Those results imply that tyrosine-20 is the second most reactive. Our results, in conjunction with the NMR results of the Oxford group,[11-14] suggest that the second most reactive tyrosine (as judged by the decrease in intensity of the resonances upon nitration) is 53. Our NMR data for nitrotyrosyllysozyme suggest that about 30–40% of the tyrosine-53 molecules have been nitrated and that tyrosine-20 has not been modified. The above discussion, of course, depends on the validity of the NMR assignments that were based on Gd(III)-induced broadening. Since X-ray studies have been published for the lysozyme–Gd complex[17] and since tyrosine-53 (of the three available) is the only one near the position of the two Gd(III) ions being bound, we accept the NMR assignments. Three possibilities exist: (i) the chemical modification study assigned 20 instead of 53;[4] (ii) ^{1}H NMR assignment of 20 and 53 is indeed reversed; and (iii) less likely, the nitration in this study modified tyrosine-53; that by Atassi and Habeeb,[4] tyrosine-20. We feel that the third possibility is extremely unlikely; unfortunately our results cannot distinguish between the first and second choices. It is abundantly clear from both the resonance Raman and ^{1}H NMR data that the most reactive tyrosine is in an aqueous environment. The resonance Raman data further indicate that the second tyrosine to be modified is in a very hydrophobic and strongly hydrogen-bonded environment. The utility of resonance Raman spectroscopy for discerning the nature of the environment around tyrosine residues is demonstrated.

Acknowledgment. We are grateful to the Charles and Johanna Busch Biomedical Fund (Rutgers University) for financial support and to Dr. David Ruben for advice and help with the NMR. The high-field NMR experiments were performed at the NMR facility for Biomolecular Research located at the F. Bitter National Magnet Laboratory, MIT. The NMR Facility is supported by Grant No. RR 00995 from the Division of Research Resources of the NIH and by the National Science Foundation under Contract No. C-670.

Registry No. 3-Nitrotyrosine, 3604-79-3; E.C.3.2.1.17, 9001-63-2.

---

(17) (a) Kurachi, K.; Sieker, L. C.; Jensen, L. H. *J. Biol. Chem.* **1975**, *250*, 7663–7667. (b) Perkins, S. J., Johnson, L. W., Machin, P. A.; Phillips, D. C. *Biochem. J.* **1979**, *181*, 21–36.

Macromonocyclic Polyamines as Biological Polyanion Complexons. 2.[1] Ion-Pair Association with Phosphate and Nucleotides

Eiichi Kimura,*[2a] Mutsuo Kodama,[2b] and Takashi Yatsunami[2a]

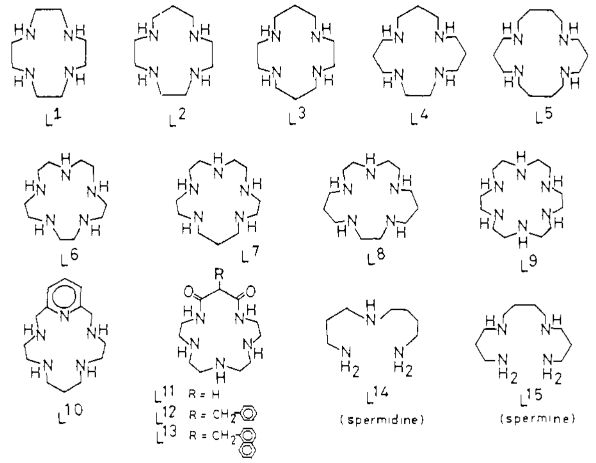
Contribution from the Department of Medicinal Chemistry, Hiroshima University School of Medicine, Kasumi, Hiroshima 734, Japan, and Department of Chemistry, College of General Education, Hirosaki University, Bunkyo, Hirosaki 036, Japan. *Received August 6, 1981*

Abstract: The interaction of 15- to 18-membered macromonocyclic tetra-, penta-, and hexaamines with biologically important polyanions, such as phosphate, AMP, ADP, and ATP, in neutral pH solutions has been studied using polarographic methods and ^{1}H NMR shift measurements. The results established 1:1 ion pairings between the polycationic forms of the macrocycles and the polyanionic forms of the phosphates. The inorganic phosphate anion is bound to the macrocycles as strongly as the previously studied polycarboxylate anions. The greater stability constants β_L for the 1:1 nucleotide complexes imply the presence of other binding sites, such as adenine moieties, in the nucleotide molecules. These β_L values are greater than those reported for the nucleotide complexes of physiological linear polyamines (e.g., spermidine and spermine) or of divalent metal cations (e.g., Mg^{2+}). The macrocyclic polyamines, moreover, interact with Mg^{2+}– and Ca^{2+}–nucleotide complexes to yield the ternary complexes. Significant biological implications are discussed.

Introduction

Recognition and binding of ionic substrates to organic host molecules (e.g., enzymes, antibodies, or membrane transporters) are of vital importance in biological reactions. Model studies so far have centered mainly on the recognition of cations using synthetic host compounds, such as macrocyclic polyethers[3-8] and polyamines.[9,10]

Chart I. Macromonocyclic Polyamines and Dioxo Polyamines Studied for the Complexation with Phosphate and Nucleotides



Recently, anion complexons have been drawing much attention. The anion receptors possess appropriate anion binding sites, like

---

(1) Part 1: Kimura, E.; Sakonaka, A.; Yatsunami, T.; Kodama, M. *J. Am. Chem. Soc.* **1981**, *103*, 3041.

(2) (a) Hiroshima University. (b) Hirosaki University.

(3) Izatt, R. M.; Hansen, L. D.; Eatough, D. J.; Bradshaw, J. S.; Christensen, J. J. In "Metal-Ligand Interactions in Organic Chemistry and Biochemistry", Pullmann, B.; Goldblum, N., Eds.; D. Reidel: Dordrecht, Holland, 1977; pp 337–361.

(4) Ovchinnikov, Yu. A.; Ivanov, V. T.; Shkrob, A. M. In "Membrane-Active Complexones"; Elsevier: New York, 1974; pp 53–72 and 184–198.

(5) Lamb, J. D.; Izatt, R. M.; Christensen, J. J.; Eatough, D. J. In "Coordination Chemistry of Macrocyclic Compounds"; Melson, G. A., Ed.; Plenum Press: New York, 1979.

(6) Izatt, R. M.; Lamb, J. D.; Izatt, N. E.; Rossiter, B. E., Jr.; Christensen, J. J.; Haymore, B. L. *J. Am. Chem. Soc.* **1979**, *101*, 6273–6276, and references therein.

(7) Cram, D. J.; Cram, J. M. *Acc. Chem. Res.* **1978**, *11*, 8–14.

