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The Complexation Equilibria of Mercury(II) Ions with Macromonocyclic 16-Membered Dioxopentamine, 18-Membered Dioxohexamine, and Their Related Compounds

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We have investigated the equilibria of complexation reactions of mercury(II) ions with 16-membered macrocyclic dioxopentamine (dioxo[16]aneN$_5$), 18-membered macrocyclic dioxohexamine (dioxo[18]aneN$_6$), and their related compounds by employing a polarographic method and pH-metric titration. Dioxo[16]aneN$_5$, dioxo[18]aneN$_6$, and other dioxo ligands except dioxotetramines were found to form solely 1 : 1 ratio mercury(II) complexes designated as $HgH_{-1}L^+$ with displacement of one amide proton, while the macrocyclic 16-membered monooxopentamine(monooxo[16]aneN$_5$) forms HgL^{2+} in addition to $HgH_{-1}L^+$. The substitution of amine nitrogen of dioxo[16]aneN$_5$ by the sulfur donor unexpectedly gave no increase in the stability of mercury(II) complex of dioxo ligand, $HgH_{-1}L^+$.

The macrocyclic dioxopolyamines possess novel ligand properties of saturated macrocyclic polyamines blended with oligopeptide features. They can accommodate certain metal ions such as Cu^{2+}, Ni^{2+}, and Co^{2+} in the macrocyclic cavities with simultaneous dissociation of the amide protons to afford 1 : 1 ratio complexes generally designated as $MH_{-1}L^+$ or $MH_{-2}L$.[1-3] However in reactions with Zn^{2+}, Cd^{2+}, and Pb^{2+} they undergo no deprotonation, giving a ML^{2+} complex.[4]

The mercury(II) ion is generally harmful to biological systems and an understanding of its action is of great importance. Its toxic action may be ascribable to the formation of protein complexes, which may block the enzymatic activity or change the conformation and the solubility of proteins. For a more detailed understanding of the toxic effect of mercury(II) ion on living systems, it is necessary to know the nature of the Hg(II)-protein interaction. It is not well known whether the mercury(II) ion can bind to the amide nitrogen atom of the peptide group, promoting the dissociation of the amide proton. In this paper we studied systematically the complexation reactions of mercury(II) ions with macrocyclic dioxopentamine and dioxohexamine using polarographic and pH-metric methods.

Though care should be taken in drawing conclusion about the Hg(II)-protein interaction from studies on low-molecular weight systems, the present equilibrium study on the complexation reactions of Hg(II) ions with macrocyclic dioxopolyamines might shed more light on the properties of Hg(II)-peptide interaction.

Experimental

Reagents. The macromonocyclic 18-membered dioxohexamine L^8 was synthesized as an intermediate to a saturated macrocyclic 18-membered hexamine, [18]aneN$_6$, by employing the method proposed by Tabushi et al.[5] All monooxo and dioxo ligands used in this study (Chart 1) were synthesized according to the reported method.[3]

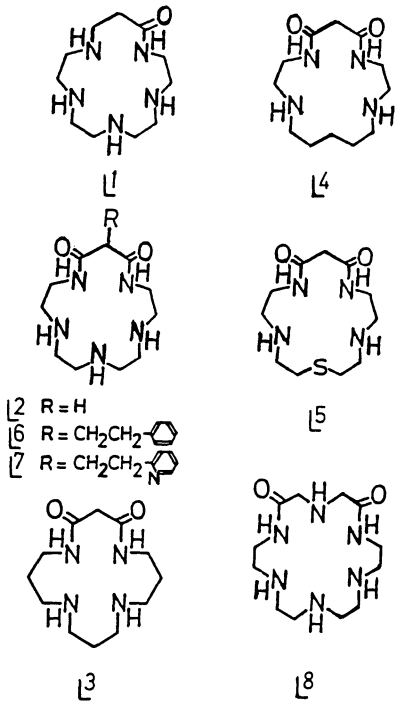

Chart 1.

