Macromonocyclic Polyamines as Specific Receptors for Tricarboxylate-Cycle Anions

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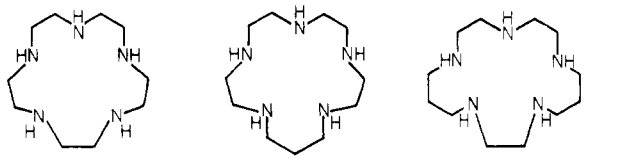
Abstract: Macromonocyclic penta- and hexaamines specifically interact at neutral pH with polycarboxylates having the two CO_2^-s at short distances such as succinate, malate, citrate, malonate, and maleate, but they are inert toward the other dicarboxylates fumarate, aspartate or glutarate and also toward the monocarboxylates acetate or lactate. Thus, they are selective receptors for most of the tricarboxylic acid-cycle intermediates and their analogues. The polarographic method showed 1:1 ion-pair association between the macrocyclic polyamines in the triprotonated form and the polycarboxylates in the dianion form (or the trianion form with citrate) at neutral pH. When the association constants for various membered cyclic and relevant acyclic ligand systems are compared, the macrocyclic effect and the ring size effect are demonstrated. The fact that the ion-pair formation depends on the structural features of both polyamines and carboxylates indicates a steric controlled interaction. The structural relevance is inferred to the receptor sites of biological transporters for the tricarboxylate-cycle polyanions.

Host-guest interaction results from a complementary stereoelectronic arrangement of binding sites in the host and the guest.[2] Host-guest interactions have recognized importance in many biological processes, including enzyme catalysis and inhibition, antibody-antigen reactions, and membrane transport. Macrocyclic molecules of polyethers,[3,4] polyamines,[5,6] and peptides[7,8] are well-known as selective hosts for spherical (e.g., metal ions) and sometimes symmetrical cations (e.g., protonated amines.)[9]

Recently much attention is received on anion-binding complexes. Typical examples are macrobicyclic[10-12] and macrotricyclic amines,[13-15] which interact with anions by including them into cavities lined with the appropriate anion-binding sites capable of forming ionic hydrogen bonds like those of protonated amines: N^+-H···X^-. The electronic and geometric features of anions should be complementary to those of the (protonated) receptor molecules to be specifically recognized: the spherical halide anions associate with the spherical macropolycycles[10,11,13] and linear triatomic anions such as N_3^- with a cylindlical macrobicycle.[12] For complexons of nonsymmetrical polyanions such as polycarboxylates and phosphates, cyclic[16] and linear polyguanidinium salts[17] and linear polyammonium salts[17] have been reported very recently. Both the stability and the selectivity of these complexones are primarily governed by electrostatic forces. Interesting prospects have been discussed for guanidinium salts as anion complexions.[17]

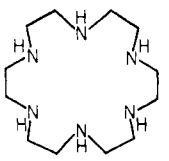
In previous works[18] we found that macromonocyclic polyamines take up several protons (depending on the N donor number and ring size) to confine them into the central cavities. The highly densed positive charges and the presence of encircling hydrogens attached to the secondary amines may facilitate strong hydrogen bonds with polyoxyanions. Herein we report that the penta- (L^1-L^3) and hexaamines (L^4) depicted below among the macrocyclic polyamines tested indeed function as polyoxyanion receptor molecules. Most significantly, our receptor models are selective to special polycarboxylates such as succinate, malate, maleate, malonate, and citrate. They associate neither with the monocarboxylates acetate or lactate nor with the dicarboxylates fumarate, glutarate, or aspartate. Namely, they recognize only the dicarboxylates having suitable geometry and electronic arrangement. Thus our macrocyclic polyamine system offers a new type of receptor molecules for the selective binding of polycarboxylates.