(8) van Bergen, T. J.; Kellogg, R. M. *J. Am. Chem. Soc.* **1977**, *99*, 3882–3884.

0002-7863/82/1504-3182$01.25/0 © 1982 American Chemical Society

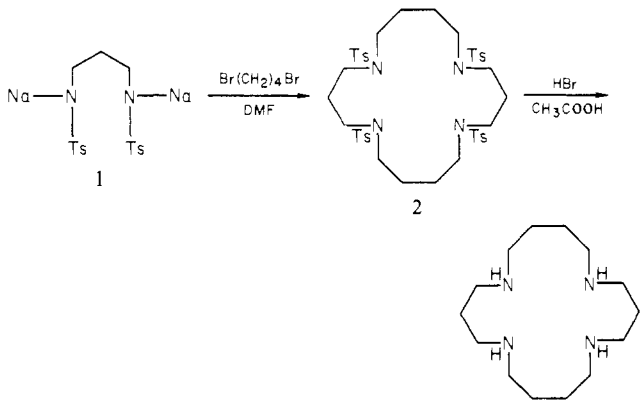
guanidinium or ammonium cation groups, on properly aligned linear[11] and macrobi-[12,13] or -tricyclic molecular skeletons.[14,15] These cationic molecules interact with halide, phosphate, or carboxylate anions by inclusion, electrostatic forces, and/or hydrogen bonding.

In previous studies,[10,16,17] we noticed that the macromonocyclic polyamines depicted in Chart I were multiproton acceptors at neutral pH to accommodate more than two protons into their central cavities. Hence, it was anticipated that the high-charged ammonium cations may be anion complexons. Indeed, the macrocyclic penta- and hexaamines L^6–L^9 displayed highly selective complexing abilities at neutral pH toward polycarboxylate anions.[1,18] Interestingly, these macrocyclic polyamines form 1:1 complexes only with polycarboxylates occurring in the catabolic tricarboxylate cycle (i.e., succinate, malate, citrate, malonate, and maleate) so as to discriminate against monocarboxylates (e.g., acetate) or other dicarboxylates (e.g., fumarate and aspartate). Thus, they mimic the active center of polycarboxylate carrier proteins. The substrate specificities and the 1:1 complexation constants were determined by the electrophoresis technique and polarographic method. The results were interpreted in terms of electrostatic attractions and hydrogen bondings between the triprotonated polyamines and polycarboxylate anions. The tetraamine macrocycles L^1–L^4 bear only two protons at neutral pH and, thus, fail to interact with the polycarboxylates. These findings led us to the idea that such an interaction might also occur with phosphate groups having polyoxyanion characters.

Indeed, we have now discovered strong ion pairings between inorganic phosphate, AMP, ADP, and ATP and a series of macromonocycles composed of various ring sizes and N donor atom numbers. These synthetic macrocyclic polyamines are to be compared to the intracellular metal ions Mg^{2+} and Ca^{2+} [19–21] or to the organic polyamines spermidine L^{14} and spermine L^{15},[22,23] which all bind tightly to the nucleotides to participate in numerous biological reactions. The formation constants for some of our polyamines[24] are found to be larger than those for Mg^{2+}, Ca^{2+}, and the biogenic polyamine cations. Several unique complexation features are also presented.

Recently, complexation[25] and liquid membrane transport[26] of AMP and ATP with bicyclic diammonium salts have been reported. After the present work was completed, a communication[27] appeared reporting complexations of AMP, ADP, and ATP with 24- to 32-membered macrocyclic hexa- and octaamines. Their 1:1 formation constants are considerably different from ours.

Scheme I



1

2

L^5
(as 4HBr salt)

Experimental Section

Materials. Syntheses of a number of the macrocyclic polyamines, L^1–L^4,[28] L^6–L^8,[16] L^9,[10] and L^{10},[29] were described elsewhere. The macrocyclic dioxo polyamines L^{11}–L^{13} were prepared according to a modified procedure of Tabushi et al.[30] Synthesis of tetraamine L^5 is described below. The purities were checked by melting points and the suitable analytical method developed recently in our laboratory.[18] Nucleotides were purchased from Nakarai Chemicals, spermidine trihydrochloride, and spermine tetrahydrochloride from Sigma, and D_2O (99.75% D), NaOD (40% in D_2O, 99% D), and DCl (20% in D_2O, 99% D) from Merk. All the other chemicals were of analytical grade and were used without further purification.

Synthesis of L^5. The disodium salt **1** (Scheme I), prepared by mixing a bis(*p*-toluenesulfonamide) derivative of 1,3-diaminopropane (69 g) with 2 equiv of sodium methoxide, was treated with 1 equiv of 1,4-dibromobutane in boiling DMF (1.5 L) for 4 h. A usual workup and recrystallization from benzene eliminated a major 1:1 cyclization product. Chromatography of the mother liquid and subsequent recrystallization from benzene–ethanol gave the desired product **2** having 2:2 cyclization stoichiometry: yield 1.67 g; mp 227–230 °C; ^1H NMR ($CDCl_3$) δ 1.60 (8 H, br s), 1.70–2.10 (4 H, m), 2.41 (12 H, s), 2.90–3.30 (16 H, m), 7.33 and 7.66 (16 H, AB q); mass spectrum, *m/e* 872 (M^+), 717, 562. Detosylation of **2** with HBr–acetic acid (1:1, v/v) and recrystallization from HBr–acetic acid afforded L^5 (670 mg) as the tetrahydrobromide salt: mp >300 °C; ^1H NMR (D_2O, pD 8.0) δ 1.6–2.0 (4 H, br s), 2.0–2.4 (2 H, t), 2.9–3.4 (8 H, m). The mixed protonation constants are 10.36, 9.97, 7.00, and 6.70 at $I = 0.2$ M ($NaClO_4$) and 25 °C.

Polarographic Method. The polarographic procedures are the same as those applied to the previous macrocyclic polyamine–polycarboxylate systems.[1] The special features of the dropping mercury electrode (DME) and of all the other apparatus were described elsewhere.[28] The mixed protonation constants (K_i) of L^6–L^9 used for the calculation are described in the previous report.[1] Log K_i values for L^{10} and L^{11} were determined by potentiometric titrations: 9.48, 8.56, 5.83, and <2 for L^{10} and 9.10, 8.47, and ~2 for L^{11} at $I = 0.2$ M and 25 °C. In all the polarographic measurements, ionic strength (I) was adjusted to 0.2 M ($NaClO_4$).

