Interaction between Quaternary Ammonium Ions and Dipeptides: Positive Anion Allosteric Effect

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The binding properties of dipeptides possessing aromatic residues towards quaternary ammonium ions have been investigated by ^{1}H-NMR spectroscopy. The intermolecular hydrogen bonding between exchangeable protons (OH and NH) of aromatic residues of dipeptides and the counter anion of ammonium ion is the primary force. After the formation of the intermolecular hydrogen bonding, two aromatic residues of dipeptides can provide π-base cavity to interact with the quaternary ammonium moiety.

Noncovalent interactions are of fundamental importance in determining the highly dimensional structures and fundamental properties of molecular systems in biology, chemistry, and materials sciences. Relatively strong non-covalent bonding such as salt bridge and hydrogen bonding has been extensively investigated. In contrast, weak noncovalent bonding such as π–π, CH–π and cation–π interaction is now known to play important roles in the stabilization of the complexes[1—3] but is not well understood, because the weak noncovalent bond is usually cancelled behind the strong noncovalent bonding. However, there is much attention on cation–π interaction in a biological event such as recognition of biological relevant quaternary ammonium ions, for example, the neurotransmitter acetylcholine, by receptors and enzyme.[4] Therefore, several research groups have devoted considerable effort to the synthesis of receptor molecules to investigate binding properties of quaternary ammonium cations.[5—10]

In spite of many reports of synthetic receptors for binding quaternary ammonium ions, we are unaware of any on acyclic compounds acting as a receptor.[11] Recently, we reported a study on the recognition of quaternary ammonium ion using acyclic phenol-formaldehyde dimer as a receptor, and found that quaternary ammonium cation is entrapped in the π cavity which is constructed of two phenol units; its counter ion participates in the recognition process by coordination *via* hydrogen bonding of the two hydroxyl groups.[12]

As part of this study, we investigated the interaction between quaternary ammonium ions and dipeptides having the aromatic amino acid residues tyrosine and tryptophane, which have exchangeable protons (OH and NH) and aromatic surfaces.

Dipeptides (**1**) were prepared in good yield by the condensation reactions of *N*-Boc L-amino acid with L-amino acid methyl ester monohydrochloride in the presence of *N*-methyl morphorine in CHCl$_3$ using DCC (1,3-dicyclohexylcarbodiimide) as a condensation reagent according to the literature (Fig. 1).[13]

The interaction behavior of dipeptides (**1**) and quaternary ammonium ions (**2**) was studied by using ^{1}H-NMR spectroscopy at 20 °C. When benzyl trimethyl ammonium chloride (**2a**) was added into a CDCl$_3$ solution of Boc-Tyr-Tyr-OMe (**1a**) at the ratio of **1a** : **2a**=1 : 1 ([**1**]=[**2**]=10 mM), all peaks of **2a** moved to a higher field due to the ring current ef-

fect of the aromatic rings of **1a** during formation of the complex as shown in Fig. 2. Especially, higher induced chemical shifts of the N$^+$–CH$_2$ and N$^+$–CH$_3$ protons of **2a** were observed, suggesting that the ammonium moiety is located close to the aromatic surface of **1a**.[5—12] The same phenomenon was also observed in the mixture of Boc-Trp-Trp-OMe (**1b**) and **2a** in CDCl$_3$. In contrast, the chemical shifts of **2a** scarcely changed in the presence of Boc-Phe-Phe-OMe (**1c**), indicating that no complexation occurs between **1c** and **2a**. Although Boc-Tyr-Phe-OMe (**1d**) and Boc-Trp-Phe-OMe (**1e**) having a phenol or an indole group can interact with ammonium ion (**2a**), the induced chemical shifts are smaller than those of **1a** and **1b**. We also observed a similar induced chemical shift pattern in the complexation of peptides (**1**) with other quaternary ammonium salts (**2b**, **c**) as shown in Table 1.

