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Interaction of dopamine and acetylcholine with an amphiphilic resorcinarene receptor in aqueous micelle system

Makoto Demura,[a,*] Tsutomu Yoshida,[a] Takatsugu Hirokawa,[b] Yasuhiro Kumaki,[a] Tomoyasu Aizawa,[a] Katsutoshi Nitta,[a] Isván Bitter[c] and Klára Tóth[d]

[a]*Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan*
[b]*Computational Biology Research Center, National Institute of Advanced Industrial Science and Technology, Aomi Frontier Building 17F, 2-43 Aomi, Koto-ku, Tokyo 135-0064, Japan*
[c]*Department of Organic Chemical Technology, Budapest University of Technology and Economics, H-1111, Budapest, Muegyetem rpt.-3, Hungary*
[d]*Research Group for the Technical Analytical Chemistry of the Hungarian Academy of Sciences, Institute of General & Analytical Chemistry, Budapest University of Technology and Economics, H-1111 Budapest, Szt. Gellért 4, Hungary*

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Abstract—The molecular recognition of neurotransmitters, dopamine and acetylcholine with an amphiphilic resorcinarene receptor was investigated in an aqueous sodium dodecylsulfate (SDS) micelle system by ^{1}H NMR measurements. The interaction distances of these neurotransmitters from the hydrophilic cavity of the amphiphilic receptor were estimated based on the calculation of the ring current shift using the atomic coordinates obtained from molecular dynamics calculation.

Resorcinarenes are readily available molecular platforms that have been widely used for the synthesis of artificial receptors as well as self-assembling structures.[1] A number of research projects are involved in modifying these macrocycles in order to create unique cavities and capsules for specific guests.[2–6] Recently, the advantage of the incorporation of a novel resorcinarene (actually pyrogallarene) in planar freely-suspended bilayer lipid membranes (BLMs), composed of phosphatidylcholine (PC) and dipalmitoyl phosphatidic acid, for the rapid selective detection of dopamine has been reported.[7] In addition, resorcinarene derivatives have been applied in biosensors for the detection of metals.[8–10] Recent work has investigated the selectivity of these resorcinarene receptors towards catecholamines.[7,10] However, direct observations about the molecular interactions of neurotransmitters with resorcinarene derivatives in amphiphilic environments, such as micelles and bilayer membranes, have not yet been published.

The present work was concentrated about the ^{1}H NMR observation of neurotransmitters, dopamine and acetylcholine (guests) with a pyrogallolarene receptor (host) in order to elucidate the molecular recognition and binding selectivity of the neurotransmitters in aqueous sodium dodecylsulfate (SDS) micelle system. In addition, the distance between these guests and the hydrophilic cavity of the amphiphilic host were estimated based on the chemical shift calculation of the ring current effect. Solution NMR is a useful method to observe the molecular interactions in a solution state.[5–8] As a mimic of the lipid membrane phase, the micelle system have been frequently used for solution NMR measurements.[11,12] A highly lipophilic host[13] (2,8,14,20-tetraundecylpyrogallol[4]arene, Fig. 1) was used to form a stable receptor pocket at the amphiphilic interface. The solid host synthesized here easily dissolved in the organic solvent, while this powder could not be solubilized in the aqueous SDS micelle system under mild stirring only. However, the solubilization in the aqueous micelle was achieved by a sonication treatment (3 s × 3 times) and standard solution NMR techniques[14] could be applied to this solubilized sample.

The ^{1}H NMR spectra of the host-guest pairs in the micelle system exhibited a complex pattern. The chemical

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* Corresponding author. Tel./fax: +81 11 706 2771; e-mail: demura@sci.hokudaia.ac.jp