^1H NMR Measurements. The ^1H NMR spectra were recorded on a Hitachi R-40 spectrometer (90 MHz) in concentric NMR tubes using Me_4Si as an external reference. The pD of a sample solution was obtained by adding 0.40 to the pH reading using a Hitachi-Horiba F-7 pH meter equipped with a Horiba microprobe combination electrode (Model 6028-10T). Mixtures of nucleotides and polyamines were lyophilized two times with each 2 mL of D_2O to minimize the water content. The chemical shifts were measured as least three times with reproducibility of ±0.5 Hz.

Results and Calculation

Polarographic Studies. (a) Interactions between Macrocyclic Polyamines L^1–L^{10} (Except for L^5) and AMP, ADP, and ATP.

Complex formations between L^1–L^{10} (except for L^5) and the nucleotides were analyzed in an identical manner as those pre-

---

[9] Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1978**, 1081–1085 and references therein.

[10] Kodama, M.; Kimura, E.; Yamaguchi, S. *J. Chem. Soc., Dalton Trans.* **1980**, 2536–2538.

[11] Dietrich, B.; Fyles, D. L.; Fyles, T. M.; Lehn, J. M. *Helv. Chim. Acta* **1979**, *62*, 2763–2787.

[12] Lehn, J. M.; Sonveaux, E.; Willard, A. K. *J. Am. Chem. Soc.* **1978**, *100*, 4914–4916.

[13] Park, C. H.; Simmons, H. E. *J. Am. Chem. Soc.* **1968**, *90*, 2431–2432.

[14] Graf, E.; Lehn, J. M. *J. Am. Chem. Soc.* **1976**, *98*, 6403–6405.

[15] Schmidtchen, F. P. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 720–721.

[16] Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1978**, 104–110.

[17] Kimura, E.; Kodama, M. *Yuki Gosei Kagaku Kyokaishi* **1977**, *35*, 632–641.

[18] Yatsunami, T.; Sakonaka, A.; Kimura, E. *Anal. Chem.* **1981**, *53*, 477–480.

[19] Sigel, H., Ed. In "Metal Ions in Biological Systems"; Marcel Dekker: New York, 1973–1980; Vol. 1–11.

[20] Pezzano, H.; Podo, F. *Chem. Rev.* **1980**, *80*, 365–401.

[21] Izatt, R. M.; Christensen, J. J.; Rytting, J. H. *Chem. Rev.* **1971**, *71*, 439–481.

[22] Cohen, S. S. In "Introduction to the Polyamines"; Prentice-Hall: Englewood Cliffs, NJ, 1971.

[23] Tabor, C. W.; Tabor, H. *Annu. Rev. Biochem.* **1976**, *45*, 285–306.

[24] It should be noticed that L^5 has a unique structure, namely, a cyclic dimer of spermidine or a cyclic derivative of spermine.

[25] Tabushi, I.; Imuta, J.; Seko, N.; Kobuke, Y. *J. Am. Chem. Soc.* **1978**, *100*, 6287–6288.

[26] Tabushi, I.; Kobuke, Y.; Imuta, J. *J. Am. Chem. Soc.* **1980**, *102*, 1744–1745.

[27] Dietrich, B.; Hosseini, M. W.; Lehn, J. M.; Sessions, R. B. *J. Am. Chem. Soc.* **1981**, *103*, 1282–1283.

[28] Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1976**, 2335–2338.

[29] Kimura, E.; Kodama, M.; Machida, R.; Ishizu, K. *Inorg. Chem.*, in press.

[30] Tabushi, I.; Taniguchi, Y.; Kato, H. *Tetrahedron Lett.* **1977**, 1049–1052.

Table I. 1:1 Association Constants for Phosphate and Nucleotide Complexation with Polyamines and Metal Ions at 25 °C

ligand	species	AMP^{2-}	ADP^{3-}	ATP^{4-}	HPO_4^{2-}
$L^{5\,a,c}$	H_4L^{4+}	$6._9 \times 10^3$	$3._2 \times 10^4$	$4._5 \times 10^6$	
$L^{6\,a,c}$	H_3L^{3+}	$1.5_6 \times 10^3$	$8.7_2 \times 10^3$	$1.0_3 \times 10^4$	
$L^{7\,a,c}$	H_3L^{3+}	$1.3_0 \times 10^3$	$1.4_7 \times 10^3$	$4.2_3 \times 10^3$	1.1×10^2
$L^{8\,a,c}$	H_3L^{3+}	$6.8_6 \times 10^2$	$1.0_0 \times 10^3$	$5.1_2 \times 10^3$	
$L^{9\,a,c}$	H_3L^{3+}	$1.7_7 \times 10^3$	$4.4_7 \times 10^5$	$2.5_0 \times 10^6$	$1.3_8 \times 10$
$L^{10\,a,c}$	H_2L^{2+}	$3.3_4 \times 10^2$	$2.2_1 \times 10^2$	$1.7_8 \times 10^2$	
$L^{11\,a,d}$	HL^+	$5.0_3 \times 10^2$	$1.2_1 \times 10^2$	$4.7_7 \times 10^2$	
L^{14} (spermidine)^a		$2.3 \times 10^{2\,e}$	$3.3 \times 10^{2\,e}$	$9.0 \times 10^{2\,e}$	
L^{15} (spermine)^a		$3.6 \times 10^{2\,e}$	$1.3 \times 10^{3\,e}$	$9.5 \times 10^{3\,e}$	
$Mg^{2+\,b}$		9.33×10^f	$2.19 \times 10^{3\,g}$	$1.66 \times 10^{4\,h}$	
$Ca^{2+\,b}$		7.08×10^f	$7.24 \times 10^{2\,f}$	$9.33 \times 10^{3\,h}$	

[a] $\beta_L = [H_iL^{i+}\text{–}A^{b-}]/[H_iL^{i+}][A^{b-}]$ (M^{-1}). [b] $K_{MA} = [M^{2+}\text{–}A^{b-}]/[M^{2+}][A^{b-}]$ (M^{-1}). [c] $I = 0.2$ M (Tris buffer, 0.1 M). [d] $I = 0.2$ M (borate buffer 0.06 M). [e] 0.05 M Tris buffer, pH 7.5 (ref 34). [f] $I = 0.1$ M (KNO_3). Taqui Khan, M. M.; Martell, A. E. *J. Am. Chem. Soc.* 1962, *84*, 3037. [g] 0.085 M tributylethylammonium bromide + 0.025 M triethanolamine, pH 7.9. Burton, K. *Biochem. J.* 1959, *71*, 388. [h] $I = 0.1$ M (KNO_3). Taqui Khan, M. M.; Martell, A. E. *J. Am. Chem. Soc.* 1966, *88*, 668.

viously applied to the carboxylate complexes with L^6–L^9,[1] wherein the theory was described in detail.