Interestingly, the induced chemical shifts were dramatically reduced by the replacement of counter anion of ammonium from chloride (**2a**) to iodide (**2d**). Another important observation was that the ^{1}H-NMR signals for exchangeable protons (NH and OH) of aromatic amino acid residues of **1** were considerably downfield-shifted and extensively broadened upon addition of **2**.[12,14] These results could be explained by hypothesizing an important role for the anion that interacts with the OH or NH groups of **1**. This hypothesis is supported by the lack of efficiency shown by Boc-Phe-Phe-OMe (**1c**) of which the absence of the exchangeable proton prevents any participation of the anion in the binding event.

We estimated the stoichiometry of the complex (**1**–**2**) using the Job plot method.[15] The 1 : 1 stoichiometry of the complexes (**1**–**2**) was confirmed by the plot, which contains a maximum at the molar ratio of 0.5 in all cases (Fig. 3). The binding affinities of peptide (**1**) to quaternary ammonium ions (**2**) were determined by nonlinear least-squares fitting method of the saturation curves obtained from ^{1}H-NMR titration (Fig. 4).[16] During the titration by varying concentrations of **1** and **2** in CDCl$_3$, the signals for the protons of ammonium moiety of **2** are gradually upfield-shifted.[17] The obtained association constants (K_a) of the complexes (**1**–**2**) indicated that **2** was bound by tyrosine peptides (**1a**, **d**) more efficiently than tryptophane peptides (**1b**, **e**). It is also clear that the association constants are strongly dependent on the nature of the anion of **2**. Although Boc-Tyr-Tyr-OMe (**1a**) and Boc-Tyr-Phe-OMe (**1d**) had similar association constants, the

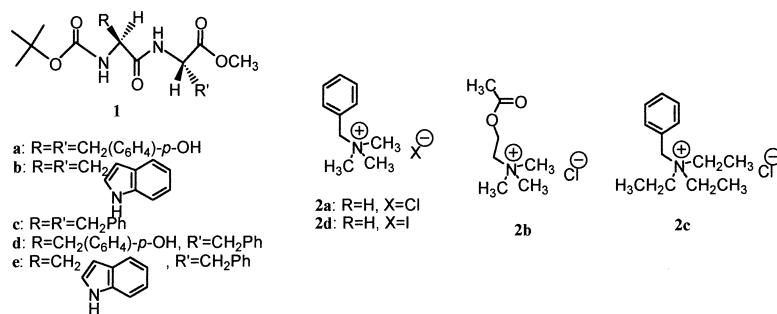Fig. 1

Table 1. Chemical Shift Changes ($\Delta\delta$) in **2** Induced by Added **1**[a)]

Ammonium ion (**2a**)/[ppm]

Peptide	$N(CH_3)_3$		NCH_2		o-proton		m-proton		p-proton	
	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
None	3.42	—	5.04	—	7.66	—	7.49	—	7.52	—
1a	3.17	−0.25	4.68	−0.36	7.51	−0.15	7.43	−0.06	7.49	−0.03
1b	3.14	−0.28	4.73	−0.31	7.51	−0.15	7.44	−0.05	7.49	−0.03
1c	3.42	0.00	5.04	0.00	7.66	0.00	7.49	0.00	7.52	0.00
1d	3.34	−0.08	4.91	−0.13	7.62	−0.04	7.48	−0.01	7.51	−0.01
1e	3.38	−0.04	4.99	−0.05	7.63	−0.03	7.48	−0.01	7.52	0.00

Ammonium ion (**2b**)/[ppm]

Peptide	$N(CH_3)_3$		NCH_2		OCH_2		$COCH_2$	
	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
None	3.57	—	4.57	—	4.19	—	2.13	—
1a	3.28	−0.29	4.42	−0.15	3.85	−0.34	2.05	−0.08
1b	3.28	−0.29	4.39	−0.18	3.86	−0.33	2.13	−0.05
1c	3.55	0.02	4.57	0.00	4.17	0.02	2.10	0.00
1d	3.48	−0.13	4.48	−0.09	4.04	−0.15	2.11	−0.03
1e	3.42	−0.09	4.51	−0.06	4.09	−0.10	2.11	−0.02