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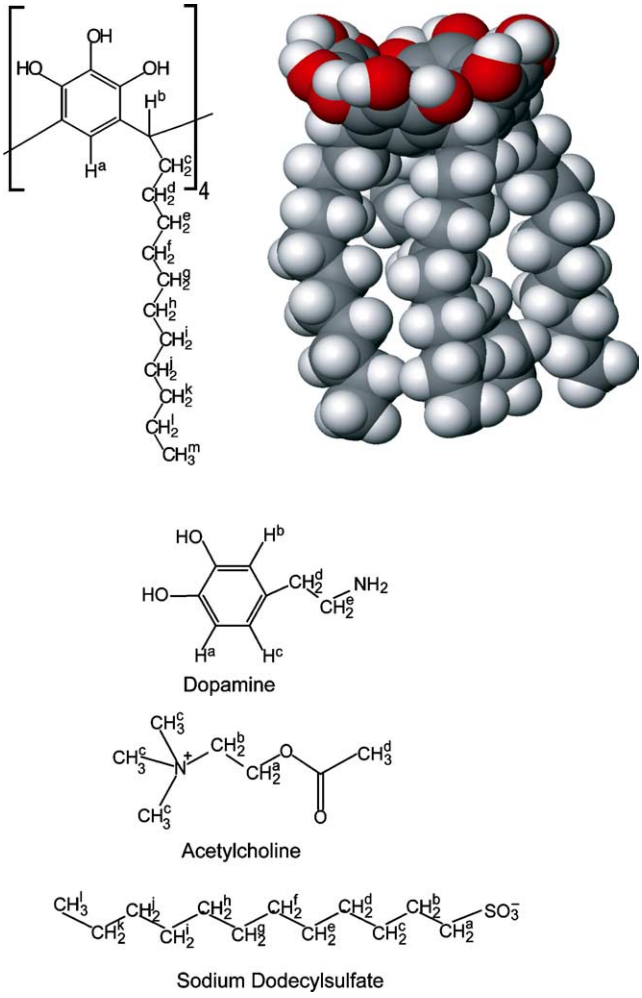

Figure 1. (Upper) molecular structure of the host (2,8,14,20-tetraundecylpyrogallol[4]arene). Space-filling model indicates the averaged structure, which was extracted from the molecular dynamics calculation. (Lower) structures of the guests (dopamine and acetylcholine) and sodium dodecylsulfate (SDS).

shifts of resorcinarene in the fully deuterated SDS-d_{25} micelle (a–m in Fig. 2) were similar to those in the organic solvent (chloroform-*d* and methanol-d_4, 1:1). However, the NMR peaks of the host with and without guest molecules in the SDS micelle system indicated a line-broadening because of the slower molecular motion[12] of the host, which is incorporated in the SDS micelles than in the isotropic organic solvent as shown in Figure 2.

Assignments of Aa–Ad (Fig. 2, upper) and Da–De (Fig. 2, lower) represent the proton peaks of acetylcholine and dopamine, respectively, and are summarized in Table 1. As listed in Table 1, the chemical shifts of both acetylcholine and dopamine with the resorcinarene were significantly upfield shifted. These results agree with the previous report on electrochemical sensing of dopamine by the resorcinarene, which is incorporated in the lipid bilayer.[7] The largest upfield shifts were observed for the Dd and De protons (−0.3 to −0.4 ppm) and for the Aa–Ac protons (−0.4 to −1.0 ppm). These upfield shifts originated from the ring current effect of the