The half-wave potentials, $E_{1/2}$, of the anodic dissolution waves at DME due to the polyamines L^6–L^{10} shifted commonly to more positive values upon addition of AMP, ADP, or ATP in Tris buffers. The amount of shifts, $\Delta E_{1/2}$, increased with an increase in the concentration of the added anions. The wave heights decreased as the anion concentration increased. These and all the other polarographic behaviors were identical with those observed for the carboxylate systems, leading us to conclude the complex formation between L^6–L^{10} and the nucleotides. Typical data are given in Table S1 (see paragraph at the end of paper concerning supplementary material). Interactions between small-sized macrocyclic tetraamines L^1–L^4 and the nucleotides were negligible, as concluded by the very small $\Delta E_{1/2}$ values. The stoichiometries and stability constants of the L^6–L^{10} complexes were determined from the $\Delta E_{1/2}$ values.

If assuming formation of 1:1 complexes H_iL^{i+}–A^{b-} from a protonated polyamine H_iL^{i+} and an anion A^{b-}, one can express $\Delta E_{1/2}$ by eq 1 at a given pH,[1] where $\beta_L = [H_iL^{i+}\text{–}A^{b-}]/[H_iL^{i+}][A^{b-}]$.

$$\Delta E_{1/2} = 0.0296[\log\,[(\alpha_H)_L + \beta_L K_1 K_2 ... K_i [H^+]^i [A^{b-}]] - \log\,(\alpha_H)_L] \quad (1)$$

Equation 1 is rearranged to eq 2 (the same as eq 9 in ref 1).

$$[\text{antilog}\,(\Delta E_{1/2}/0.0296) - 1](\alpha_H)_L = \beta_L K_1 K_2 ... K_i [H^+]^i [A^{b-}] \quad (2)$$

The plots on the left hand of eq 2 in logarithm against pH at constant $[A^{b-}]$ are all linear, with slopes corresponding to $-i$. Further, the plots on the left hand of the equation against $[A^{b-}]$ at constant pH afforded linear lines passing through the origin. The β_L values are calculable from the slopes. Here, $[A^{b-}]$ is put equal to the total concentration of the added nucleotide, since AMP, ADP, and ATP exist exclusively as completely dissociated AMP^{2-}, ADP^{3-}, and ATP^{4-}, respectively, at the experimental pH range of 7.1–9.1 (the reported pK_a values for the final dissociation of phosphate residues are in the range of 6.2–6.4 for AMP, 6.1–6.7 for ADP, and 6.0–6.9 for ATP[31]). All of the experimental results are compatible with eq 2, validating the initial assumption that the 1:1 interactions occur between L^6–L^{10} and AMP, ADP, and ATP. The *i* values obtained from the slopes of the former plots were 2.9–3.2 for L^6–L^9 and ~2.0 for L^{10}, indicating the involved species as H_3L^{3+} for L^6–L^9 and H_2L^{2+} for L^{10}. The stability constants β_L are listed in Table I.

(b) Complex Formation with Inorganic Phosphate. With inorganic phosphate ion having pK_a values of 12.38, 7.20, and 2.15,[31] two anionic forms, HPO_4^{2-} and $H_2PO_4^-$, are available at neutral pH. Here, $[HPO_4^{2-}]$ and $[H_2PO_4^-]$ are given by multiplying the total phosphate concentration by $K_{a2}/([H^+] + K_{a2})$ and $[H^+]/([H^+] + K_{a2})$, respectively. The $\Delta E_{1/2}$ data with L^7- and L^9-phosphate systems were analyzed by eq 2. The results best fit the interaction between H_3L^{3+} and HPO_4^{2-} species. The stability constants thus obtained are given in Table I.

(c) Interactions between L^5 and AMP, ADP, and ATP. Unlike the cases with L^6–L^{10}, L^5 did not give the anodic dissolution waves at DME in neutral pH solutions. However, complex formations between L^5 and AMP, ADP, and ATP could be assessed by an indirect method measuring the anodic waves due to L^8 in the presence of L^5–nucleotide mixtures. Addition of a nucleotide to a solution containing both L^5 and L^8 at a given pH caused a positive shift of $E_{1/2}$ (due to L^8). Assuming that L^5 also forms complexes with the nucleotides, the concentration of the nucleotides $[A^{b-}]$ available for the complexes with L^8 should be less than the initial concentration $[A^{b-}]_0$. Using the experimentally obtained $\Delta E_{1/2}$ values, one can calculate $[A^{b-}]$ by using eq 3, which is rearranged from eq 1, where *i* is 3 and the terms $(\alpha_H)_L$, K_i, and β_L refer to L^8.

$$[A^{b-}] = [\text{antilog}\,(\Delta E_{1/2}/0.0296) - 1](\alpha_H)_L/\beta_L K_1 K_2 K_3 [H^+]^i \quad (3)$$

The values of $[A^{b-}]$ thus obtained for AMP, ADP, and ATP were, indeed, much less than those of $[A^{b-}]_0$ (Table S2, see supplemental material), lending support to the complex formation between L^5 and the anions.

The stability constants and the reactive ligand species were determined in the following way. The concentration of the L^5–nucleotide complexes, $[L^5\text{–}A^{b-}]$, and of the uncomplexed (with nucleotide) L^5, $[L^5]_F$, are given by $([A^{b-}]_0 - [A^{b-}])$ and $[[L^5]_0 - ([A^{b-}]_0 - [A^{b-}])]$ ($[L^5]_0$ is the initial concentration of L^5), respectively. Thus, the apparent stability constants, β_L' ($= [L^5\text{–}A^{b-}]/[L^5]_F[A^{b-}]$) for L^5–nucleotide complexes are calculable from eq 4. The stability constants, β_L, for a particular reactive species $(H_iL^5)^{i+}$ are expressed by eq 5.

$$\beta_L' = \frac{[A^{b-}]_0 - [A^{b-}]}{([L^5]_0 + [A^{b-}] - [A^{b-}]_0)[A^{b-}]} \quad (4)$$

$$\beta_L = \beta_L' \frac{[L^5]_F}{[(H_iL^5)^{i+}]} \quad (5)$$

Since $[L^5]_F$ and $[(H_iL^5)^{i+}]$ are given by eq 6 and 7, respectively, eq 5 can be rewritten into eq 8 and then into eq 9.

$$[L^5]_F = [L^5] + [(HL^5)^+] + ... + [(H_4L^5)^{4+}] = (\alpha_H)_L'[L^5] \quad (6)$$

$$[(H_iL^5)^{i+}] = K_1 K_2 ... K_i [H^+]^i [L^5] \quad (7)$$

$$\beta_L = \frac{\beta_L'(\alpha_H)_L'}{K_1 K_2 ... K_i [H^+]^i} \quad (8)$$

$$\log \beta_L'(\alpha_H)_L' = -i\text{pH} + \log K_1 K_2 ... K_i \beta_L \quad (9)$$

Equation 9 predicts that plots of the left hand of the equation against pH should afford linear lines. Then, the *i* values can be obtained from the slopes. This turned out to be the case for all the L^5–AMP, –ADP, and –ATP systems, with

---

[31] Dawson, R. M. C.; Elliott, D. C.; Elliott, W. H.; Jones, K. M., Eds. In "Data for Biochemical Research"; Oxford University Press: London, 1969.

the i values of all equal to 4. The β_L values calculated using eq 9 were almost constant at different pH in the neutral region, confirming the validity of the values. The mean values are listed in Table I.

