous approach including polarization effect[8a] shows that the selectivity of NMe_4^+ over NH_4^+ is enhanced in water in the presence of pyrrole receptors compared with that in the pure bulk water by 5 kcal/mol. This is highly contrasted with the same calculation in 89 chloroform molecules in which the selectivity of NH_4^+ is more favored than that of NMe_4^+.

(16) Analytical data for **4b**: mp 110−112 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.94 (s, 3H, Ph), 6.73(s, 6H, Py), 5.99(s, 6H, Py), 5.01(s, 6H, CH_2); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 139.66, 125.17, 121.26, 109.52, 53.16; MS (EI, 70 eV) *m*/*z* 315.17; Anal. Calcd for $C_{21}H_{21}N_3 \cdot 1/5H_2O$: C, 79.15; H, 6.66; N, 13.19. Found: C, 79.20; H, 6.56; N, 13.12.

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(18) Ion-selective membranes were prepared by dissolving the ionophore (**4b**), poly(vinyl chloride), and bis(2-ethylhexyl) adipate in THF at a ratio of 1:33:66 by weight. The solution was then poured onto a glass casting, which was then allowed to stand for about 2 days for the solvent (THF) to evaporate. The ISE electrode was prepared by fastening the membrane thus prepared to the end of a glass tubing, which contained a 0.10 M AChCl solution (or other ammonium ions) used as an inner filling solution and a silver chloride coated silver wire. The potentials of this indicator electrode were then measured with respect to an Orion double junction Ag|AgCl reference electrode (in saturated KCl) in solutions containing the same ions of various concentrations as those of the inner filling solution. These solutions were prepared by injecting appropriate amounts of a 1.0 M stock solution of AChCl (or other ammonium salt) into a 0.050 M tris buffer solution of pH 8.0 (and pH 9.0 and 7.4). The potentiometric selectivity coefficients were determined from the potential responses in solutions containing various concentrations of AChCl and 0.10 M interferents, i.e., NH_4^+ using the fixed interference method.[17] The concentrations of cations, ACh, and ammonium salts were increased by injecting concentrated solution every 100 s from 10^{-6} M to 10^{-1} M. Expectedly, the electromotive force (EMF) increased with an increase in the cation concentration. Considering pK_a of guest cations (NH_4^+ 9.24; $MeNH_3^+$ 10.64; $Me_2NH_2^+$ 10.73; Me_3NH^+ 9.81), the partially deprotonated ratio of guest molecules is less than 6% at pH 8.0. The selectivity of **4b** to ACh over NH_4^+ at pH 7.4 is almost the same as that of pH 8.0.

(19) We investigated the selectivity of **4b** for ACh over alkali metal cations (Na^+, K^+). The selectivities for alkali metal cations are similar to that for NH_4^+. To find out if the receptor **4b** binds ACh in its cavity, we synthesized *N*-benzylpyrrole **5** and carried out an ISE experiment. The selectivity of **5** for ACh over NH_4^+ (log $K_{ACh/NH_4^+} = -0.95$) is reduced compared with that of **4b** (log $K_{ACh/NH_4^+} = -1.38$). The difference in the selectivities of **4b** and **5** indicates that the higher affinity of **4b** with ACh should arise from four cation-π interaction sites by ACh with a benzene moiety and three pyrrole subunits, in contrast to the case of **5** interacting with ACh for which there are only two cation-π interaction sites by ACh with a benzene moiety and one pyrrole subunit. Since the hydrophobic effects by **4b** and **5** are considered to be similar as a result of the same benzene and pyrrole moieties present in both receptors, their significant difference in affinity would be explained by their difference in the number of cation-π interaction sites.

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(21) The solution (1 mM) of acetylcholine chloride in chloroform-*d* was titrated by chloroform-*d* solution (20 mM) of **4b** at 298 K. The addition of AChCl led to slight upfield shift (ca. 0.1 ppm) of the N-CH_3 protons of ACh, indicating that ACh is inside the cavity of **4b** by the cation-π interactions. Data analysis was made using eqnmr program.[20] The binding site of ACh was confirmed to be the tetramethylammonium group (Figure 2). The NMR analysis in water was not feasible, because **4b** was almost insoluble in water.

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high miscibility between ACh and chloroform, that in the aqueous solution is high as a result of the poor miscibility of ACh with water. Since the association constant of **4b** with NH_4^+ in water is measured to be 1300 M^{-1} in extraction experiments[23,24] and the ISE experiment shows 24-fold selectivity of ACh over NH_4^+ in buffer solutions, the association constant of **4b** with ACh in water is estimated to be up to ~30 000 M^{-1} (or binding free energy of 6.1 kcal/mol).

In conclusion, in the buffer solution, 1,3,5-tris(pyrrolyl)-benzene (**4b**) is a good receptor for ACh, NMe_4^+, NH_3Me^+, $NH_2Me_2^+$, and $NHMe_3^+$ over NH_4^+, while ACh and NMe_4^+ are slightly favored over NH_3Me^+, $NH_2Me_2^+$, and $NHMe_3^+$. The present approach to design novel receptors with selectivity would aid design of novel functional molecular systems and biologically important chemosensors based on ISE. Our results would be useful in molecular recognition studies of nanostructures in the gas phase wherein the origin of pure interaction forces is elucidated.[25]

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