Macrocyclic Polyazacycloalkane-poly-*N*-carboxylate Anion as a Receptor for Amino Acid

Mutsuo Kodama

Department of Chemistry, College of General Education, Hirosaki University, 1, Bunkyo-cho, Hirosaki 036

(Received May 7, 1996)

The doubly-protonated 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetracarboxylate anion (H_2teta^{2-}), 1,4,7,10,13-pentaazacyclopentadecane-1,4,7,10,13-pentacarboxylate anion (H_2pepa^{3-}), and 1,4,7,10,13,16-hexaazacyclooctadecane-1,4,7,10,13,16-hexacarboxylate anion (H_2heha^{4-}) have been shown to form stable 1 : 1 ratio complexes with amino acids in aqueous solutions. Their association constants, β_L, were determined polarographically. The greater association constants obtained indicate that H_2teta^{2-} and H_2pepa^{3-} anions selectively interact with an amino acid which has a planar aromatic moiety. The results show the importance of hydrophobic interaction, such as CH–π interaction between the aliphatic CH_2 residue of acetate pendant of receptor and the planar aromatic moiety of substrate, amino acid, in holding the protonated macrocyclic polyazacycloalkane-poly-*N*-polycarboxylate anion and amino acid together. Thus, they act as selective receptors for phenylalanine, tyrosine, tryptophan(e), and their derivatives.

Earlier studies have discovered that the macromonocyclic penta- and hexa-amines accomodating three protons within their macrocyclic cavities show selective complex formation abilities towards polycarboxylate anion,[1] nucleotide anions,[2] carbonate ion,[3] and catechols,[4] forming exclusively 1 : 1 ratio receptor–substrate (R–S) complexes in neutral pH solution. Their substrate specificities and formation constants for the 1 : 1 ratio complexes determined by the conventional polarographic method could be interpreted in terms of ionic charge and hydrogen-bonding interactions between the receptor and substrate.

As a continuation of equilibrium studies concerning the formation reactions of receptor–substrate complexes involving the macrocyclic polyamines, the reactions of macrocyclic polyazacycloalkane-poly-*N*-carboxylic acids depicted below, which have both anion- and cation-seeking functions, with amino acids were investigated using a conventional polarographic method. The macrocyclic polyazacycloalkane-poly-*N*-carboxylic acids studied were indeed found to be selective, especially to the amino acids possessing a planar aromatic moiety (Charts 1 and 2). The stability and selectivity of their receptor–substrate (R–S) complexes with an amino acid might be governed by concerted binding action of the electrostatic (including the hydrogen-bonding) and hydrophobic forces. In the complexation reaction, the ring size effect on the complex stability was also observed.

Although the chemical aspects of the amino acid recognition and carrier mechanism are not well known yet, the present system may offer a new model for binding sites involving amino and carboxylate residues of carrier proteins.

Experimental

Materials. The synthetic procedures of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA, H_4dota, H_4L, [12]-ane-$N_4(CH_2COOH)_4$, 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA, H_4teta, H_4L, [14]ane$N_4(CH_2COOH)_4$), 1,4,7,10,13-pentaazacyclopentadecane-1,4,7,10,13-pentaacetic acid (PEPA, H_5pepa, H_5L, [15]ane$N_5(CH_2COOH)_5$), and 1,4,7,10,13,16-hexaazacyclooctadecane-1,4,7,10,13,16-hexaacetic acid (HEHA, H_6heta, H_6L, [18]ane$N_6(CH_2COOH)_6$) as HCl salts were prepared employing the methods reported in the previous papers.[5—9] Other polyaza macrocycles used were also synthesized and purified by using the methods given previously.[10—13] Amino acids and their derivatives used in this study were purchased from Peptide Institute Inc. (4-1-2, Ina, Minoh, Osaka 562, Japan) and used without further purification.

Polarographic Method. The pieces of apparatus and the procedures used for the polarographic measurements are the same as those applied to the study of the mercury(II)–macrocyclic dioxopolyamine complexation.[14] The half-wave potentials, $E_{1/2}$, of reversible anodic waves due to the uncomplexed macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion or its parent macrocyclic polyamine in the presence of amino acid shifted in the same manner as those observed in the previous polyamine–polycarboxylate, –phosphate, and –catechol systems.[1—4] Hence, identical procedures and theories were applied to the present macrocyclic polyazacycloalkane-poly-*N*-carboxylate–amino acid system. In all polarographic measurements, 5.0×10^{-2} mol dm^{-3} CH_3COOH–CH_3COONa, 5.0×10^{-2} mol dm^{-3} Tris. –$HClO_4$, or 3.0×10^{-2} mol dm^{-3} borate buffer solutions containing 3.0×10^{-4} mol dm^{-3} macrocyclic polyazacycloalkane-poly-*N*-carboxylate or its parent macrocyclic polyamine and an ample excess of anion with an ionic strength (I) of 0.20 mol dm^{-3} $NaNO_3$ were used. These buffers had practically no effect on the half-wave potentials of reversible anodic wave due to the uncomplexed receptor (macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion or its parent macrocyclic polyamine).

The pH values of test solutions used for the polarographic measurements were determined using a glass-electrode pH-meter (a Hitachi F8-AT).