(d) Interactions between Macrocyclic Dioxo Polyamines L^{11}–L^{13} and AMP, ADP, and ATP. Macrocyclic dioxo polyamines L^{11}–L^{13} also gave well-defined anodic waves due to two-electron reversible oxidation at DME in borate-buffered solution. The $E_{1/2}$ values of the anodic waves were not affected by the bulk concentrations of the dioxo polyamines and of the borate buffer but shifted to more negative values with an increase in the solution pH to fit a linear relation (eq 10). These facts support that L^{11}–L^{13} form

$$\Delta E_{1/2}/\Delta \log (\alpha_H)_L a_{H^+} = 30 \text{ mV} \tag{10}$$

1:1 complexes with Hg^{2+} on the electrode either as in the form of eq 11 or 12 with the $E_{1/2}$ expressed as in eq 13. Provided that

$$Hg + L \rightleftarrows HgH_{-1}L^+ + H^+ + 2e^- \tag{11}$$

$$Hg + L \rightleftarrows HgL(OH)^+ + H^+ + 2e^- \tag{12}$$

$$E_{1/2} = \text{constant} + 0.0296 \log (\alpha_H)_L a_{H^+} \tag{13}$$

L^{11}–L^{13} (in the H_iL^{i+} forms) interact with AMP, ADP, and ATP with 1:1 stoichiometries, $E_{1/2}$ would shift to more positive potentials $E'_{1/2}$ upon addition of the nucleotides according to eq 14. Then,

$$E'_{1/2} = \text{constant} + 0.0296 \log a_{H^+}[(\alpha_H)_L + \beta_L K_1 K_2 ... K_i [H^+]^i [A^{b-}]] \tag{14}$$

the $\Delta E_{1/2}$ measuring the effect of the added nucleotides is given as in eq 1. The experimental data for L^{11} nicely fitted eq 1, showing 1:1 complex formation. The i value obtained graphically was 1 for every L^{11}–nucleotide system, which proves the active species $(HL)^+$ in the interactions. With L^{12} and L^{13}, little shifts of $E_{1/2}$ were observed on addition of the anions.

(e) Effects of Metal Cations on the Macrocyclic Polyamine–Nucleotide Interactions. Since alkaline earth cations tend to bind to nucleotides,[19-21] an addition of such a metal ion (M^{2+}) to a polyamine–nucleotide solution may diminish the concentration of the nucleotide available for complexation with the polyamine.

If the resulting metal–nucleotide complex is postulated to no longer interact with the polyamine, then one expects the anodic half-wave potential $(E_{1/2})_1$ to shift to a more negative value $(E_{1/2})_2$. The concentration of free (from the metal) nucleotide $[A^{b-}]_F$ was derived as eq 18 by an appropriate combination of equations (15–17) for the metal–nucleotide equilibria, where K_{MA} is the 1:1

$$K_{MA} = \frac{[MA^{2-b}]}{[M^{2+}]_F [A^{b-}]_F} \tag{15}$$

$$[A^{b-}]_0 = [A^{b-}]_F + [MA^{2-b}] \tag{16}$$

$$[M^{2+}]_0 = [M^{2+}]_F + [MA^{2-b}] \tag{17}$$

$$[A^{b-}]_F = [-(K_{MA}\Delta C + 1) + [(K_{MA}\Delta C + 1)^2 + 4[A^{b-}]_0 K_{MA}]^{1/2}]/2K_{MA} \tag{18}$$

stability constants for metal–nucleotide complexes, $[A^{b-}]_0$ and $[M^{2+}]_0$ are, respectively, the initial concentrations of nucleotides and metals, $[M^{2+}]_F$ is the concentration of uncomplexed metal ions, and $\Delta C = [M^{2+}]_0 - [A^{b-}]_0$. The K_{MA} values used are included in Table I.

Then, the theoretical shifts $(\Delta E_{1/2})_{calcd}$ can be estimated by eq 19 where i is 3 for L^6–L^9. Table S3 (supplementary material)

$$(\Delta E_{1/2})_{calcd} = (E_{1/2})_2 - (E_{1/2})_1 = 0.0296 \log [(\alpha_H)_L + \beta_L K_1 K_2 ... K_i [H^+]^i [A^{b-}]_F] - 0.0296 \log [(\alpha_H)_L + \beta_L K_1 K_2 ... K_i [H^+]^i [A^{b-}]_0] \tag{19}$$

compares the $(\Delta E_{1/2})_{calcd}$ values with the experimental values $(\Delta E_{1/2})_{obsd}$ for L^6–L^9 systems. The solutions containing Mg^{2+} and Ca^{2+} showed large discrepancies without exception, with $(\Delta E_{1/2})_{obsd}$ values being more positive than $(\Delta E_{1/2})_{calcd}$.

Table **II.** Chemical-Shift Changes (in Hertz) of ^{1}H NMR Resonances of Nucleotides (0.12 M) on Addition of Macrocycles and/or Mg^{2+} in D_2O at pD 8.0[a]

	chemical shift,[c] δ (ppm)						
additive[b]	H-8	H-2	H-1'	H-2'	H-3'	H-4'	H-5'
			AMP				
none δ(ppm)[c]	8.59	8.19	6.15	4.83	4.58	4.42	4.10
L^1	0	−1	0	−1	0	0	0
L^5	+3	−4	−1.5	−5.5	+1.5	−1.5	0
L^9	+2	−4	0	−5.5	+2.5	0	0
L^{14}	+1.5	+1	+1	0	+0.5	0	−0.5
			ATP				
none δ(ppm)[c]	8.55	8.21	6.16	4.86	4.70	4.46	4.35
L^1	0	−0.5	0	+0.5	+1	0	0
L^5	+0.5	−3	−1	−3.5	+1	−3.5	−3.5
L^9	+1.5	−3	−1	−3	+4	−1.5	−1
L^{14}	+2.5	+1.5	+1	+1	+3	+2	−1
$MgCl_2$	+9	+9	+6.5	+6.5	+9	+6	+2
$L^9 + MgCl_2$	+6	+4.5	+4	+1.5	+6.5	−2	−3.5

[a] Values on 90-MHz spectra at 35 °C. Upfield is positive. Precision is ±0.5 Hz. [b] Each 0.12 M. [c] From external Me_4Si.