Ammonium ion (**2c**)/[ppm]

Peptide	$(CH_3)_3$		$(CH_2)_3$		NCH_2		o-proton		m-proton		p-proton	
	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
None	1.48	—	3.49	—	4.85	—	7.57	—	7.46	—	7.50	—
1a	1.42	−0.06	3.32	−0.17	4.58	−0.27	7.46	−0.11	7.44	−0.02	7.50	0.00
1b	1.36	−0.12	3.29	−0.20	4.60	−0.25	b)		7.50	0.00
1c	1.48	0.00	3.49	0.00	4.85	0.00	7.57	0.00	7.46	0.00	7.50	0.00
1d	1.46	−0.02	3.45	−0.02	4.79	−0.06	7.54	−0.03	7.45	−0.01	7.50	0.00
1e	1.47	−0.01	3.46	−0.03	4.81	−0.04	7.55	−0.02	7.45	−0.01	7.50	0.00

Ammonium ion (**2d**)/[ppm]

Peptide	$N(CH_3)_3$		NCH_2		o-proton		m-proton		p-proton	
	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
None	3.38	—	4.90	—	7.63	—	7.53	—	7.53	—
1a	3.30	−0.08	4.78	−0.12	7.57	−0.06	7.51	−0.02	7.52	−0.01
1b	3.31	−0.07	4.82	−0.08	7.59	−0.04	7.51	−0.02	7.52	−0.01
1c	3.38	0.00	4.90	−0.00	7.63	0.00	7.53	0.00	7.53	0.00
1d	3.34	−0.04	4.83	−0.07	7.61	−0.02	7.52	−0.01	7.52	−0.01
1e	3.35	−0.03	4.87	−0.03	7.63	0.00	7.53	0.00	7.53	0.00

a) At 20 °C in CDCl$_3$, [**1**]=[**2**]=10 mM, — denotes the shift to higher magnetic field. b) The aromatic protons of **2d** are overlapped with the protons of **1b**.
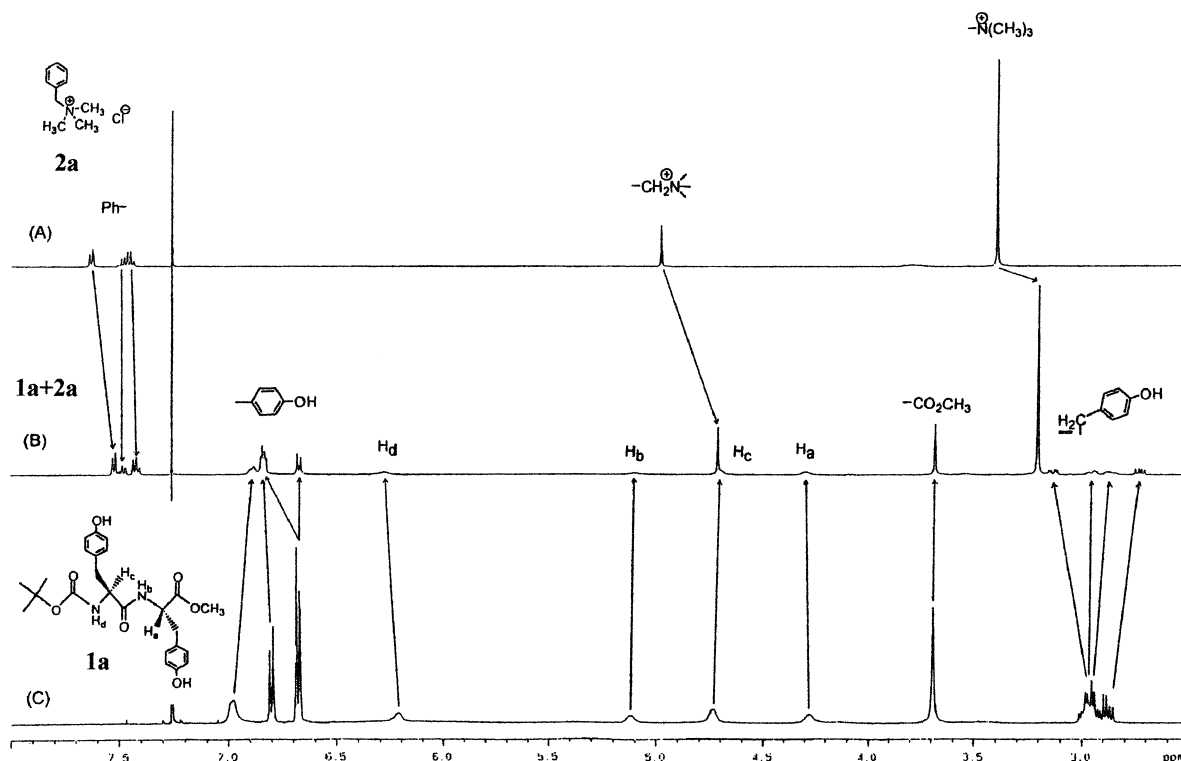
Fig. 2. Partial ^{1}H-NMR Spectra for **2a** (A), **1a**+**2a** (B), **1a** (C) at 20 °C in CDCl$_3$
[**1a**]=[**2a**]=10 mM.