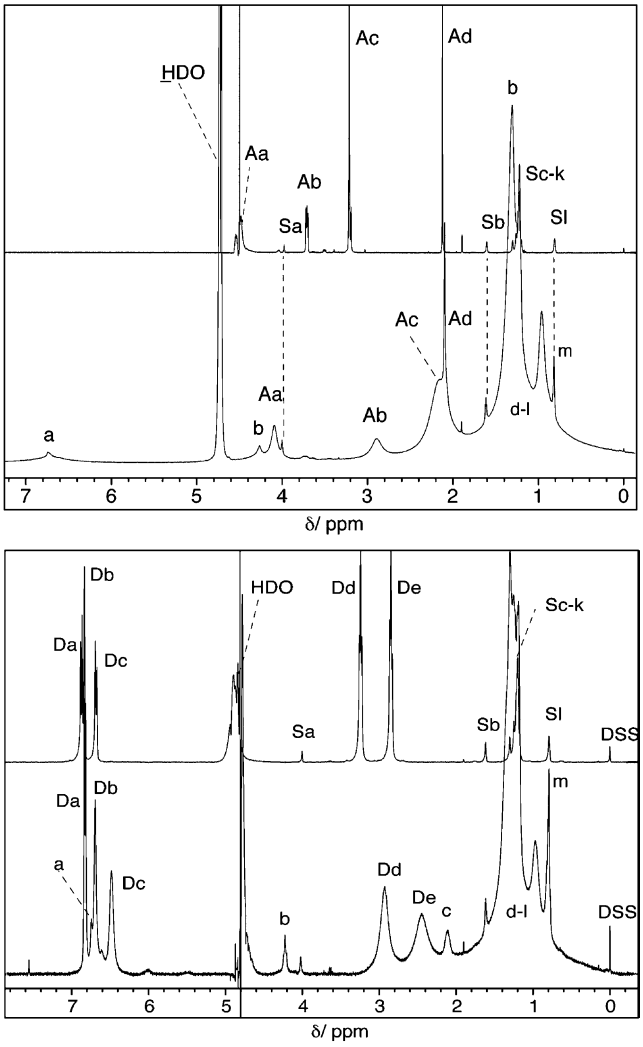

Figure 2. ^{1}H NMR spectra of acetylcholine (upper) and dopamine (lower) in 50 mM SDS-d_{25} micelle aqueous solution in the presence and absence of 5 mM resorcinarene. Dopamine-HCl and acetylcholine chloride (purchased from Wako Pure Chemical, Japan) were used. The molar ratio of the neurotransmitters and resorcinarene was approximately 2:1. Assignments of acetylcholine (Aa–Ad), dopamine (Da–De), SDS (Sa–Sl) and resorcinarene (a–m) correspond to the protons of each molecule presented in Figure 1.

Table 1. ^{1}H chemical shifts (ppm[a]) of the neurotransmitters in the SDS-d_{25} micelle system

Proton[b]	δ without resorcinarene	δ with resorcinarene	$\Delta\delta$
Dopamine			
Da	6.873	6.828	−0.045
Db	6.833	6.694	−0.139
Dc	6.681	6.483	−0.198
Dd	3.241	2.929	−0.312
De	2.846	2.441	−0.405
Acetylcholine			
Aa	4.545	4.093	−0.452
Ab	3.727	2.890	−0.837
Ac	3.227	2.167	−1.060
Ad	2.135	2.097	−0.038

[a] Parts per million from DSS at 30 °C. SDS: 50 mM, resorcinarene: 5 mM. [Dopamine]/[Resorcinarene] and [Acetylcholine]/[Resorcinarene] = 2.
[b] The proton labels are represented in Figure 1.

four-pyrogallol rings of the host. In addition, in the SDS micelle, the line width of both neurotransmitters was broader in the presence of resorcinarene than in the absence of the host, suggesting a slow exchange of neurotransmitters between the free and bound states. Temperature dependency of the ^{1}H chemical shift changes of acetylcholine and dopamine in the presence of the host (Fig. 3) supported the above consideration. Arising temperature brings about a faster exchange of neurotransmitters between the free and bound states. Thus, the apparent chemical shift changes decreased, as shown in Figure 3. In addition, a slight decrease of the line width of the guest molecules by increasing temperature was observed, due to faster molecular motion within the NMR time scale (data not shown).

In general, ^{1}H chemical shift (shielding effect) of protons (σ) may be calculated as[15]

$$\sigma = \sigma^{dia} + \sigma^{ani} + \sigma^{E} + \sigma^{ring} \quad (1)$$

where σ^{dia} is the diamagnetic shift, σ^{ani} is the shift due to anisotropy arising from locally induced currents on neighbouring atoms, σ^{E} is the polar effect arising from the electric fields created by polar groups in the molecule, and σ^{ring} is the ring current effect from aromatic systems. σ^{dia} depends on the atomic charge on the proton. In proteins and peptides, it is known that the secondary ^{1}H chemical shifts of $C^{\alpha}H$ ($\sigma^{obsd} - \sigma^{random\ coil}$) can be calculated from $\sigma^{ani} + \sigma^{E} + \sigma^{ring}$ by ignoring the term of σ^{dia} and the dominant effect comes from the σ^{ring} term such as phenylalanine and tyrosine.[15,16] The Fortran program SHIFTCALC is available for this purpose.[16] In the present study, the chemical shift changes of the guest molecules upon binding by the resorcinarene are considered to originate predominantly from the ring current shift caused by the four aromatic (pyrogallol) rings. Therefore, the distance between the host and guest molecules can be estimated by the ^{1}H chemical shift changes of the guest molecule measured in the presence of resorcinarene.