The positive shifts of $E_{1/2}$ in the presence of Mg^{2+} and Ca^{2+} are best accounted for by assuming that the Mg^{2+}– and Ca^{2+}–nucleotide complexes interact with the macrocycles (i.e., ternary complex formation). Ternary complex formation was proved by the typical experiment with the L^7–Mg^{2+}–ATP system. A plot of the left hand part of eq 2 (where $[ATP^{4-}]$ is replaced by $[MgATP^{2-}]$ in the presence of a large excess of Mg^{2+}) in logarithm against pH at constant $[MgATP^{2-}]$ was linear with a slope of 3. A plot of the left hand portion of the same equation against $[MgATP^{2-}]$ at constant pH was also linear, passing through the origin. These two results strongly support the 1:1 association between $(H_3L^7)^{3+}$ and the $MgATP^{2-}$. The stability constant for the ternary complex $(H_3L^7)^{3+}$–$MgATP^{2-}$ was calculated as 5.6×10^4 M^{-1} at 25 °C. Moreover, the following NMR measurement also demonstrates the ternary complex formation.

^{1}H NMR Studies. The complex formation could also be shown by ^{1}H NMR spectroscopy (90 MHz) in D_2O solution. An addition of macrocyclic hexaamine L^9 to a 0.12 M solution of ATP and AMP at pD 8.0 caused shifts of nonexchangeable proton resonances of adenine and ribose (Table II). Signal assignments were made with reference to the works by Bunce and Kong[32] and by Davies and Danyluk.[33] Downfield shifts of adenine H-2 and ribose H-2′ resonances and upfield shifts of those of H-8 and H-3′ were obvious with both AMP and ATP. Similar trends were found for the interaction of ATP with L^5, along with lower shifts of H-4′ and H-5′ resonances. On the other hand, almost no change of the ATP or AMP spectrum was observed when small-sized macrocyclic tetraamine L^1 was added. This result is in good agreement with the polarographic analysis that showed no shift of $E_{1/2}$. Thus, the ^{1}H NMR shift measurement can be a good probe for evaluating the present interactions.

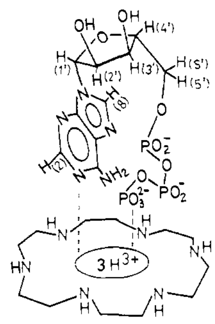
Another noteworthy result was that in the presence of L^9 the resonance shifts of AMP or ATP were much larger than in the presence of linear triamine spermidine L^{14} known to be strongly bound to the nucleotides.[34] This fact supports greater stabilities of the L^9–nucleotide complexes compared with those for the L^{14} complexes (the stability constants are $1.7_7 \times 10^3$ M^{-1} for L^9–AMP and $2.5_0 \times 10^6$ M^{-1} for L^9–ATP as against the reported values[34] of 2.3×10^2 M^{-1} for L^{14}–AMP and 9.0×10^2 M^{-1} for L^{14}–ATP at 25 °C).

The signal movements after the addition of an equimolar amount of L^9 and Mg^{2+} were different from those after the addition of L^9 or Mg^{2+} alone, which is compatible with the preceding postulation of formation of a ternary complex, L^9–Mg–ATP.

---

(32) Bunce, S.; Kong, E. S. W. *Biophys. Chem.* **1978**, *8*, 357-368.
(33) Davies, D. B.; Danyluk, S. S. *Biochemistry* **1974**, *13*, 4417-4434.
(34) Nakai, C.; Glinsmann, W. *Biochemistry* **1977**, *16*, 5636-5640.

Chart II. Proposed Model for the 1:1 Complex of L^9 with ATP



Finally, a more careful inspection of the ATP ^1H NMR spectrum is useful to get information about the L^9–ATP complex structure. We figure a model in which the nucleotides interact with L^9 both through the phosphate and adenine sites by adopting a bent conformation and the base–sugar torsion in a syn form (Chart II). This model explains the marked downfield shifts of the adenine H-2. The relative positioning of the sugar H-2′ and H-3′ above the face of the aromatic ring will account for the downfield and upfield shifts, respectively.

Discussion

We have now established that 15- to 18-membered macrocycles, L^5–L^{10}, and 16-membered dioxopentaamine, L^{11}, form stable 1:1 complexes with inorganic phosphate, AMP, ADP, and ATP at physiological pH. Hence, these macrocyclic polyamines will become useful phosphate sequestering agents. Of particular interest with the present results is that the macrocyclic polyamines may serve as a model to elucidate the mode of phosphate binding to a biological anion carrier, whose chemistry is little explored,[35] although many of the membrane transport proteins have been known.[36-40] Our macrocyclic polyamine L^6–L^{11} has two distinct features as probes to study the phosphate binding. First, they exhibit reversible, well-behaved anodic polarograms in neutral pH buffers, which enable simple identification and characterization of the binary and tetrary complexes. This is especially true with L^7. Second, as typically shown by L^7, the protonation constants are distinctly separated between log K_3 (= 7.28) and log K_4 (= 1.7), which means the resolution of the pH-dependence data is relatively simple. In the study of biological phosphate transport, on the other hand, one has to consider that the receptor sites are gravely affected by the slightest pH change. We will discuss our findings of the mode of the protonated polyamine–phosphate interactions, which may have biological relevances.

Anion Binding Sites. Table I indicates that, as in the dicarboxylate complexes,[1] phosphate complex formation is governed mainly by electrostatic forces. Thus, more negative nucleotides are more strongly attracted to the protonated polyamine macrocycles: the stability sequence is on the order of $ATP^{4-} > ADP^{3-} > AMP^{2-}$ for every L^5–L^9 system. Despite the same PO_4^{2-} charges, AMP forms 10–10^2 times more stable complexes with L^7 and L^9 than inorganic phosphate. The extra stability for the AMP complexes comes from an additional interaction of the adenine base. Interestingly, the active species of inorganic phosphate to be transported in the membrane of rat-liver mitochondria is HPO_4^{2-},[39] the same divalent anion form as determined in our model system.