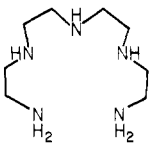
L^1 1,4,7,10,13-pentaazacyclopentadecane

L^2 1,4,7,10,13-pentaazacyclohexadecane

L^3 1,4,7,11,14-pentaazacycloheptadecane



L^4 1,4,7,10,13,16-hexaazacyclooctadecane



L^5 1,11-diamino-3,6,9-triazaundecane

Dicarboxylate (e.g., succinate or malate) transport carrier proteins have been long postulated[19-21] and recently isolated.[22-24]

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The chemical aspects of the polyanion recognition and carrier mechanisms are totally unknown yet. Our system may serve as a new model for anion-binding sites involving amine residues (e.g., lysine) of carrier proteins, just as the polyguanidinium ligands offered a model for anion-binding sites by arginine residues of proteins.[16,17]

Experimental Section

Preparation of the polyamines was described earlier.[6,25] Their purity was checked by elemental analysis and gas chromatography.[26] Their mixed protonation constants, K^i, used for the present calculation are summarized in Table I. Standard solutions of polyamines were prepared by dissolving known amounts of them into redistilled water. All other chemicals were of an analytical grade and were used without further purification. To obtain neutral pH, we used Tris buffer (0.1 M), which was ascertained to have no influence on $E_{1/2}$. The polarographic apparatus and procedures are the same as those applied at Hg^{2+}–macrocyclic polyamine complexation.[5,6,27] Typical primary data are given in Table II.

Electrophoresis. Electrophoresis was performed by using a Gelman semimicroelectrophoresis chamber and an Atto VC stabilizer, SJ-1051. Gelman Sepraphore 111 (6 × 22 cm) was used as the supporting medium for each run. The electrophoresis strips were connected to the electrode vessels by bridges consisting of Toyo No. 50 filter papers. After equilibration of the supporting medium in the buffer system described below for 15 min, one microliter of aqueous solutions containing 10 $\mu g/\mu L$ of the free polyamine bases or salts was applied for elution by specified potential. The polyamine spots were detected by staining the strips with iodine vapor. Electrolyte solutions (I = 0.1 M) and the elution potentials with the time that was used were as follows: (a) malonate, pH 5.9, 200 V, 6 min; (b) maleate, pH 5.9, 200 V, 7 min; (c) fumarate, pH 5.8, 200 V, 7 min; (d) *m*-phthalate, pH 5.4, 200 V, 8 min; (e) malate, pH 5.7, 200 V, 7 min; (f) *o*-phthalate, pH 6, 300 V, 8 min; (g) succinate, pH 5.8, 300 V, 5 min; (h) citrate, pH 6, 300 V, 10 min. Table I summarizes the relative mobilities. 1,4,8,11-Tetraazacyclotetradecane was chosen as the reference, because its mobility is little influenced by the electrolytes used.

Polarographic Results and Calculation

As previously observed in valinate buffers (pH ~9),[5,6,27] all the tetra-, penta-, and hexaamines (except for 16- and 17-membered tetraamines) gave well-defined anodic waves at DME in the Tris-buffered neutral pH region. Their limiting currents were proportional to the bulk concentration of polyamines and also to the square root of the effective pressure on the DME. Plots of $\log [i/(i_l - i)]$ against the dc potential, E, gave invariably straight lines with reciprocal slopes falling in the range of −28 to −33 mV over the entire range of pH values covered, showing a two-electron reversible oxidation at DME. The dc half-wave potentials $E_{1/2}$ of the anodic dissolution waves were independent of the bulk concentration of polyamines and of the Tris buffer. However, they shifted to more negative potentials by increasing the solution pH, obeying the relation 1, where $(\alpha_H)_L$ is defined by (2). These

$$\Delta E_{1/2}/\Delta \log (\alpha_H)_L = -30 \text{ mV} \quad (1)$$

$$(\alpha_H)_L = [L]_F/[L] = 1 + K_1[H^+] + K_1K_2[H^+]^{2+} + \ldots \quad (2)$$

facts imply that 1:1 Hg^{2+}–L complex formed is to be represented by the reaction 3. The half-wave potential $(E_{1/2})_L$ is, then

$$Hg + H_iL^{i+} \rightleftharpoons HgL^{2+} + iH^+ + 2e^- \quad (3)$$

$$(E_{1/2})_L = \epsilon^0_{Hg} + 0.0296 \log f_{Hg^{2+}} - 0.0296 \log K_{HgL}/(\alpha_H)_L \quad (4)$$