Electrophoresis. Experimental procedures and apparatus

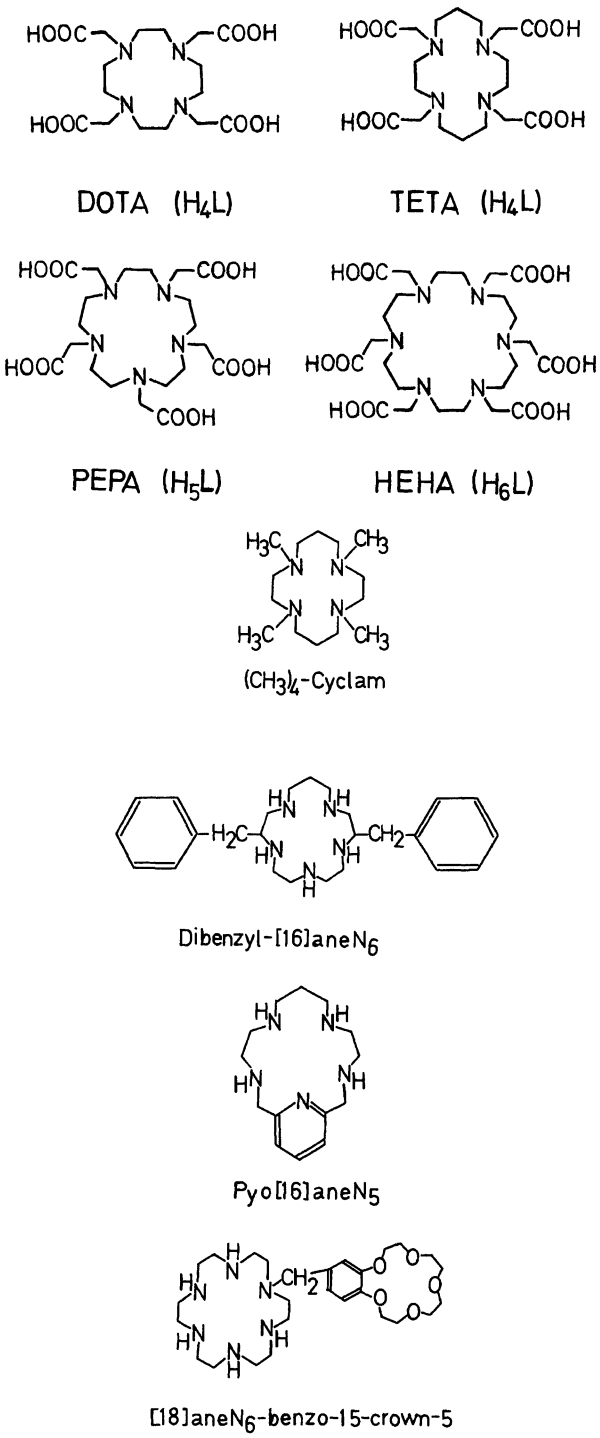


Chart 1.

Amino acid (R, H_2N-CH-COOH)	-R
phenylalanine	$-CH_2-C_6H_5$
tyrosine	$-CH_2-C_6H_4-OH$
tryptophan	$-CH_2-C_8H_6N$
histidine	$-CH_2-C_3H_3N_2$
methionine	$-CH_2-CH_2-S-CH_3$
leucine	$-CH_2-CH(CH_3)_2$
arginine	$-CH_2-(CH_2)_2-NH-C(=NH_2^+)-NH_2$
threonine	$-CH(OH)-CH_3$

Related Compounds	Structure
3-(4-hydroxyphenyl)-propionic acid	HO-C_6H_4-CH_2-CH_2-COOH
tyramine	HO-C_6H_4-$CH_2-CH_2-NH_2$
p-cresol	HO-C_6H_4-CH_3
tyrosine	HO-C_6H_4-$CH_2-CH(NH_2)-COOH$

Chart 2.

employed in the electrophoresis were the same as those applied to the previous macrocyclic polyamine–polycarboxylate[1] and 15,15′-trimethylne-bis(1,4,7,10,13)-pentaazacyclohexadecane–inorganic and organic systems.[15] Macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion spots were detected by staining the finished strips with copper(II) solution.

^{1}H NMR Measurements. The ^{1}H NMR spectra were recorded on a JEOL GSX-400 spectrometer operating at 400 MHz in concentric NMR tubes using sodium 3-(trimethylsilyl)propanesulfonate (DSS) as an internal reference at 308 °K. The pD value of each sample solution was corrected for a deutrium by adding 0.40[2] to the pH readings using a Hitachi–Horiba F-21 pH meter equipped with a Horiba microprobe combination electrode (model 6366-10D). Mixtures of macrocyclic polyazacycloalkane-poly-*N*-carboxylate and amino acid were lyophilized two times with each 2.0 ml of D_2O to minimize the water content. The chemical shifts were measured at least three times with reproducibility of ±0.5 Hz.

The values of $-\log[H^+]$ used for the calculation of association constants, β_L, for the R–S complexes were estimated from pH readings at $I = 0.20$ mol dm^{-3}: $\log(1/[H^+]) = \mathrm{pH} - 0.13$.[14]

Results and Discussion

Unless otherwise indicated, the amino acids used in this study are L-form. As was previously observed in the anodic polarography of polyaza macrocycle–polycarboxylate,[1] –nucleotide,[2] –carbonate,[3] and –catechol[4] systems, at a given pH the half-wave potentials of the reversible anodic dissolution waves at the dropping mercury electrode (DME) due to macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion[16] shifted to more positive values upon addition of most amino acids, except glycine, alanine, threonine, and arginine. The positive potential-shift increased with an increase in the concentration of the added amino acid. On the other hand, the wave-height decreased as the amino acid concentration increased.