Polarographic Method. All the polarograms were obtained with a Yanagimoto P-8 pen-recording polarograph or a manual polarograph similar to that of Kolthoff and Lingane.[6] The dropping mercury electrode (DME) had the open-circuit characteristics m=0.928 mg s^{-1} and t_d=4.75 s. in an air-free 0.10 mol·dm^{-3} $NaClO_4$ solution at a column height of 60 cm at 25°C. The polarographic procedures were the same as those applied to the previous mercury(II) complexes of macromonocyclic tetramine[7] and pentamines.[8] In order to maintain the solution pH constant, acetate (5.80>pH>4.00), tris. (8.20>pH>7.00), and borate (10.00>pH>8.20) buffers were used in this study.

Potentiometric Method. Potentiometric (pH-metric) titrations were performed with a Mettler automatic titrator at 25±0.10 °C under a nitrogen or argon atmosphere. The

mixed protonation constants (pK_a's) of the new dioxo ligands, L^3 and L^8, were determined by titrations with 0.100$_0$ mol·dm^{-3} carbonate-free tetraethylammonium hydroxide (TEAOH) or NaOH solution using a sample solution containing 10^{-3} mol·dm^{-3} ligand with an ionic strength made up to 0.20 mol·dm^{-3} using $NaClO_4$. The pK_a values for L^3 were 9.70 and 8.05, and those for L^8 were 8.70, 7.10, ca.2, and ca.1. The cumulative formation constants for the dioxopentamine and dioxohexamine complexes were also determined by titration with 0.100$_0$ mol·dm^{-3} TEAOH or NaOH solution of 10^{-3} mol·dm^{-3} equimolar mixture solution of Hg(II) and L^2·3HCl or L^8·4HCl salt. The change in the Na^+ concentration had no effect on the titration curves and polarograms. The values of $-\log[H^+]$ (for calculation of formation constants) were estimated from pH readings at an ionic strength (I)=0.20; $-\log[H^+]$=pH−0.13.

Results and Discussion

In tris.(0.050 mol·dm^{-3}) and borate (0.030 mol·dm^{-3}) buffer solutions all the macromonocyclic monooxopentamine, dioxopentamines, and dioxohexamine studied gave well defined anodic waves at DME. A typical polarogram obtained for the dioxo[16]aneN$_5$ (L^2) at pH=8.36 is shown in Fig. 1. Their polarographic behavior exept for the pH dependence of the half-wave potential were the same as those of the saturated macromonocyclic tetramines[7] and pentamines.[8] The reversible nature of the electrode processes was also confirmed by the ac polarographic method.[7,9]

The half-wave potential, $(E_{1/2})_L$, for the dioxo[16]aneN$_5$ shifted to more negative values on increasing the solution pH according to the relation (1) (Table 1 and Fig. 2), where $(\alpha_H)_L$ is defined as $1+[H^+]/K_3+[H^+]^2/K_3\cdot K_2+[H^+]^3/K_3\cdot K_2\cdot K_1$.[3]

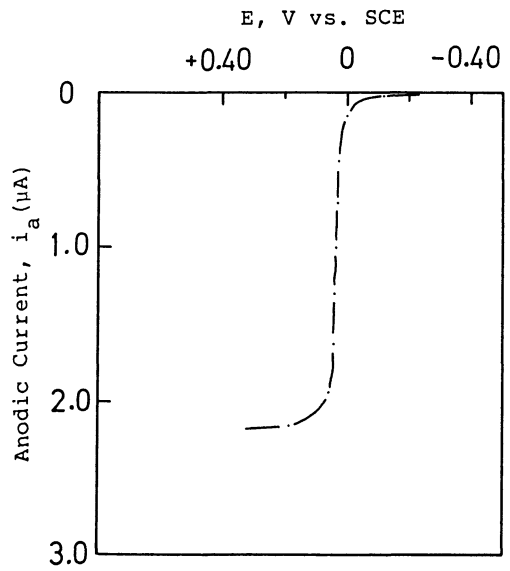

Fig. 1. Anodic dissolution wave at DME due to dioxo[16]aneN$_5$.
[dioxo[16]aneN$_5$]=0.40 mM, borate buffer 0.060 M, pH=8.36, I=0.20 M, 25 °C.