[28] on the reasonable assumption that the diffusion coefficients of Ht^{2+}–L complex and of the various forms of H_iL^{i+} are equal to one another.[29]

An addition of citrate, succinate, malate, maleate, or malonate shifted $E_{1/2}$ to more positive values at a given pH (see Table II). The shift is greater with an increasing concentration of the polycarboxylate anions. The wave heights decreased as the carboxylate concentration increased. $E_{1/2}$ shifted to more negative potentials by increasing the L–carboxylate mixing solution pH. Meanwhile addition of fumarate, acetate, lactate, glutarate, or aspartate did not virtually cause the $E_{1/2}$ shift. If postulated that only the first group of carboxylic acids A associate with polyamines L and that the resulting ion-pair complexes do not interact with Hg^{2+}, then the observed $E_{1/2}$ shifts are well explained. To be elaborate, only the following treatment fitted the experimental data.

With the assumption that a protonated polyamine H_iL^{i+} and a carboxylate anion A^{b-} form a 1:1 ion-pair complex H_iL^{i+}–A^{b-}, the concentration of the uncomplexed (with Hg^{2+}) ligand species $[L]_F$ at DME is expressed as

$$\begin{aligned}[L]_F &= [L] + [HL^+] + [H_2L^{2+}] + \ldots + [H_iL^{i+}\text{–}A^{b-}] \\ &= (\alpha_H)_L[L] + [L]\beta_L[H^+]^iK_1K_2K_3[A^{b-}] \\ &= (\alpha_H)_L'[L] \end{aligned} \quad (5)$$

where β_L is the 1:1 ion-pair association constant.

$$\beta_L = [H_iL^{i+}\text{–}A^{b-}]/[H_iL^{i+}][A^{b-}] \quad (6)$$

Then, eq 4 is modified to eq 7 when a carboxylate is added. The

$$(E_{1/2})_{LA} = \epsilon^0_{Hg} + 0.0296 \log f_{Hg^{2+}} - 0.0296 \log K_{HgL}/(\alpha_H)_L' \quad (7)$$

shift of $E_{1/2}$ upon addition of a carboxylate at constant temperature 25 °C is

$$\begin{aligned}\Delta E_{1/2} &= (E_{1/2})_{LA} - (E_{1/2})_L \\ &= 0.0296[\log (\alpha_H)_L' - \log (\alpha_H)_L] \end{aligned} \quad (8)$$

Equation 8 is rearranged to eq 9. Since $(\alpha_H)_L'$ is greater than

$$[\text{antilog } (\Delta E_{1/2}/0.0296) - 1](\alpha_H)_L = \beta_LK_1K_2K_3[H^+]^i[A^{b-}] \quad (9)$$

$(\alpha_H)_L$, $\Delta E_{1/2}$ should be positive, which agrees with the observation. Equation 9 predicts that at constant $[A^{b-}]$ the plotting logarithm of the left hand against pH should afford linear lines. This has turned out to be the case as shown in Figure 1A. The i values can be directly determined from the gradients, which were practically 3 for all the measured systems. Equation 9 also predicts that at constant pH the plots of the left hand against $[A^{b-}]$ should afford linear lines passing through the origin. This was also confirmed experimentally; see Figure 1B. From the gradients, β_L was determined. All the results are listed in Table III.