I found that amino acids which bear a planar aromatic moiety, such as tyrosine and tryptophan(e) are often adsorbed at the surface of DME. In polarography, adsorption of such a substance often decreases the limiting current, increases the shift of half-wave potential, and in some cases obliterates the entire polarographic wave at the potential range where its adsorption occurs. In addition, it can cause minima or deceivingly split waves by hindering the approach of the reacting particle to the electrode surface or by creating the interface conditions that are unfavourable either for chemical reactions prior to and after the discharge or for the electron transfer itself.[17] By comparing the electrocapillary curve obtained in the solution containing surface-active amino acid with the normal one obtained in the absence of amino acid, the adsorption of amino acid at the DME surface could be studied. Typical results obtained for tyrosine and tryptophan systems are reproduced in Fig. 1. Curves B and C, the tops of which are flat, might suggest that the formally neutral species of these amino acids are adsorbed in the potential range from −0.40 to −1.10 V vs. SCE. Considering that all the anodic dissolution waves due to the free macrocyclic polyazacycloalkane-poly-*N*-carboxylate studied appear at potentials more positive than −0.30 V vs. SCE under the experimental conditions employed, one can safely conclude that the above positive shift of half-wave potential due to the amino acid added can be ascribed to the formation of receptor–substrate (R–S) complexes between macrocyclic polyazacycloalkane-poly-*N*-carboxylate and amino acid. The effects of amino acid concentration (at a given pH) and of pH (at a given amino acid concentration) on the anodic half-wave potential, $E_{1/2}$, for macrocyclic polyazacycloalkane-poly-*N*-carboxylate were all found to fit a theoretical Eq. 1 derived for the 1 : 1 ratio complexation (for the derivation of Eq. 1 see Refs. 2, 3, and 4).

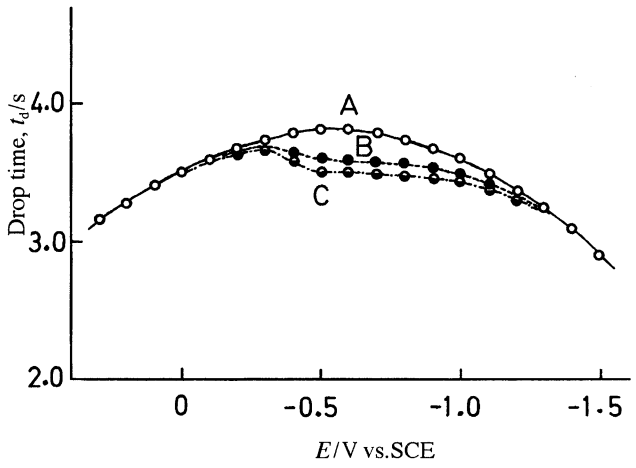


Fig. 1. Electrocapillary curves. $I = 0.20$ mol dm^{-3}, 25 °C, borate buffer 0.030 mol dm^{-3}, pH = 9.08. (A) no amino acid, (B) tyrosine 4.0 mmol dm^{-3}, (C) tryptophan(e) 10.0 mmol dm^{-3}.

$$\left[\text{Antilog}\left(\frac{\Delta E_{1/2}}{0.0296}\right) - 1\right](\alpha_H)_L(\alpha_H)_A = \beta_L[A]_f[H^+]^{a+b}K_1K_2\cdots K_aK_1'K_2'\cdots K_b' \quad (1)$$

The symbols used in Eq. 1 are defined by Eqs. 2—6.

$$K_i = \frac{[H_iL^{(i-a)}]}{[H_{i-1}L^{(i-1-a)}][H^+]} \quad (2)$$

$$K_j' = \frac{[H_jA^{(j-b)}]}{[H_{j-1}A^{(j-1-b)}][H^+]} \quad (3)$$

$$\beta_L = \frac{[H_nL^{n-a}H_mA^{m-b}]}{[H_nL^{(n-a)}][H_mA^{(m-b)}]} \quad (4)$$

$$(\alpha_H)_H = [L]_{un}/[L^{a-}] = 1 + K_1[H^+] + K_1K_2[H^+]^2 + \cdots + K_1K_2\cdots K_a[H^+]^a \quad (5)$$

$$(\alpha_H)_A = [A]_{un}/[A^{b-}] = 1 + K_1'[H^+] + K_1'K_2'[H^+]^2 + \cdots + K_1'K_2'\cdots K_b'[H^+]^b \quad (6)$$

$[L]_{un}$ and $[L^{a-}]$ denote concentrations of uncomplexed and completely-deprotonated macrocyclic polyazacycloalkane-poly-*N*-carboxylic acid, respectively, and $[A]_{un}$ and $[A^{b-}]$, those of uncomplexed and completely-deprotonated amino acid, respectively. $\Delta E_{1/2}$ means the shift of half-wave potential. Thus, all the β_L values were determined from the $\Delta E_{1/2}$ values employing relation (1).

Just as earlier checked for the 1 : 1 macrocyclic polyamine–polycarboxylate complexation,[1] the β_L values thus determined were assessed from the limiting current changes by using Eq. 7,[18,19] where β_L' is the conditional association constant expressed in terms of β_L by Eq. 8:

$$\beta_L'[A]_f = \frac{(i_l)_o^2 - (i_l)_{ob}^2}{(i_l)_{ob}^2 - (i_l)^2} \quad (7)$$

$$\beta_L' = \frac{\beta_L}{(\alpha_H)_L(\alpha_H)_A}[H^+]^{n+m}K_1K_2\cdots K_nK_1'K_2'\cdots K_m' \quad (8)$$

Symbols used in the right-hand side of Eq. 7 have the same meanings as those used previously.[1] The β_L value determined from the limiting-current change using Eq. 7 were in satisfactory agreement with that estimated from the $E_{1/2}$ shift. Although results were not shown here, the quantitatively established macrocyclic polyazacycloalkane-poly-*N*-carboxylate–amino acid association could be qualitatively supported by a paper electrophoretic measurement.