$$\frac{\Delta E_{1/2}}{\Delta\log(\alpha_H)_L\cdot[H^+]} = 30\ \text{mV} \quad (1)$$

The findings evidently indicate that only a 1 : 1 ratio mercury(II)-dioxo[16]aneN$_5$ complex designated as $HgH_{-1}L^+$ is formed in the Eq. 2 with simultaneous dissociation of one amide proton.

$$Hg + H_iL^{i+} \rightleftarrows HgH_{-1}L^+ + (i+1)H^+ \quad (2)$$

The half-wave potential is then expressed as in Eq. 3.

$$(E_{1/2})_L=\varepsilon^0_{Hg}+0.0296[\log f_{Hg}{}^{2+}-\log K_{HgH_{-1}L}+\log(\alpha_H)_L\cdot[H^+]]+0.0296\log(k_L/k_{HgH_{-1}L}) \quad (3)$$

Table 1. Effects of Ligand and Buffer Concentrations and Solution pH on the Half-Wave Potential, $E_{1/2}$.
I=0.20 M, 25 °C

pH	[ligand]$_f$	[buffer]	$E_{1/2}$	$\log(\alpha_H)_L\cdot[H^+]$
	mM	M	V vs. SCE	
7.96[a]	0.40	0.060	+0.0730	−6.18$_6$
8.36	0.40	0.060	+0.0393	−7.22$_7$
8.86	0.40	0.060	+0.0092	−8.32$_3$
9.31	0.40	0.060	−0.0143	−9.07$_8$
9.82	0.40	0.060	−0.0370	−9.74$_1$
10.30	0.40	0.060	−0.0533	−10.27$_0$
8.86	0.40	0.015	+0.0100	
8.86	0.40	0.030	+0.0085	
8.86	0.20	0.060	+0.0096	
8.86	1.20	0.060	+0.0087	

a) Tris. buffer (0.060 M)

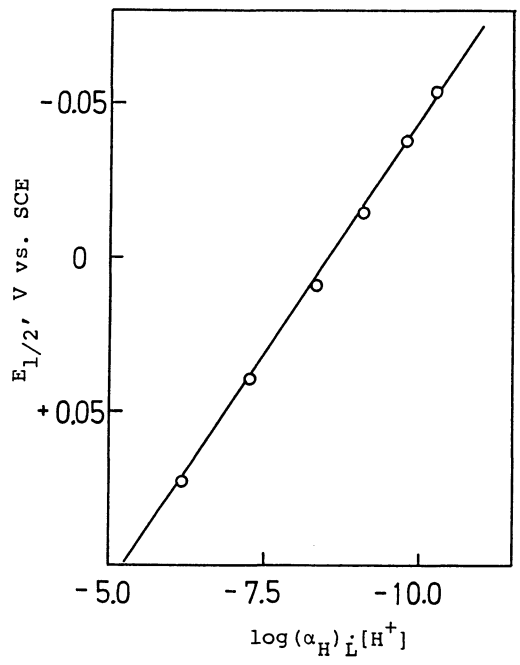

Fig. 2. Plots of half-wave potential, $(E_{1/2})_L$, against $\log(\alpha_H)_L\cdot[H^+]$.
[dioxo[16]aneN$_5$]=0.40 mM, borate buffer 0.060 M, I=0.20 M, 25 °C.

Here, the diffusion current coefficients of the mercury(II)-dioxo[16]aneN$_5$ complex, $k_{HgH_{-1}L'}$ and of the various forms of dioxo[16]aneN$_5$, $k_{L'}$ can be assumed equal for approximation. In Eq. 3, ε^0_{Hg} is the ordinary formal standard potential[10] and a cumulative formation constant, $K_{HgH_{-1}L'}$ is defined as $[HgH_{-1}L^+]\cdot[H^+]/[Hg^{2+}]\cdot[L]$. In a similar way as those applied to the Hg(II)-saturated macrocyclic polyamine complexes,[7,8] the cumulative formation constant can be determined from the $E_{1/2}$ difference from the EDTA (Y^{4-}) system,[11] $\Delta E_{1/2}$, using the Eq. 4. Here, $(\alpha_H)_Y$