The validity of β_L was checked by using eq 10 as derived earlier

$$K_{LA}[A] = \frac{(i_d)_L^2 - (i_d)_{ob}^2}{(i_d)_{ob}^2 - (i_d)_{LA}^2} \quad (10)$$

for 1:1 metal–L complexation,[30] where K_{LA} (=[L–A]/[L][A]) corresponds to $\beta_L[H^+]^3K_1K_2K_3/(\alpha_H)_L$. For the typical case of L^4 interacting with citrate(3−) anion at pH 8.66, [Tris buffer] = 0.1 M, I = 0.20 M, T = 25 °C, $(i_d)_L = (i_d)_{ob}$ (at [A] = 0) = 13.9 (cm), $(i_d)_{ML} = (i_d)_{ob}$ (at [A] = ∞) = 12.6_0 (cm), and $(i_d)_{ob}$ = 13.0 (cm) (at [A] = 20.0 mM). Substitution of these values

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Table I. Mixed Protonation Constants (at 25 °C, I = 0.2 M) and Electrophoretic Mobilities[a] of Polyamines

	ligand				
	L^1	L^2	L^3	L^4	L^5
mixed protonation constants	10.85, 9.65, 6.00, 1.74, 1.16[b]	10.64, 9.49, 7.28, 1.71, 1.45[b]	10.32, 9.62, 7.36, 4.10, 2.38[b]	10.19, 9.23, 8.73, 4.09, ~2, ~1[c]	10.36, 9.65, 8.50, 4.70, 2.40[b]
electrolytes					
acetate	0.9	1.1	0.9	1.1	1.2
lactate	1.0	1.0	0.9	0.9	1.1
malonate	0.7	0.7	0.6	0.4	0.9
succinate	0.7	0.7	0.7	0.5	0.8
malate	0.7	0.8	0.7	0.5	0.8
tartrate	0.6	0.7	0.5	0.0	0.6
maleate	0.7	0.8	0.7	0.4	0.8
fumarate	0.9	0.9	1.0	0.8	1.0
o-phthalate	0.6	0.9	0.5	0.3	0.5
m-phthalate	0.8	0.9	0.8	0.7	1.0
citrate	0.3	0.0	−0.2	−0.9	0.0

[a] Relative values (at 25 °C and I = 0.1 M) with respect to 1,4,8,11-tetraazacyclotetradecane. [b] Reference 24. [c] Reference 6.

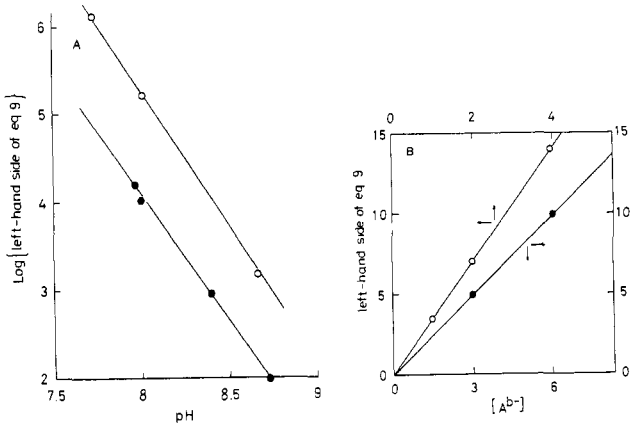


Figure 1. A. Plots of logarithmic (left-hand side of eq 9) against pH at [A] = 0.04 M. B. Plots of (left-hand side of eq 9) against [carboxylate] at pH 8.66: ●, for L^4–succinate; ○, for L^4–citrate.

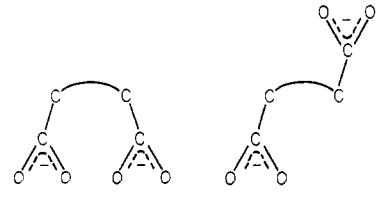
into the right-hand side of eq 10 gives a value of 2.36, which is in satisfactory agreement with the calculated value (=2.48) by using the above determined β_L value ($=2.3_7 \times 10^2$). The substitution of $(i_d)_{ob} = 12.8_5$ (cm) (at [A] = 40.0 mM) gives a value of 4.42, as compared to the calculated value of 4.9_6. The agreement of the two independent measurements is a good support for the postulated H_3L^{3+}–citrate^{3-} ion-pair formation.