Inorganic phosphate ions are bound to the polyamines with stability constants [$\beta_L = 1.1 \times 10^2$ (L^7) and $1.3_8 \times 10$ M^{-1} (L^9)] nearly comparable to those found for dicarboxylate anions, such as succinate ($\beta_L = 1.2 \times 10^2$ M^{-1} for L^7 and 1.8×10 M^{-1} for L^9) and malate ($\beta_L = 5.0 \times 10$ M^{-1} for L^7 and 1.5×10 M^{-1} for L^9) where the structurally analogous two singly charged CO_2^- groups are involved.[1] This implies an operation of a similar electrostatic force in phosphate and dicarboxylate systems. It is of interest to note that in biological anion-transport systems, these dicarboxylate anions are often exchanged for phosphate anions.[41,42]

Cation Binding Site. The anion coordination normally requires highly positive charges on the polyamines. This is evident from the finding that among L^1–L^9 polyamines tested, only those capable of incorporating more than three protons at neutral pH (L^5–L^9, see their protonation constants in ref 1) formed the stable complexes under the experimental condition studied. The polarographic method revealed the triprotonated form (H_3L^{3+}) for L^6–L^9 and the tetraprotonated form (H_4L^{4+}) for L^5 as the reactive cation species. Full protonation of L^5 is a result of negligible electrostatic repulsion between four ammonium cations separated by long methylene units. By contrast, the 12- to 15-membered macrocyclic tetraamines L^1–L^4, which exist as diprotonated forms in neutral solutions (log K_1 = 10.7, 11.1, 11.5, and 11.2; log K_2 = 9.7, 10.1, 10.2, and 10.1, respectively; log K_3 and log K_4 are all less than 2),[28] showed little complexing ability. The phosphate binding with our model reminds us of a phosphate-transport protein in red blood cells that is supposed to contain three positive charges (by protonated amines) at the anion-recognition site.[38] A requirement of the high-charged polyamines has been similarly observed in the previous dicarboxylate complexations.[1]

For macrocycles L^{10} (comprising a pyridine ring) and L^{11} (two amide groups), the reactive form is doubly protonated (L^{10}) and singly protonated species (L^{11}) and, accordingly, the electrostatic forces that they execute are weaker than those for the tripositive macrocycles (cf. β_L values in Table I). Despite the smaller positive charges, each characteristic group should provide additional binding sites. It is well recognized that the nucleotides self-associate or interact with certain heteroaromatics, such as 2,2′-bipyridyl, at the adenine moiety site.[43,44] In the dioxo pentaamine L^{11} molecule, the relatively acidic two protons of the amides may be available for hydrogen bondings with phosphate anions. The finding that L^{12} and L^{13} bearing bulky blocking groups show no complexing ability illustrates the importance of steric effects on the anion interaction.

Of the equally (+3) charged polyamines L^6–L^9, L^9 complexes with ADP and ATP are the most stable, and yet its complexes with inorganic phosphate and AMP show comparable stabilities with those for L^6–L^8. The outstanding stability of L^9–ADP and –ATP probably results from the favorable complemental bindings between the largest (18-membered) ring sized hexaamine macrocycle and the flexible nucleotides (see Chart II).

Comparison of stability constants for macrocyclic polyamine L^5 ("cyclic spermine") and for the relevant linear polyamines, spermidine L^{14} and spermine L^{15}, reveals the macrocyclic effect on the anion bindings: the complexes with L^5 are at least one or two orders of magnitude more stable.

Competition of Macrocyclic Polyamines for Phosphate Anions and Metal Cations. Recently,[10] we found that L^9 has an appreciable affinity to Ca^{2+} (but not to Mg^{2+}; log K_{CaL} = 2.5 at 35 °C) in an aqueous solution, a property hitherto unknown with other macrocyclic or linear polyamine systems. This fact, along with the present discovery that these macrocycles bind to the nucleotides with comparable or even much greater affinities than Ca^{2+} and Mg^{2+}, led us to question whether the macrocycles can disrupt the metal–nucleotide interactions as is expressed by eq 20 or 21. The

$$M^{2+}\text{–nucleotide} + H_iL^{i+} \rightleftharpoons M^{2+}\text{–L} + \text{nucleotide} + iH^+ \quad (20)$$

$$M^{2+}\text{–nucleotide} + H_iL^{i+} \rightleftharpoons H_iL^{i+}\text{–nucleotide} + M^{2+} \quad (21)$$

---

(35) A molecular mechanism is proposed for ADP and ATP recognition and subsequent transfer by a carrier in rat liver mitochondria: Schlimme, E.; Book, K.; Groot, E. J. *Biochemistry* **1981**, *19*, 5569–5574.

(36) Ho, M. K.; Guidotti, G. *J. Biol. Chem.* **1975**, *250*, 675–683.

(37) Hadvary, P.; Kadenbach, B. *Eur. J. Biochem.* **1976**, *67*, 573–581.

(38) Rothstein, A.; Cabantchick, Z. I.; Knauf, P. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1976**, *35*, 3–10.

(39) Freitag, H.; Kadenbach, B. *Eur. J. Biochem.* **1978**, *83*, 53–57.

(40) Wehrle, J. P.; Cintron, N. M.; Pedersen, P. L. *J. Biol. Chem.* **1978**, *253*, 8598–8603.

(41) Robinson, B. H.; Williams, G. R. *Biochim. Biophys. Acta* **1970**, *216*, 63–70.

(42) Palmieri, F.; Prexioso, G.; Quagliariello, E.; Klingenberg, M. *Eur. J. Biochem.* **1971**, *22*, 66–74.

(43) Naumann, C. F.; Sigel, H. *J. Am. Chem. Soc.* **1974**, *96*, 2750–2756.

(44) Scheller, K. H.; Hofstetter, F.; Mitchell, P. R.; Prijs, B.; Sigel, H. *J. Am. Chem. Soc.* **1981**, *103*, 247–260, and references therein.

Table III. Selectivity Ratios for the Nucleotide Complexations[a]

ligand	AMP	ADP	ATP
L^5	1	5	700
L^6	1.0	5.6	6.6
L^7	1.0	1.1	3.3
L^8	1.0	1.5	7.5
L^9	1.0	250	1400
L^{10}	1.0	0.66	0.53
L^{11}	1.0	0.24	0.95
L^{14} (spermidine)	1.0	1.4	3.9
L^{15} (spermine)	1.0	3.6	26
Mg^{2+}	1.0	23	180
Ca^{2+}	1.0	10	130

[a] Relative stability constants $\beta_L{}^{AXP}/\beta_L{}^{AMP}$ calculated using Table I.

results with L^6–L^9–Mg^{2+} and –Ca^{2+} systems indicated the formation of the ternary complexes (eq 22). The multiple cation

$$\mathrm{M^{2+}\text{–}nucleotide} + \mathrm{H}_i\mathrm{L}^{i+} \rightleftharpoons \mathrm{M^{2+}\text{–}nucleotide\text{–}H}_i\mathrm{L}^{i+} \quad (22)$$

binding sites on the nucleotides would permit the accommodation of both cations simultaneously. The fact that L^7 (as the triprotonated species) binds to $MgATP^{2-}$ more strongly ($\beta_L = 5.6 \times 10^4\ M^{-1}$) than to ATP^{4-} itself ($\beta_L = 4.2 \times 10^3\ M^{-1}$) is an indication of the Mg^{2+} binding unconflicting with the L^7 cation binding. We propose that the Mg^{2+} ion attaches to the free oxyanion atoms in a 1:1 L^7–ATP complex (cf. Chart II).