Table 2. Association Constants K_a [M^{-1}] for 1 : 1 Complexes Measured in CDCl$_3$ of Quaternary Ammonium Salts (**2a–d**) with Dipeptides (**1a–e**) at 20 °C

Dipeptide	Ammonium ions			
	2a	**2b**	**2c**	**2d**
1a	100±13	130±3	125±12	22±1
1b	69±4	80±9	55±2	10±1
1c	<1	<1	<1	<1
1d	70±6	87±8	59±1	13±1
1e	18±2	30±2	6±1	2±<1

Fig. 4. ^{1}H-NMR Titration of **1a** with **2** in CDCl$_3$ at 20 °C

($\delta_f - \delta$) indicates a change in chemical shift of N$^+$(CH$_3$)$_3$ or N$^+$CH$_2$ protons of **2** as a result of added **1a**. Points are experimental and curves are calculated by non-linear regression.

Fig. 3. Job Plots in the Complexation of **1a** with **2**

induced chemical shifts were quite different. Considering that association constants are mainly governed by the intermolecular hydrogen bonding, the large induced chemical

shifts observed in the complexes (**1a**–**2**) can be rationalized by assuming that cation–π interaction exists. Similar large induced chemical shifts were also observed on the complexes of Boc-Trp-Trp-OMe (**1b**) with **2**, implying the participation of the cation–π interaction on binding **2**.