The ring current shift was calculated according to the Haigh and Mallion method[17,18] using the SHIFTCALC program, with the atomic coordinates of the host (Fig. 1), which were determined with the MOE molecular dynamics calculation.[19] Figure 4 shows the contour map of the ring current shift at the cross-section along a centre of the molecule. The labelled numbers on the contour lines represent the chemical shift values. The negative (solid line) and positive (broken line) values indicate the upfield and downfield shift, respectively. As shown in Figure 4, the cross-section of the hydrophilic cavity of the resorcinarene (upper half of this figure) indicates a large upfield shift and a gradual decrease. Contrary, the opposite side of the host indicates a lowfield shift. Acetylcholine (the molecular length between the acetyl and ammonium carbons is about 6–6.5 Å) shows chemical shift changes from about −1.0 to −0.05 ppm on binding to resorcinarene (Table 1). This range allowed us to conclude a 2–7 Å proximity to the hydrophilic surface of the aromatic cavity and the molecular recognition due to cation-π interaction between the choline group of acetylcholine and the hydrophilic cavity composed of four-pyrogallol rings. In contrast, dopamine (it has about a 7.5 Å length from N to *para*-OH) shows −0.4 to −0.05 ppm changes, suggesting long-range interaction when compared with acetylcholine.

In order to clarify the selectivity (affinity) of the resorcinarene towards the two kinds of neurotransmitters, the competitive binding experiments based on the chem-

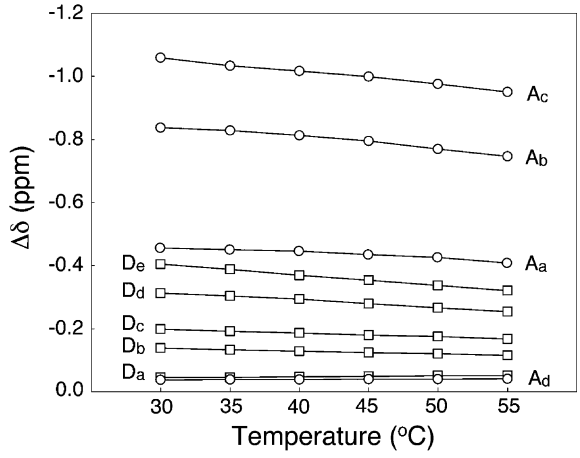

Figure 3. Temperature dependencies of the ^{1}H chemical shift changes of acetylcholine (As–Ad) and dopamine (Da–De) interacting with the host, resorcinarene in the SDS micelle system.