Discussion

The present investigation of ion-pair formation stemmed from the paper on electrophoretic analysis[26] of macrocyclic polyamines (N_3 to N_6) where we found that certain carboxylate anions used as a buffer or an electrolyte strangely influenced the sequence and migration distances of some polyamines. In monocarboxylate solutins such as acetate and lactate (at pH ~6), all the polyamines tested moved normally: they moved as protonated cations toward the cathode with more or less similar rates. On the other hand in citrate buffers at the same pH, some of the polyamines showed unusually slow movements. The polyamines outstandingly dragged were larger macrocycles: 16-membered N_4 (1,4,8,13-tetraazacyclohexadecane),[31] 17-membered N_4 (1,5,9,14-tetraazacycloheptadecane),[31] 15–17-membered N_5 (L^1–L^3), and an 18-membered N_6 (L^4). On the other hand, smaller macrocycles (e.g. 12- to 14-membered N_4) were virtually unaffected. We proposed ion-pair formation through favorable hydrogen bonds between large sized macrocyclic polyamine cations and the tricarboxylate anion. Such ion-pair equilibria could be used to effect separations of polyamines having various sizes by electrophoresis. This technique has been first employed to examine the steric and electronic effects of polycarboxylate anions on the ion-pair formation with macrocyclic polyamines L^1–L^4[32] and a relevant linear polyamine L^5.

The electrophoretic results summarized in Table I seem compatible with the "ion-pair complexation" hypothesis. The dicarboxylates investigated might possibly be classified into two groups. With group A the movement of macrocycles tends to be detained. Anions in this group are citrate, succinate, malate, tartrate, malonate, maleate, and *o*-phthalate, which may strongly interact with those polyamines. With group B the macrocycles are not so remarkably detained as with group A. Anions in this group are fumarate and *m*-phthalate, which may not have special interaction with macrocycles.

Comparison of the movements with *o*- and *m*-phthalates is instructive. Since these anions are all doubly charged at the pH studied, they would associate with the polyamine cations to the same extent if only the electrostatic force plays an important role in the interaction. Of them only the ortho isomer influences specifically the larger macrocyclic polyamines. Furthermore, the movement patterns of polyamines in doubly charged *m*-phthalate anions are similar to those observed in singly charged anion acetate or lactate. Thus the *m*-phthalate anions seems to interact with polyamines as a monovalent anion. A paralleling situation was seen with maleate and fumarate: the former (cis dicarboxylate) works like *o*-phthalate and the latter (trans dicarboxylate) like *m*-phthalate. These facts indicate that a geometrical factor is important for the effective ion-pair formation. The skeleton I is



I II

essential to the strong interaction, while II has weak interaction. The syn-type dicarboxylate anions (I) may offer more donor atoms for effective hydrogen-bonding complexation with protonated polyamines than the anti-type dicarboxylates (II).[33]

It is of interest to remind that tris(ethylenediamine)cobalt(III) $[Co(en)_3]^{3+}$ strongly interacts with special dicarboxylates to form stable outer-sphere complexes in aqueous solutions.[34-39] The

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(32) Although the cyclic spermines are considered to have strong associaion with polycarboxylates on the basis of the electrophoresis study, they unfortunately did not give reversible polarographic waves in the quantitative second test. Accordingly, we have excluded them from the present discussion.

(33) A reviewer kindly suggested that as a consequence of the spatial distance of the carboxylates, the negative charge density is much larger in I than in II; thus the observed binding sequence follows the strength of the purely electrostatic force. The explanation of the charge accumulation is also compatible with the experimental observations.