$$\log K_{HgH_{-1}L}=\frac{\Delta E_{1/2}}{0.0296}+\log K_{HgY}-\log(\alpha_H)_Y+\log(\alpha_H)_L\cdot[H^+] \quad (4)$$

is the (α_H) value for the EDTA system. With the aid of Eq. 4, $\log K_{HgH_{-1}L}$ value for the Hg(II)-dioxo[16]aneN$_5$ was determined to be 10.14. The reported K_{HgY}(=$[HgY^{2-}]/[Hg^{2+}]\cdot[Y^{4-}]$) and dissociation constants of EDTA (H_4Y^0) at ionic strength I=0.10 mol·dm^{-3} were corrected to I=0.20 mol·dm^{-3} by using the activity coefficients of the ions derived from the Davies relation.[12]

The complexation reactions of dioxo ligands with mercury(II) ions were also studied using the potentiometric method. A typical pH-metric titration curve for the 1.00×10^{-3} mol·dm^{-3} equimolar mixture of mercury(II) ion and dioxo[16]aneN$_5$ ($L^2\cdot3HCl$) is shown in Fig. 3. The complexation reaction occurs in a buffered region of pH 3.0—4.0. The titration data were found to be in best agreement with the concomitant formation of $HgH_{-1}L^+$ (Fig. 4). As was discussed in connection with the reactions of copper(II) ions with macrocyclic dioxotetramine,[13] the following theoretical relation can be expected for the $HgH_{-1}L^+$ formation. β_H in Eq. 5 has the same meaning as those used previously.[3] C_L and α are the

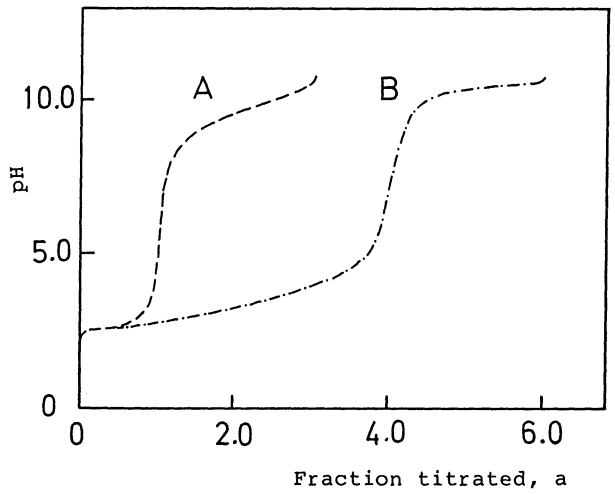

Fig. 3. Titration curves.
I=0.20 M, 25 °C. (A) Dioxo[16]aneN$_5$ 3HCl salt 1.00 mM. (B) Dioxo[16]aneN$_5$ 3HCl salt 1.00 mM+Hg (II) 1.00 mM.

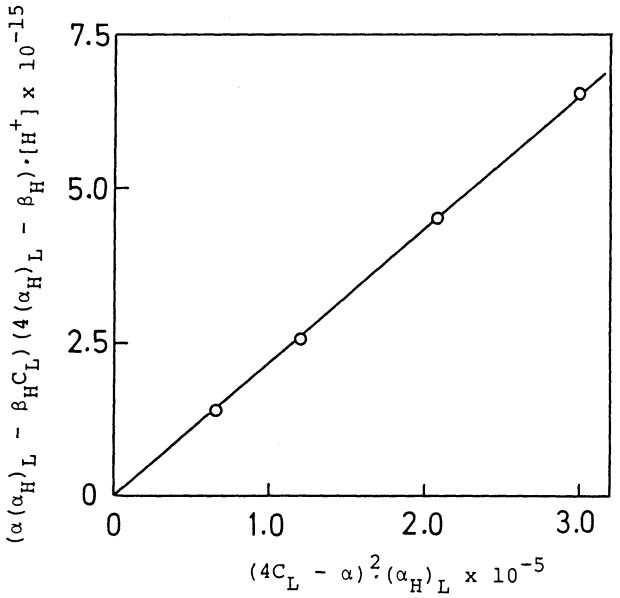

Fig. 4. Plots of $(\alpha(\alpha_H)_L-\beta_H\cdot C_L)\ (4(\alpha_H)_L-\beta_H)\ [H^+]$ against $(4C_L-\alpha)^2\cdot(\alpha_H)_L$.
[dioxo[16]aneN$_5$ 3HCl]=1.00 mM, I=0.20 M, 25 °C.