^{1}H NMR Studies. The complex formation could also be demonstrated by ^{1}H NMR spectroscopy (400 MHz) in D_2O solution. Typical results obtained in the [14]-aneN$_4$(CH$_2$COOH)$_4$ (X)–phenyalanine (A) and –3-(4-hydroxyphenyl)-propionic acid (B) systems are given in Table 1. An addition of phenylalanine to a 2.0×10^{-2} mol dm^{-3} [14]aneN$_4$(CH$_2$COOH)$_4$ solution at pD=9.1±0.1 caused high field shifts for H2, H3, and H4 ($\Delta\delta$=0.015—0.039 ppm) of receptor, X, while low field shifts were found for H3 (δ=−0.026) of A and Hl (δ=−0.148), H2 (δ=−0.072), and H6 (δ=−0.024) of B. A similar trend was also observed in the [15]aneN$_5$(CH$_2$COOH)$_5$ and [18]aneN$_6$(CH$_2$COOH)$_6$ systems. Kobayashi et al. mentioned in their ^{1}H NMR and CD spectroscopic studies on the receptor–substrate association between monols and acetylated compounds and resorcinol cyclic tetramer[20] that the association causes high field shifts of the ^{1}H NMR signals for the bound substrate, which is ascribable to the ring-current effect of the receptor molecule, resorcinol cyclic tetramer. In the way just indicated, the above-mentioned high field shifts for H2, H3, and

Table 1. The Chemical Shift of the 1 : 1 Mixtures[a,b] of Tetraazacyclotetradecane-Tetraacetic Acid (X) and *p*-Hydroxyphenyl-propionic Acid (A) or L-Phenylalanine (B)

Signal	Proton numbering[d]	Compound X	A	B	A–X		B–X	
X	1	1.895[m]			1.892[m]	(−0.003)	1.905[d]	(+0.010)
	2	3.072[t]			3.088[t]	(+0.016)	3.100[s]	(+0.038)
	3	3.143[s]			3.158[s]	(+0.015)	3.174[s]	(+0.031)
	4	3.392[s]			3.422[s]	(+0.030)	3.431[s]	(+0.039)
A	1		2.798[t]		2.789[t]	(−0.009)		
	2		2.430[t]		2.422[t]	(−0.008)		
	3		6.819[d]		6.793[d]	(−0.026)		
	4		7.147[d]		7.145[d]	(−0.002)		
B	1			3.842[q]			3.694[q]	(−0.148)
	2			3.034[q]			2.962[c]	(−0.072)
	3			3.193[q]				
	4			7.300[t]			7.291[t]	(−0.008)
	5			7.402[t]			7.388[t]	(−0.014)
	6			7.348[c]			7.320[c]	(−0.024)

a) Concentration=2×10^{-2} M, pD=9.0. b) () shows the deviation of chemical shift. c) Overlap with other peaks. d) Proton numbering:

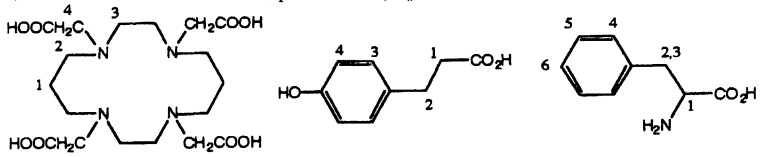


Table 2. The β_L Values for [15]aneN$_5$(CH$_2$COOH)$_5$ and [15]aneN$_5$ Complexes with Amino Acids[a] I=0.20 mol dm^{-3}, 25 °C

Substrate	β_L/dm^3 mol^{-1}			
	[15]aneN$_5$(CH$_2$COOH)$_5$		[15]aneN$_5$	
	H_2L^{3-}–H_mA^{-}	H_2L^{3-}–$H_{m+1}A^{\circ}$	H_2L^{2+}–H_mA^{-}	H_2L^{2+}–$H_{m+1}A^{\circ}$
Tyrosine	$(5.7\pm0.6)\times10^5$	$(4.9\pm0.7)\times10^5$	$(1.9\pm0.2)\times10^2$	No ev.
Phenylalanine	$(6.3\pm0.7)\times10^5$	$(1.2\pm0.2)\times10^4$	No ev.	No ev.
Tryptophan(e)	$(6.4\pm0.8)\times10^5$	$(3.6\pm0.5)\times10^5$	$(1.7\pm0.2)\times10^2$	$(1.4\pm0.2)\times10^1$
Methionine	$(9.4\pm1.0)\times10^2$	$(2.2\pm0.5)\times10^2$	No ev.	No ev.
Leucine	$(1.8\pm0.2)\times10^2$	$(1.3\pm0.2)\times10^2$	No ev.	No ev.
Histidine	$(4.7\pm0.5)\times10^2$	$(2.1\pm0.3)\times10^2$	$(9.3\pm1.0)\times10^1$	$(3.9\pm0.5)\times10^1$
Aspartic acid	$(2.1\pm0.5)\times10^1$	$(1.2\pm0.6)\times10^1$	$(2.8\pm0.3)\times10^2$	$(2.5\pm0.4)\times10^2$
Glycine	No ev.	No ev.	No ev.	No ev.
α-Alanine	No ev.	No ev.	No ev.	No ev.
Threonine	No ev.	No ev.	No ev.	No ev.
Arginine	No ev.	No ev.	No ev.	No ev.
Gly-L-phenylalanine	$(2.0\pm0.2)\times10^4$	$(1.7\pm0.2)\times10^4$	No ev.	No ev.

a) "No ev." indicates no evidence for complexation.