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Table II. Typical Data of the Effects of Carboxylate and pH on Half-Wave Potentials at I = 0.2 M and 25 °C ([Tris Buffer] = 0.10 M)

	10^3[L], M	pH	10^3 × [carboxylate], M	$E_{1/2}$, vs. SCE	$\Delta E_{1/2}$, mV
			Citrate		
L^1	0.4	7.18	40.0		$11._0$
	0.4	7.18	20.0		$6._7$
	0.4	7.49	40.0		$6._0$
	0.4	7.86	40.0		$2._6$
			Citrate		
L^2	0.48	7.15	40.0		$24._5$
	0.48	7.38	20.0		$15._0$
	0.48	7.38	40.0		$21._7$
	0.48	7.88	40.0		$14._4$
			Succinate		
L^2	0.48	7.25	40.0		$15._4$
	0.48	7.36	40.0		$12._2$
	0.48	7.36	20.0		$7._5$
	0.48	7.82	40.0		$9._1$
			Malonate		
L^2	0.48	7.25	40.0		$10._9$
	0.48	7.40	40.0		$9._8$
	0.48	7.40	20.0		$5._7$
	0.48	7.82	40.0		$5._9$
			Citrate		
L^3	0.40	8.15	40.0		$21._4$
	0.40	8.52	40.0		$15._7$
	0.40	8.90	20.0		$4._7$
	0.40	8.90	40.0		$8._5$
			Succinate		
L^3	0.40	7.25	40.0		$12._8$
	0.40	7.40	40.0		$10._9$
	0.40	7.84	20.0		$4._4$
	0.40	7.84	40.0		$7._7$
			Malonate		
L^3	0.50	7.25	40.0		$5._6$
	0.50	7.40	40.0		$5._0$
	0.50	7.40	20.0		$2._8$
	0.50	7.84	40.0		$2._6$
			Citrate		
L^4	0.40	7.74	0	-0.185_0	
	0.40	7.74	40.0	-0.150_0	35
	0.40	8.01	0	-0.212_4	
	0.40	8.01	40.0	-0.181_3	31
	0.40	8.66	0	-0.246_9	
	0.40	8.66	10.0	-0.236_7	10
	0.40	8.66	20.0	-0.230_9	16
	0.40	8.66	40.0	-0.224_7	23
			Succinate		
L^4	0.40	7.96	60.0		$7._0$
	0.40	8.02	60.0		$7._0$
	0.40	8.73	30.0		$2._7$
	0.40	8.73	60.0		$4._9$
			Malonate		
L^4	0.40	7.34	20.0		$6._3$
	0.40	7.34	40.0		$10._5$
	0.40	7.74	40.0		$9._6$
	0.40	8.01	40.0		$9._6$
			Citrate		
L^5	0.52	7.22	40.0		$10._0$
	0.52	7.22	20.0		$5._7$
	0.52	7.37	40.0		$9._7$
	0.52	7.56	40.0		$8._5$
	0.52	7.70	40.0		$9._0$

skeleton of $^-O_2CCCCO_2^-$ with two carboxyl groups adjacent to each other is required for stereospecific H bondings with three axial amine protons in the complex cation. Thus, maleate, malonate, succinate, *o*-phthalate, etc. strongly interact with $[Co(en)_3]^{3+}$, while *m*- and *p*-phthalate have little association. The most well-known application of such outer-sphere complexes is resolution of racemic $[Co(en)_3]^{3+}$ as well as *d*-tartrate salts. The separation of the optical isomers was achieved by means of electrophoresis in the *d*-tartrate electrolyte.[34-36] Those having no N–H, e.g., $[Co(dip)_3]^{3+}$ and $[Co(o\text{-phen})_3]^{3+}$ cannot form outer-sphere complexes, resulting in failure of the optical resolution of electrophoresis.[35] The interacting pattern of the dicarboxylates with our protonated macrocycles (on electrophoresis) is astonishingly similar. We thus propose a similar hydrogen-bonded structure for the macrocyclic polyamine–dicarboxylate ion-pair association.