The present ternary complexes can find an interesting appliation. It has been postulated that certain enzyme reactions (e.g., myosin ATPase[45] and adenylate kinase[46,47]) involve the metal–ATP–enzyme ternary complexes. Several ternary complex models have been investigated using 1,10-phenanthroline-2-carbinol,[48] 2,2′-bipyridyl,[43,49] or synthetic peptide[50] as substitutents for amino acid residues of the active sites of enzymes. Our systems would add a new model to these enzyme reactions.

Relevance to the Intracellular Cations. It is well known that naturally occurring polyammonium cations, such as spermidine L^{14} and spermine L^{15}, or divalent metal ions, such as Mg^{2+} and Ca^{2+}, are necessary for a number of biophysical and biochemical functions,[19-23] including stabilization of nucleic acid structures, DNA replication, and protein synthesis. It is widely believed that the specific bindings of these cations to anionic sites (e.g., phosphate groups) of the biomacromolecules are the origins of their specific functions. Moreover, most of the intracellular ATP is bound to divalent metal ions, such as Mg^{2+}, and the resulting metal–ATP complexes are presumed to act as real substrates or cofactors in number of enzyme reactions, such as phosphate transfer and phosphate hydrolysis.[19,46] It has also been suggested that in vivo levels of metal–ATP complexes may be regulated by the natural polyamines by their association with the ATP.[34] It will be of interest to see whether our cyclic spermine L^5 and other relevant macrocyclic polyamines perturb the regular biochemical and biological functions of the natural polyamines.[51]

Our macrocycles might display unique biological activities due to the following features that we have revealed in the present study. They also aid in the understanding of the roles of the natural polyamines and metal ions in biological systems. First, the macrocyclic polyamines, especially 18-membered tetra- (L^5) and hexxamines (L^9), have much higher affinities to either AMP, ADP, or ATP than spermidine, spermine, Mg^{2+} or Ca^{2+} (see Table I). Second, some macrocycles exhibit selectivities toward the nucleotides appreciably different from those of the natural polyamines and metal ions (see Table III). For instance, L^9 is highly selective toward ADP and ATP, and L^5 is highly selective toward ATP. Thus, the selectivity ratios $\beta_L{}^{ATP}/\beta_L{}^{AMP}$ and $\beta_L{}^{ADP}/\beta_L{}^{AMP}$, respectively, reach 1400 and 250 with L^9 and 700 and 5 with L^5, in contrast to the values of 26 and 3.6 with spermine and 180 and 23 with Mg^{2+}. On the other hand, L^{10} and L^{11} prefer AMP over ADP or ATP, although its specificity is low. Third, our macrocycles have structural properties different from natural polyamines or metal ions. Mg^{2+} and Ca^{2+} cations possess point charges and interact with the nucleotides at only one site of the phosphate residues.[52,53] On the other hand, the organic polyamines L^{14} and L^{15} have linear structures to bear extended charges along the chains[34] and, hence, interact with nucleotides both at adenine and phosphate moieties.[32] Both types of positive charges are necessary to stabilize the three-dimensional structure of a tRNA molecule.[54] Our macrocycles, which have relatively fixed and concentrated positive charges and yet supply multiple binding sites for the nucleotides, may play a mixed role with the two types of natural cations. Fourth, the interaction of L^6–L^9 with the metal–ATP complexes would influence the various enzyme reactions involving ATP.

Macromonocyclic polyamines presented herein have very simple structures with limited variations in ring sizes and donor atom numbers. Further variation would provide a number of biologically useful ammonium cations which may also cast great potentials to anion coordination chemistry in general.

Registry No. 1, 53118-69-7; **2**, 81316-28-1; L^1, 294-90-6; $L^5 \cdot 4HBr$, 81316-29-2; $L^6 \cdot 3H^+$, 81316-30-5; $L^7 \cdot 3H^+$, 81316-31-6; $L^8 \cdot 3H^+$, 81316-32-7; $L^9 \cdot 3H^+$, 81316-33-8; $L^{10} \cdot 2H^+$, 81316-34-9; $L^{11} \cdot H^+$, 81316-35-0; $L^{14} \cdot 3HCl$, 334-50-9; $L^{15} \cdot 4HCl$, 306-67-2; AMP^{2-}, 6042-43-9; ADP^{3-}, 52322-03-9; ATP^{4-}, 13265-06-0; $HPO_4{}^{2-}$, 29505-79-1; *N,N′*-1,3-propanediylbis(4-methylbenzenesulfonamide), 53364-99-1.

Supplementary Material Available: Table S1, data of the effects of phosphate anions and solution pH on half-wave potentials; Table S2, data for complex formation of L^5 with nucleotides; and Table S3, effects of metal ions on half-wave potentials of macrocyclic polyamine complexes with nucleotides (8 pages). Ordering information is given on any current masthead page.

---

(45) Schneider, P. W.; Brintzinger, H. *Helv. Chim. Acta* **1964**, *47*, 1717.

(46) Mildvan, A. S. *Enzymes, 3rd Ed.* **1970**, *2*, 445–536.

(47) O'Sullivan, W. J.; Noda, L. *J. Biol. Chem.* **1968**, *243*, 1424–1433.

(48) Sigman, D. S.; Wahl, G. M.; Creighton, D. J. *J. Biol. Chem.* **1972**, *11*, 2236.

(49) Buisson, D. H.; Sigel, H. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 1025.

(50) Barany, G.; Merrifield, R. B. *Cold Spring Harbor Symp. Quant. Biol.* **1972**, *37*, 121.

(51) We have already reported the synthesis of a series of "cyclic spermidine" and "cyclic spermine" and their effects on various reactions influenced by natural polyamines in vitro. Kimura, E.; Yatsunami, T. *Chem. Pharm. Bull.* **1980**, *28*, 994–997. Igarashi, K.; Kashiwagi, K.; Kakegawa, T.; Hirose, S.; Yatsunami, T.; Kimura, E. *Biochim. Biophys. Acta* **1980**, *633*, 457–464.

(52) Hammes, G. G.; Miller, D. L. *J. Chem. Phys.* **1967**, *46*, 1533.

(53) Happe, J. A.; Morales, M. *J. Am. Chem. Soc.* **1966**, *88*, 2077–2078.

(54) Quigley, G. J.; Teeter, M. M.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 64–68.