Table 3. The β_L Values for the Polyamino Polycarboxylic Macrocycle Complexes[a] I=0.20 mol dm^{-3}, 25 °C

Substrate	β_L for H_2L^{2-n}–H_mA^- reaction/dm^3 mol^{-1}			
	[12]aneN$_4$(CH$_2$COOH)$_4$	[14]aneN$_4$(CH$_2$COOH)$_4$	[15]aneN$_5$(CH$_2$COOH)$_5$	[18]aneN$_6$(CH$_2$COOH)$_6$
Tyrosine(H_2L^{2-n}–HA^-)	ca. 6×10^4	$(2.3\pm0.3)\times10^4$	$(5.7\pm0.6)\times10^5$	$(2.1\pm0.3)\times10^2$
Phenylalanie	—	$(4.4\pm0.5)\times10^3$	$(6.3\pm0.7)\times10^4$	$(3.4\pm0.5)\times10^2$
Gly-L-phenylalanine	—	ca. 1.0×10^3	$(2.0\pm0.2)\times10^4$	No ev.
ADP^{3-}	—	$(2.8\pm0.5)\times10^4$	$(7.0\pm0.7)\times10^5$	$(3.3\pm0.6)\times10^2$
Tryptophan(e)	—	$(1.7\pm0.5)\times10^5$	$(6.4\pm0.8)\times10^5$	$(3.4\pm0.4)\times10^2$
Histidine	—	$(8.8\pm1.0)\times10^1$	$(4.7\pm0.5)\times10^2$	$(2.2\pm0.3)\times10^2$
Aspartic acid	—	$(1.6\pm0.2)\times10^1$	$(2.1\pm0.5)\times10^1$	No ev.

a) "No ev." indicates no evidence for complexation. "—" means not determined.

Table 4. β_L Values for [15]aneN$_5$(CH$_2$COOH)$_5$ Complexes I=0.20 mol dm^{-3}, 25 °C

Substrate	β_L (for the H_2L^{3-}–H_mA^- reaction) dm^3 mol^{-1}
Tyrosine (H_2L^{3-}–HA^-)	$(5.7\pm0.6)\times10^5$
3-(4-Hydroxyphenyl)propionic acid (H_2L^{3-}–HA^-)	$(4.4\pm0.5)\times10^5$
Tyramine (H_2L^{3-}–HA°)	$(1.5\pm0.2)\times10^3$
p-Cresol (H_2L^{3-}–HA°)	$(1.0\pm0.2)\times10^4$
Phenylalanine	$(6.3\pm0.7)\times10^4$

Table 5. Association constants, β_L, for H_2L^{2-n}–H_mA^- reaction[a] I=0.20 mol dm^{-3}, 25 °C

Substrate	β_L/dm^3 mol^{-1}		
	[14]aneN$_4$(CH$_2$COOH)$_4$	(CH$_3$)$_4$[14]aneN$_4$	[14]aneN$_4$
Tyrosine (H_2L^{2-n}–HA^-)	$(2.3\pm0.3)\times10^4$	$(1.4\pm0.2)\times10^4$	ca. 4×10^2
3-(4-Hydroxyphenyl)propionic acid (H_2L^{2-n}–HA^-)	$(2.4\pm0.3)\times10^4$	$(1.7\pm0.2)\times10^3$	$(1.0\pm0.2)\times10^2$
Tyramine (H_2L^{2-n}–HA°)	$(7.8\pm0.9)\times10^2$	ca. 1.8×10^2	No ev.
p-Cresol (H_2L^{2-n}–HA°)	$(7.9\pm0.9)\times10^3$	$(2.7\pm0.3)\times10^3$	No ev.
Phenylalanine	$(4.4\pm0.5)\times10^3$	$(2.7\pm0.3)\times10^3$	No ev.
Tryptophan(e)	$(1.7\pm0.2)\times10^5$	$(1.2\pm0.1)\times10^4$	7×10^1

a) "No ev." indicates no evidence for complexation.

H4 of [14]aneN$_4$(CH$_2$COOH)$_4$ can be ascribed to the ring current effect of benzene ring of amino acid involved in the R–S complexation with [14]aneN$_4$(CH$_2$COOH)$_4$. The low field shifts of H3 of A and H1, H2, and H6 of B in the presence of [14]aneN$_4$(CH$_2$COOH)$_4$ give further support for the above R–S complexation. On the other hand, almost no change of receptor, [15]aneN$_5$(CH$_2$COOH)$_5$, spectrum was observed when glycine, alanine, threonine, or arginine was added. These results are clearly in good agreement with those obtained by the polarographic analysis.

As stated before, we noticed that only the triply-protonated macrocyclic polyamines can interact with organic and inorganic oxygen anion, including polycarboxylates, phosphates (phosphate, AMP^{2-}, ADP^{3-}, and ATP^{4-}) and carbonate anion at neutral pH. In the case of macrocyclic polyazacycloalkane-poly-*N*-carboxylate system, the anions accomodating two protons into their central cavities have the efficient and selective amino acid functions. All the β_L and $(n+m)$ values for [15]aneN$_5$(CH$_2$COOH)$_5$ system estimated as before[2–4] are summarized in Table 2, together with those for the parent [15]aneN$_5$ system. Table 2 shows that the extent of association reactions of protonated macrocyclic polyazacycloalkane-poly-*N*-carboxylate anions with amino acids are strongly affected by the nature of the organic moiety, R, involved in the substrate molecule, amino acid. The introduction of anionic acetate (carboxylate) moieties into the macromonocyclic polyamine molecules produced an extra stability in their R–S complexes with amino acid having an planar aromatic moiety such as a phenyl group. In spite of the great steric crowdedness, they show an extraordinary high ability for the recognition of phenylalanine, tyrosine, tryptophan(e), and glycyl-L-phenylalanine. In the complexation reactions of parent polyaza macrocycles with these amino acids, the doubly-protonated forms also act as receptors in an alkaline solutions (Table 2).

It was also revealed that the macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion accomodates the amino acid in a size-selected manner (Table 3). The largest 18-membered ring system, [18]aneN$_6$(CH$_2$COOH)$_6$, is the poorest amino acid receptor, which is probably due to the loose structure of its macrocyclic N_6 ring and to electrostatic repulsion among the six anionic carboxylate groups.