The polarographic results of the $E_{1/2}$ shifts suggested the association between macrocyclic penta- and hexaamines with succinate, maleate, malonate, and citrate but not with acetate, lactate, fumarate, or *m*-phthalate. The trivial $\Delta E_{1/2}$ for macrocyclic tetraamines indicated negligible interactions with any of the polycarboxylates. Hence, the electrophoretic and polarographic results are in good agreement. The analyzed interaction data consistently obeyed the formula for 1:1 association of the triprotonated polyamine cations with the di- or tricarboxylic (in the case of citrate) anions.

At pH ~7 the macrocyclic hexaamine (and pentaamines, too) can accommodate three protons into their cavities, whereby the macrocyclic conformation may be fixed (like the $[Co(en)_3]^{3+}$ complex) so as to best adapt the H–H distance of amine protons to the oxygen–oxygen distance of the two carboxyl groups. the 12- to 15-membered macrocyclic tetraamines contain only two protons,[18] which would not be sufficient to evoke the electrostatic attraction to carboxylate anions. In addition the small ring sizes may be disadvantageous for the ion-pair hydrogen bondings.

Comparison of the data on pentaamines L^1–L^3 presented in Table III reveals some effect of the macrocyclic ring size. The smallest 15-membered ring cation is the poorest anion acceptor, which is probably due to steric crowdness marring the hydrogen bondings for the ion-pair formation. The 16- and 17-membered N_5 have similar orders of affinity to dicarboxylate. The tricarboxylic citrate anions bind more strongly to the polyamines than any of the dicarboxylate anions, suggesting that electrostatic interaction is fundamental to the ion-pair complexation. The linear N_5 (L^5) detectably forms a complex only with citrate trianion. This is supported by electrophoretic data, too. Probably the linear pentaamine has a lesser recognition ability of the carboxylate anions than the macrocyclic homologues. This fact shows the operation of the macrocyclic effects on the stability of the anion complexes, as well as the cation complexes.[5,6,25] The higher stability may come from the presence of closed and (relatively) inflexible macrocyclic structure which contribute to better holding of the dense $^+$N–H binding sites, efficient hindrance to hydration of the $^+$N–H sites, and resistance to deformation. It is to be noted that the chelate effect is seen for linear polycationic ligands (guanidine or amine) interacting with polyanions.[17]

The polarography showed that aspartate and glutarate have little interaction with the polyamines. Apparently, the electronic effect, i.e., zwitterion character of the amino acids, weakens the ion-pair association.

The present study may be generalized in that the three positively charged amino groups at neutral pH can, if their N–H's are arranged in sterically suitable environments, bind with polyoxyanions having proper geometrical requirements. Our system may offer a simplified chemical model of transporters for tricarboxylate-cycle anions. Carrier-mediated transport of malate, succinate, and citrate is postulated by saturation kinetics of the concentration-dependence or by the competitive inhibition caused by characteristic analogues.[19-24] A dicarboxylate carrier in rat liver mitochondria competitively uptakes succinate, malonate, and malate on a common binding site. Their uptake is not inhibited by "permeant" anions such as acetate, glutarate, or aspartate.[21]

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Table III. 1:1 Association Constants β_L (with Confidence Limits) for Polyamines with Carboxylates at 25 °C and $I = 0.2$ M[a]

	ligand				
	L^1	L^2	L^3	L^4	L^5
carboxylate (A)					
citrate[b]	$(5.5 \pm 0.6) \times 10^1$	$(2.5 \pm 0.3) \times 10^2$	$(1.0 \pm 0.1) \times 10^3$	$(2.4 \pm 0.2) \times 10^2$	$(3 \pm 0.3) \times 10$
succinate[c]	negligible[d]	$(1.2 \pm 0.1) \times 10^2$	$(9.2 \pm 1.0) \times 10$	$(1.8 \pm 0.2) \times 10$	negligible[d]
malonate[c]	negligible[d]	$(6.6 \pm 0.7) \times 10$	$(2.5 \pm 0.3) \times 10$	$(3.3 \pm 0.3) \times 10$	negligible[d]
malate[c]	slightly[e]	$(5.0 \pm 0.5) \times 10$	$(2.6 \pm 0.3) \times 10$	$(1.5 \pm 0.2) \times 10$	negligible[d]
maleate[c]		$(7.6 \pm 0.8) \times 10$		$(2.9 \pm 0.3) \times 10$	
fumalate		slightly[e]		negligible[d]	