In order to elucidate the complex structure, we carried out a 2D NOE experiment (ROESY) using a 1 : 1 molar mixture of **1a** and **2a** ([**1a**]=[**2a**]=10 mM in CDCl$_3$). ROESY spectrum showed the cross peaks between N$^+$(CH$_3$)$_3$ of **2a** and aryl protons of **1a**, indicating that N$^+$(CH$_3$)$_3$ moiety of **2a** is located close to the aryl surfaces of **1a** (Fig. 5). This agrees with a situation where the N$^+$(CH$_3$)$_3$ moiety is inside the π-cavity created through the hydrogen bonding between anion and the peptide phenol groups. We also investigated whether
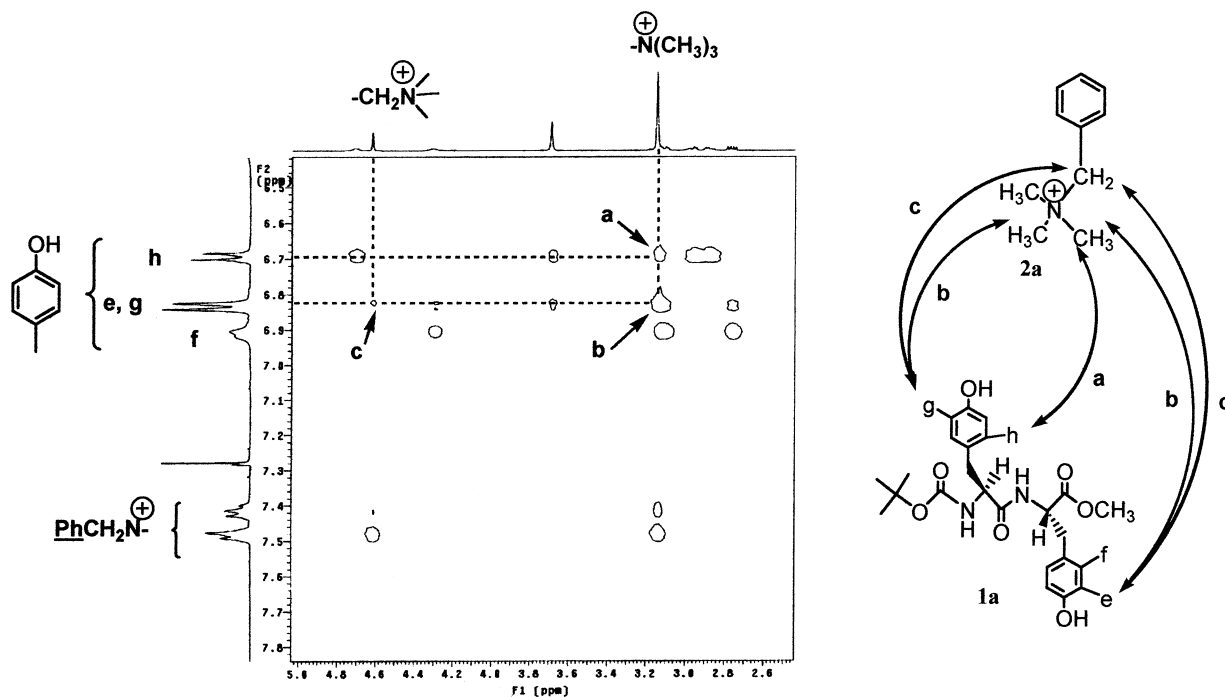
Fig. 5.   ROESY (500 MHz, Mixing Time=300 ms) Spectrum for **1a** and **2b** in CDCl₃ at 20 °C
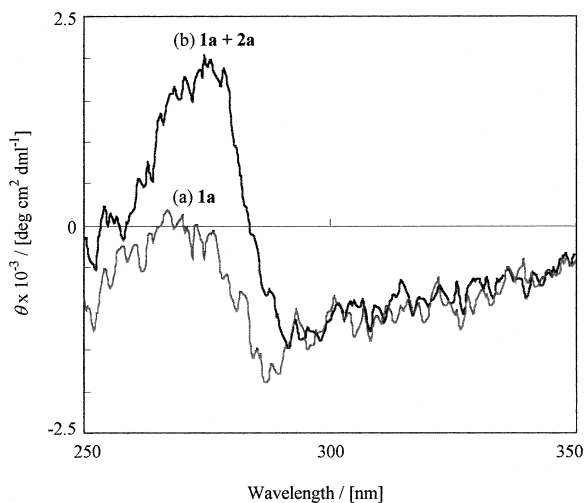[**1a**]=[**2a**]=10 mM.

Fig. 6.   CD Spectrum of **1a** in the Absence (a) and in the Presence of **2a** (b) in CHCl₃ at 20 °C
[**1a**]=[**2a**]=1.0 mM.