Generally, in the formation of R–S complex, a receptor molecule interacts with a substrate through non-covalent

Table 6. The Effect of Planar Aromatic Moiety in the Polyaza Macrocycle on Its Complexation with Glycyl-L-phenylalanine I=0.20 mol dm^{-3}, 25 °C

Receptor	β_L (for the H_2L^{2-n}–H_mA^- reaction)
[16]aneN$_5$	$(3.5\pm0.5)\times10^2$
Dibenzyl-[16]aneN$_5$	$(1.7\pm0.5)\times10^1$
Pyo-[16]aneN$_5$[2)b)]	No ev.
[18]aneN$_6$	4×10^1
[18]aneN$_6$-benzo-15-crown-5[c)]	No ev.

a) "No ev." indicates no evidence for complexation. b) 3,6,10,13,19-pentaazabicyclo[13,3,1]-nonadeca-1 (19),15,17-triene. c) 4′-(1″, 4″, 7″, 10″, 13″, 16″-hexaazacyclooctadecan-1″-ylmethyl)benzo-15-crown-5.

molecular forces, such as ionic charge attractions and van der Waals, hydrogen-bonding and hydrophobic interactions (including π–π stacking between aromatic moieties).[21—27] In order to obtain much fuller and more accurate information on the molecular mechanism of macrocyclic polyazacycloalkane-poly-*N*-carboxylates for the amino acid recognition, the association reactions of [15]aneN$_5$ with 3-(4-hydroxyphenyl)-propionic acid, tyramine and *p*-methylphenol (*p*-cresol) were also studied. The β_L values determined are listed in Table 4 together with those of tyrosine and phenylalanine. The association reactions of [14]aneN$_4$(CH$_2$COOH)$_4$, its parent macrocycle, 1,4,8,11-tetraazacyclotetradecane ([14]-aneN$_4$, cyclam), and 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane ((CH$_3$)$_4$-cyclam, (CH$_3$)$_4$-[14]aneN$_4$) with tyrosine, 3-(4-hydroxyphenyl)-propionic acid, tyramine, *p*-cresol, phenylalanine, and tryptophan were also investigated. The β_L values determined as before are listed in Table 5.

Comparison of the β_L value of [15]aneN$_5$(CH$_2$COOH)$_5$ for the inclusion of tyrosine with those of 3-(4-hydroxyphenyl)propionic acid and tyramine reveals that in the R–S complexation of macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion, tyrosinate anion is bound to the protonated macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion through its hydroxyphenyl and anionic carboxylate groups, but the amino moiety of tyrosinate anion is not practically involved in the complexation reaction. As shown by the β_L values in Table 2, the zwitter ion, R–CH(NH$_3^+$)COO$^-$, of amino acid always forms a less stable complex than the corresponding anion, R–CH(NH$_2$)COO$^-$. This might give a strong support to the above explanation that the amino moiety can take little part in stabilizing the macrocyclic polyazacycloalkane-poly-*N*-carboxylate–amino acid complex.

Table 8. Thermodynamic Parameters for 3-(4-Hydroxyphenyl)propionate Complexes I=0.20 mol dm^{-3}, 25 °C

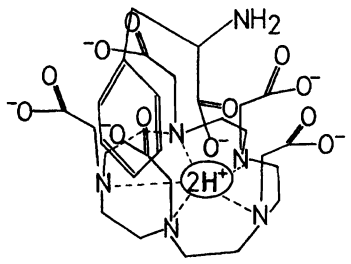
Receptor	$-\Delta H$ / kJ mol^{-1}	ΔS / JK^{-1} mol^{-1}	logβ_L
(CH$_3$)$_4$[14]aneN$_4$	16±1	9.2±1.2	3.2$_3$
[14]aneN$_4$	9.6±0.8	6.3±1.3	2.0$_0$
[14]aneN$_4$(CH$_2$COOH)$_4$	17±1	27±5	4.3$_8$
[15]aneN$_5$	10±1	6.7±1.3	2.0$_8$
[15]aneN$_5$(CH$_2$COOH)$_5$	24±2	29±5	5.6$_4$

As stated earlier, observed up-field shifts of the ^{1}H NMR signals for the doubly-protonated macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion and the down-field shifts of the ^{1}H NMR signals for amino acid in Table 1 are also strong indications for the incorporation of the electron-rich aromatic ring of amino acid into the bowl-shaped macrocyclic polyazacycloalkane-poly-*N*-carboxylate cavity, forming a stable receptor–substrate complex. The importance of electron-rich aromatic ring of amino acid in the complexation reaction with the macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion is also demonstrated by the fact that the doubly-protonated macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion can form stable complexes with *p*-cresol, phenylalanine and tryptophan(e).

As in the case of [14]aneN$_4$(CH$_2$COOH)$_4$, (CH$_3$)$_4$-cyclam forms stable R–S complexes with amino acids bearing the aromatic moiety (Table 4). This clearly means that only the CH$_2$ residue of the pendant acetate group of macrocyclic polyazacycloalkane-poly-*N*-carboxylate receptor is effective in holding the amino acid bearing the planar aromatic moiety, suggesting the general class of van der Waals interaction between CH$_2$ residue of acetate pendant and the electron-rich aromatic ring in the amino acid substrate (the CH–π interaction[20]), The largest up-field shift of ^{1}H NMR signal for the H4 of H$_2$teta^{2-} anion also supports the above explanation (Table 1). Furthermore, I found that with glycyl-L-phenylalanine the polyaza macrocycle having the aromatic moiety outside of polyamine ring, 3,11-dibenzyl-1,4,10,13-pentaazacyclohexadecane (Dibenzyl-[16]aneN$_5$) forms a less stable R–S complex than the parent [16]aneN$_5$ (Table 6). This indicates that practically no π–π stacking interaction occurs between receptor and substrate, but the bulky aromatic moieties introduced into the receptor molecule just bring about the severe steric hindrance in the association reaction with