[a] At least three separate experiments were performed for each association. [b] $\beta_L = [H_3L^{3+}\text{-}A^{3-}]/[H_3L^{3+}][A^{3-}]$ (M^{-1}). [c] $\beta_L = [H_3L^{3+}\text{-}A^{2-}]/[H_3L^{3+}][A^{2-}]$ (M^{-1}). [d] $\Delta E_{1/2}$ is less than 1 mV. [e] $\Delta E_{1/2}$ is too small (2~3 mV) to permit calculation of β_L.

These biological facts about the anion specificities may suggest a positive charge accumulated environment at the binding site similar to those in our model. A citrate carrier may possess a similar recognition site. Our simplified system, however, is not a good model for a transporter of fumarate, one of the intermediates of tricarboxylate cycle.

In biological anion-transport systems, the dicarboxylate anions are often exchanged for phosphate anions.[20,21] The common structural features of polyoxyanions favoring hydrogen bondings with N^+–H might operate in the biological recognition. A phosphate-transport protein in red blood cells is considered to contain three positive charges (by protonated amino group) at the anion-binding site.[40] Our preliminary experiment by the polarography indicated that the dicarboxylate carrier model indeed interacts with phosphate anions to form 1:1 complexes. We are currently investigating the uptake of phosphate anions (and its derivatives) by using our system.[41]

Further structural modification of fundamental structures of the present macrocyclic polyamine molecules will provide strategies for the rational design of organic anion receptors and carriers. Such results should be of interest in biology as well as in chemistry in view of the active current research on anion binding and transport in biological membranes.

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Isomer Distribution Ratios of Phenols in Aromatic Hydroxylation with the Hydroxyl Radical Generated from α-Azohydroperoxide in Anhydrous Organic Media.[1] Comparison with Fenton's Reagent[2,3]

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Abstract: New isomer distribution ratios of phenols in aromatic hydroxylation with the hydroxyl radical generated from α-azohydroperoxide (**1**) in anhydrous organic media are reported. Photodecomposition of **1** (10^{-2} M) in anisole, toluene, chlorobenzene, and nitrobenzene under argon or oxygen gas gave methoxyphenol, cresol, chlorophenol, and nitrophenol, respectively, in yields and isomer ratios listed in Table I. Photodecomposition of **1** (10^{-2} M) in acetonitrile containing toluene or nitrobenzene with various molar ratios under argon gas or under degassed conditions revealed that the isomer ratios under argon gas presented in Table I were reproducible as long as the reactions were conducted in an excess of the substrate. On the other hand, isomer ratios were varied when the reactions were caried out in an excess of **1** compared with the amount of substrate. The isomer ratios were also varied when oxygen was present in the reactants. On the basis of the isomer ratios found by this study, the degree of electrophilicity of the hydroxyl radical generated from **1** under anhydrous conditions and the mechanism of the aromatic hydroxylation are discussed in comparison with the aromatic hydroxylation with Fenton's reagent and others and with the aromatic phenylation, methylation, and trimethylsilylation reported in the literature.

The electrophilic property of the hydroxyl radical has been documented by extensive studies on the ortho:meta:para isomer ratios of phenols[1-15] and the reaction rates[16-18] in the aromatic hydroxylation with the hydroxyl radical generated from Fenton's reagent, by radiolysis of water, and by photolysis of hydrogen

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