Fig. 7.   Proposed Structure of Complex (**1a–2a**)

tion process by coordinating *via* hydrogen bonding with the OH or NH groups of tyrosine or tryptophane residue of dipeptides. As a result of the formation of the intermolecular hydrogen bonding, the two aromatic amino acid residues of dipeptides can provide a concave π-base to interact with the cation moiety of ammonium ions. This efficiency order (Cl⁻>I⁻) depends on the ability of anion to act as a hydrogen bonding acceptor, and supports the hypothesis of a positive anion allosteric effect.

Experimental

General Procedure of the Synthesis of Dipeptides (1)   To a solution of amino acid methyl ester monohydrochloride (1 mmol), *N*-Boc amino acid (1 mmol), and *N*-methyl morphorine (1 mmol) in dry chloroform (50 mmol) was added a solution of DCC (1 mmol) in dry chloroform (10 ml) over 20 min at 0 °C. After the addition was complete, the mixture was allowed to stir at room temperature for 12 h. After removal of the precipitate by filtration, the filtrate was condensed. The residue was subjected to column chromatography on silica gel using hexane–ethyl acetate 1 : 1 as an eluent to give pure dipeptide (**1**) as colorless crystals.[13]

the conformation of peptide (**1a**) changes upon interaction with ammonium ion (**2**) by CD (circular dichroism) spectroscopy, which is sensitive to conformational change. As shown in Fig. 6, CD spectral absorption of **1a** was observed at 289 nm (θ=−1870). Upon addition of **2a**, the absorption pattern was changed and the signal was observed at 291 nm (θ=−1460) and 275 nm (θ=2040), supporting the above speculation. From these results we presented the plausible structure of the complex (**1a–2**) in Fig. 7.

In conclusion, we found that dipeptides constructed from the aromatic amino acid residues tyrosine and tryptophane can serve as a receptor of quaternary ammonium ions. The counter anion of ammonium ion participates in the recogni-
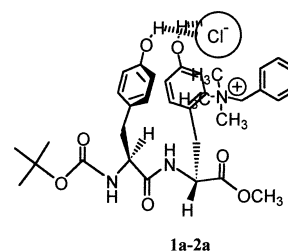
Job Plot Method[15] The ^{1}H-NMR sample solutions were made of **1**/**2** ratios under the condition that $[\mathbf{1}]+[\mathbf{2}]=10\,\text{mM}$ in CDCl$_3$, and [**1**] varies from 0 mM to 9 mM in 1 mM steps. The experimentally observed parameter is the ^{1}H-NMR chemical shift ($\Delta\delta$) of methyl groups (N$^+$–(CH$_3$)$_3$) of **2** that is sensitive to complex formation. The data were plotted in the form $\Delta\delta\times[\mathbf{2}]$ *versus* $[\mathbf{1}]/([\mathbf{1}]+[\mathbf{2}])$, and the position of the maximum indicates the stoichiometry of the complex.

^{1}H-NMR Titration Experiment[16] Association constants (K_a) were obtained by ^{1}H-NMR titration experiments, performed directly in the NMR tube using a micropipette to add known amounts (0, 10, 50, 100, 150, 200, 300, 400, 500 μl) of peptide's (**1**) stock solution (concentration=82.6 mM) in CDCl$_3$ to a solution of **2** (700 μl, concentration=11.9 mM) in CDCl$_3$. A 1:1 association of **1** and **2** was previously demonstrated, therefore, experimental data were fitted to the equation of the 1:1 binding isotherm by a nonlinear regression method.[16] All titration experiments were performed in CDCl$_3$ at 20 °C and 500 MHz.

References and Notes
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14) Addition of **2a** (1 eq) to 10 mM solution of **1** in CDCl$_3$ caused the following downfield shifts ($\Delta\delta$ ppm): OHs (0.80 ppm) for **1a**–**2a**; NHs (0.65 ppm) for **1b**–**2a**.
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17) We were unable to accurately determine K_a values using OH or NH protons of peptides because of the extensive broadening of these signals during titration.