Table 7. Thermodynamic Parameters for [15]aneN$_5$(CH$_2$COOH)$_5$ Complex (H_2L^{3-}–H_mA^- reaction) I=0.20 mol dm^{-3}, 25 °C

Substrate	$-\Delta H$ / kJ mol^{-1}	ΔS / JK^{-1} mol^{-1}	logβ_L
Tyramine (H_2L^{3-}–HA°)	13±1	17±3	3.1$_7$
Tyrosine (H_2L^{3-}–HA$^-$)	22±2	29±5	5.7$_5$
Phenylalanine	20±2	26±4	4.8$_0$
Tryptophan(e)	26±2	26±4	5.8$_0$
3-(4-Hydroxyphenyl)propionic acid (H_2L^{3-}–HA$^-$)	24±2	29±5	5.6$_4$



Proposed model

Fig. 2. Proposed model for the 1 : 1 ratio complex of [15]aneN$_5$(CH$_2$COOH)$_5$ with phenylalanine.

the amino acid bearing the aromatic ring and rather weakens the stabilizing force between them. This is equally true of complexation reactions with tyrosine, phenylalanine, and tryptophan(e).

Although the detailed results were not shown here, the linear plots of log β_L for the association reactions of [15]-aneN$_5$(CH$_2$COOH)$_5$ with tyrosine, 3-(4-hydroxyphenyl)-propionic acid, tyramine, phenylalanine, and tryptophan(e) and those of [14]aneN$_4$(CH$_2$COOH)$_4$, (CH$_3$)$_4$-[14]aneN$_4$, and [14]aneN$_4$ with 3-(4-hydroxyphenyl)-propionic acid against the reciprocal of temperature permit us to estimate the thermodynamic parameters, ΔH and ΔS. All the ΔH and ΔS values estimated are summarized in Tables 7 and 8. Making a sharp contrast to the lanthanide complexes,[28] the R–S complexes of macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion gave only a small positive entropy change, showing that their stability arises chiefly form the favourable enthalpy term. In considering the average value of hydrogen bond energy (10—21 kJ mol^{-1}),[20,29—31] ΔH values obtained (Tables 7 and 8) might suggest that the stabilizing forces in the R–S complex formation studied are provided chiefly by a combination of electrostatic forces such as a hydrogen-bonding interaction and hydrophobic interaction (including van der Waals attraction and CH–π interaction mentioned earlier).

Table 9. The pK_a Values Used in the Calculation I=0.20 mol dm^{-3}, 25 °C

Receptor or Substrate	pK_a
[12]aneN$_4$(CH$_2$COOH)$_4$	11.08, 9.72, 4.42, 4.38
[14]aneN$_4$(CH$_2$COOH)$_4$	11.04, 9.68, 4.30, 3.17
[14]aneN$_4$	11.50, 10.30, 1.5, 0.8
(CH$_3$)$_4$-[14]aneN$_4$	9.72, 9.33, 3.11, 2.66
[15]aneN$_5$(CH$_2$COOH)$_5$	10.15, 9.41, 6.14, 4.11, 3.19
[16]aneN$_5$	10.64, 9.49, 7.28, 1.71, 1.45
Dibenzyl-[16]aneN$_5$	10.39, 9.55, 7.19, 2>, ~1
Pyo-[16]aneN$_5$	9.48, 8.56, 5.83, ~2
[18]aneN$_6$(CH$_2$COOH)$_6$	10.10, 10.01, 8.92, 8.20, 4.64, 3.53
[18]aneN$_6$	10.19, 9.23, 8.73, 4.09, ~2, ~1
[18]aneN$_6$-benzo-15-crown-5	9.66, 9.13, 7.75, 4, ~2, ~1
Tyrosine	10.07, 9.02, 2.19
Phenylalanine	9.09, 2.20
Tryptophan(e)	9.31, 2.37
Methionine	9.03, 2.22
Leucine	9.55, 2.37
Histidine	9.06, 6.04, 1.8
Aspartic acid	9.56, 3.68, 1.95
Glycine	9.55, 2.38
α-Alanine	9.67, 2.32
Threonine	8.95, 2.23
Arginine	8.99, 2.07
Gly-L-phenylalanine	8.10, 3.09
3-(4-Hydroxyphenyl) propionic acid	10.10, 4.32
Tyramine	10.43, 9.43
p-Cresol	10.12
ADP(H$_3$L)	6.33, 3.86

The β_L value for the association reaction of histidine is much smaller than those of corresponding reactions of phenylalanine and tryptophan(e). This probably can be ascribed to the weak hydrophobic nature of imidazole moiety,[32] as compared with that of benzene or indole rings. Thus, because of its weak hydrophobic nature, the hydrophobic interaction of imidazole ring with the CH$_2$ residues of acetate pendants is considered to be weak. As shown by the data in Tables 2, 3, and 4 tyrosine always forms more stable R–S complexes than phenylalanine. This suggests that the hydrogen-bonding interaction involving the phenol OH group also participates in holding the receptor and tyrosine together. As in the case of triply-protonated 1,4,7,10,13,16-hexaazacyclooctadecane–dopamine system[2] the phenol OH group might interact with the protonated amino nitrogen atoms of polyaza macrocyclic ring, providing a substantial driving force for the R–S association.