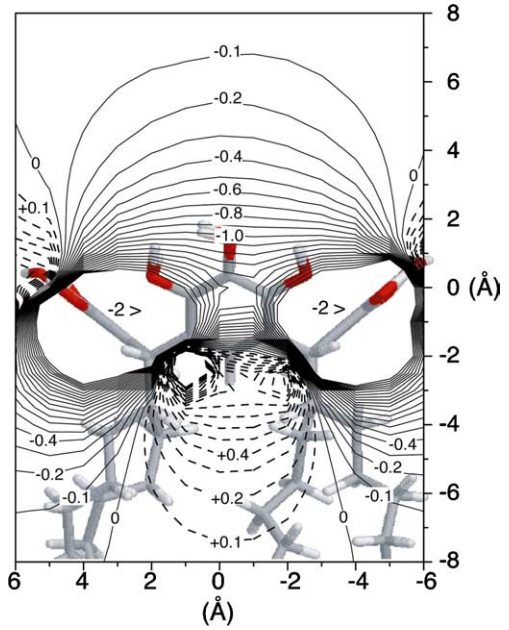

Figure 4. Contour map of ring current shifts of resorcinarene at the cross-section along a centre of the molecule including 1, 4, 13, 16-aromatic carbons. A stick model of the host is superpositioned. The ring current shifts were calculated according to the Haigh and Mallion method[17] using the SHIFTCALC program,[16] with the atomic coordinates of resorcinarene. The ring current efficiency of a pyrogallol ring was assumed to be 0.61 established for the benzene ring.[18] The labeled number on the contour lines represent the chemical shift changes. The negative (solid line) and positive (broken line) values indicate the upfield and downfield shift, respectively. Contour lines within ±2 ppm changes are shown.

Table 2. Chemical shift changes ($\Delta\delta$, ppm[a]) of neurotransmitters upon interaction with resorcinarene under single and competitive binding conditions in the SDS-d_{25} micelle

Proton	$\Delta\delta$ (Single)	$\Delta\delta$ (Competitive[b])	Residual upfield shift (%)[c]
Dopamine			
Db	−0.139	0.007	−5
Dc	−0.198	−0.001	1
Dd	−0.312	−0.011	4
De	−0.405	−0.004	1
Acetylcholine			
Aa	−0.452	−0.106	23
Ab	−0.837	−0.168	20
Ac	−1.060	−0.213	20

[a] Parts per million from DSS at 30 °C. SDS: 50 mM, resorcinarene: 5 mM. The concentration of dopamine and acetylcholine was 10 mM.

[b] In the competitive experiments, the concentration of the neurotransmitters as an inhibitor was 100 mM.

[c] Residual upfield shift (%) = $\Delta\delta$ (competitive)/$\Delta\delta$ (single) × 100. Residual upfield shifts of protons exhibiting more than 0.1 ppm differences between single and competitive conditions were listed.

ical shift perturbations were performed in the SDS micelle system (Table 2). When acetylcholine was added to the dopamine-resorcinarene system in a ratio of [acetylcholine]/[dopamine] = 10, the ring current shifts of the single binding (about −0.1 to −0.4 ppm) extremely vanished (a few percent). In contrast, in the reverse experiment (addition of dopamine to the acetylcholine-resorcinarene system in a ratio of [dopamine]/[acetylcholine] = 10), acetylcholine altered the ring current shifts of the single binding (about −0.4 to −1.1 ppm) by 20%, due to cation-π interaction between the acetylcholine ammonium cation and the aromatic cavity of resorcinarene. Thus, it was concluded that the affinity of acetylcholine to the amphiphilic host used in this experiment is higher than that of dopamine.

Acknowledgements

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14. All NMR spectra were run on JEOL A400 and A500 spectrometers operating at 400 and 500 MHz for the proton frequency, respectively, with a sample temperature of 298, 303, 308, 313, 318, 323 and 328 K. Proton peaks of DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate) in fully deuterated SDS-d_{25} micelle and chloroform (7.27 ppm) in the mixed organic solvent systems were used as the proton chemical shift standard.
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18. Williamson, M. P., private communication. The ring current efficiency of the pyrogallol rings in the chemical shift calculation was assumed to be 0.61, taken from the value of a benzene ring, that is, by adding an OH group, ring current efficiency of a benzene ring decreases by 0.13 and thus, in the case of a pyrogallol ring, 0.61 is obtained from 1 to 3 × 0.13.
19. Molecular dynamics (MD) calculations on the resorcinarene were run using the MOE program and the CHARMm22 force field in vacuo, as distributed in the MOE (Chemical Computing Group, Inc.: http://www.chemcomp.com/). In one MD simulation, a distance-dependent dielectric and a non-bonded cut-off value of 7 Å were used. The total simulation time in this case was 5 ns. In this simulation, the canonical ensemble (NVT) was used with a target temperature of 300 K. An integration time step of 1 fs was used, and the structures were saved to disk at every 100 steps. The averaged structure (atomic coordinates) was determined by the clustering of all-contacted structures with 250 structures, which were calculated by 20 ps for 5 ns. See also: Igarashi, S.; Hirokawa, T.; Sode, K. *Biomol. Eng.* **2004**, *21*, 81–89.