As stated above, the receptor–substrate complexation reactions studied in this paper gave exclusively small positive entropy cgange, ΔS. This indicates that in the complexation reactions only small parts of conformational freedoms of reactants are lost and the desolvation from their ionic moieties occurs to only a small extent. Therefore, in terms of the structural feature of the macrocyclic polyazacycloalkane-poly-*N*-carboxylate receptor, its anionic carboxylate groups of acetate pendants remain solvated and hence, they do not participate much in holding the amino acid via electrostatic interaction.

In the complexation reactions towards the D-form of amino acid, the protonated macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion always showed the same complexing ability as that towards the L-form.

Form all the findings collected and the foregoing discussion, one can conclude that the hydrophobic interaction between CH$_2$ residues of acetate pendants of receptor and the aromatic ring moiety of amino acid plays an important role in stabilizing the R–S complexes of macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion and the amino acid bearing the aromatic ring. The model illustrated in Fig. 2 may aid us in understanding the selective recognition of tyrosine, phenylalanine, and tryptophan(e) by the protonated macrocyclic polyazacycloalkane-poly-*N*-carboxylate anion and their outstanding stable complexes. The pK_a values for receptors and substrates used in this study are listed together

in Table 9.

I express my deep gratitude to Professor E. Kimura and Dr. T. Koike, Hiroshima University, School of Medicine, for the kind donation of pure macroycles. I also thank Professor Y. Fujii, Ibaraki University, for obtaining the ^{1}H NMR spectra and for his valuable discussion. I wish to express my appreciations to Misses H. Naraoka and S. Igarashi for their technical support.

References

1) E. Kimura, A. Sakonaka, T. Yatsunami, and M. Kodama, *J. Am. Chem. Soc.*, **103**, 3041 (1981).
2) E. Kimura, M. Kodama, and T. Yatsunami, *J. Am. Chem. Soc.*, **104**, 3182 (1982).
3) E. Kimura, A. Sakonaka, and M. Kodama, *J. Am. Chem. Soc.*, **104**, 4984 (1982).
4) E. Kimura, A. Watanabe, and M. Kodama, *J. Am. Chem. Soc.*, **105**, 2063 (1983).
5) H. Stetter and W. Frank, *Angew. Chem., Int. Ed. Engl.*, **15**, 686 (1976).
6) R. Delgado and J. T. R. F. Silva, *Talanta*, **29**, 815 (1982).
7) J. F. Desreux, *Inorg. Chem.*, **19**, 1319 (1980).
8) M. Kodama, T. Koike, A. B. Mahatma, and E. Kimura, *Inorg. Chem.*, **30**, 1270 (1991).
9) E. Kimura, H. Fujioka, A. Yatsunami, H. Nihira, and M. Kodama, *Chem. Pharm. Bull.*, **33**, 655 (1985).
10) M. Kodama, E. Kimura, and S. Yamaguchi, *J. Chem. Soc., Dalton Trans.*, **1980**, 2536.
11) M. Kodama and E. Kimura, *J. Chem. Soc., Dalton Trans.*, **1976**, 2335.
12) M. Kodama and E. Kimura, *J. Chem. Soc., Dalton Trans.*, **1978**, 104.
13) E. Kimura, M. Kodama, R. Machida, and K. Ishizu, *Inorg. Chem.*, **21**, 595 (1985).
14) M. Kodama and E. Kimura, *Bull. Chem. Soc. Jpn.*, **62**, 3093 (1989).
15) E. Kimura, Y. Kuramoto, T. Koike, H. Fijioka, and M. Kodama, *J. Org. Chem.*, **55**, 42 (1990).
16) M. Kodama, *Bull. Chem. Soc. Jpn.*, **67**, 2990 (1994).
17) C. N. Reilley and W. Stumm, "Adsorption in Polarography," in "Progress in Polarography," ed by P. Zuman and I. M. Kolthoff, Interscience Publishers, John Wiley and Sons, New York (1962), Vol. 1, p. 81.
18) M. Kodama and E. Kimura, *Inorg. Chem.*, **17**, 2446 (1978).
19) V. Kacena and L. Matousek, *Collect. Czech. Chem. Commun.*, **18**, 294 (1978).
20) K. Kobayashi, Y. Asakawa, Y. Kikuchi, H. Toi, and Y. Aoyama, *J. Am. Chem. Soc.*, **115**, 2648 (1993).
21) N. Dattaguta, M. Hogan, and D. M. Crothers, *Biochemistry*, **19**, 5998 (1980).
22) A. Hamilton, J-M Lehn, and J. L. Sessler, *J. Am. Chem. Soc.*, **108**, 5158 (1986).
23) S. C. Zimmerman, C. R. Lamberson, M. Corey, and T. A. Fairley, *J. Am. Chem. Soc.*, **111**, 6805 (1989).
24) M. Lin, M. Lee, K. T. Yue, and L. G. Marzilli, *Inorg. Chem.*, **32**, 3217 (1993).
25) S. Mangani, M. Ferrariu, and P. Orioli, *Inorg. Chem.*, **33**, 3421 (1994).
26) J-M Lehn, *Angew. Chem., Int. Ed. Engl.*, **27**, 89 (1988).
27) J. F. Lipskier and T. H. Tran-thi, *Inorg. Chem.*, **32**, 722 (1993).
28) M. Kodama, T. Koike, A. B. Mahatma, and E. Kimura, *Inorg. Chem.*, **30**, 1270 (1991).
29) T. Moeller, "Inorganic Chemistry," John Wiley and Sons Inc., New York (1952), p. 188.
30) K. Hayakawa, K. Shirahama, and T. Inoue, "Basic Physical Chemistry," Sankyo-Shuppan, Tokyo (1995), p. 45.
31) M. F. O'Dwyer, J. E. Kent, and R. D. Brown, "Valency," 2nd ed, Springer-Verlag, New York (1978).
32) K. Suzuki, "Energy, Environment, and Life," Kagaku Dozin, Kyoto (1990).