total concentration of L^2 and $a\cdot C_L+[H^+]$, respectively. Here, a means the fraction titrated. $K_{HgH_{-1}L}$ was determined from the slope of the straight line

$$K_{HgH_{-1}L}(4C_L-\alpha)^2\cdot(\alpha_H)_L=(\alpha\cdot(\alpha_H)_L-\beta_H\cdot C_L)\cdot(4(\alpha_H)_L-\beta_H)\cdot[H^+] \quad (5)$$

in Fig. 4 to be $10^{10.34}$ in a good agreement with that determined polarographically($10^{10.14}$).

Calculations for the simultaneous or separate formation of HgL^{2+} and $HgH_{-2}L^0$ and for the simultaneous formation of HgL^{2+} and $HgH_{-1}L^+$ were also made. However, the equation derived failed to fit the experimental data. Thus we concluded that neither HgL^{2+} nor $HgH_{-2}L^0$ is formed in this buffer region. The pH dependence of $(E_{1/2})_L$ for the anodic wave of dioxo[16]aneN$_5$ and the pH titration curve for the equimolar mixture solution of mercury(II) and dioxo[16]aneN$_5\cdot3HCl$ (shown in Fig. 3) can also be explained in terms of the formation of $HgL(OH)^+$ in which the dioxo[16]aneN$_5$ molecule might coordinate to the Hg(II) ion through its three amino nitrogens. Pure and Schwarzenbach reported that the mercury(II) ion can form a 1 : 1 : 1 ratio mixed diethylenetriamine (dien) complex including hydroxide anion, [Hg(dien)(OH)]$^+$.[14] The equilibrium constant (5.0×10^7) reported for the following reaction shows that the Hg(II)-dien complex can exist in the form of

$$[Hg(dien)(OH)]^+ + H^+ \rightleftarrows [Hg(dien)]^{2+}$$

$[Hg(dien)(OH)]^+$ only at pH's higher than 9. Thus, all the above facts lead us to choose the formation of a $HgH_{-1}L^+$ complex in which the amide group of dioxo[16]aneN$_5$ undergoes deprotonation. All the

polarographic and pH-metric behaviors of L^5, L^6, L^7, and L^8 were the same as those of the dioxo[16]aneN$_5$ system. Hence, an identical treatment of the experimental data was applied.

In the case of monooxo[16]aneN$_5$ (L^1) plots of anti-log $(\Delta E_{1/2}/0.0296+\log K_{HgY}-\log(\alpha_H)_Y+\log(\alpha_H)_L]$ against $[H^+]^{-1}$ gave a straight line with an intercept of finite value (Fig. 5). If only the $HgH_{-1}L^+$ complex is formed, the above plots should give a straight line which passes through the origin. Hence, the above linear relationship evidently indicates that under the present experimental conditions the monooxo-[16]aneN$_5$ ligand can form HgL^{2+} as well as $HgH_{-1}L^+$ as in the Reaction 6.

$$Hg+H_iL^{i+} \rightleftarrows \begin{cases} HgL^{2+}+iH^++2e^- \\ HgH_{-1}L^++(i+1)\cdot H^++2e^- \end{cases} \quad (6)$$

Thus the half-wave potential for the above electrode reaction should be given by Eq. 7. The product $K_{HgL}\cdot K^{-H}$ in Eq. 7 corresponds to $K_{HgH_{-1}L}$. From the

$$(E_{1/2})_L=\varepsilon^0_{Hg}+0.0296[\log f_{Hg^{2+}}-\log K_{HgL}\cdot(1+K^{-H}\cdot[H^+]) +\log(\alpha_H)_L+\log(k_L/k_{HgL})] \quad (7)$$

intercept and the slope of the straight line in Fig. 5,

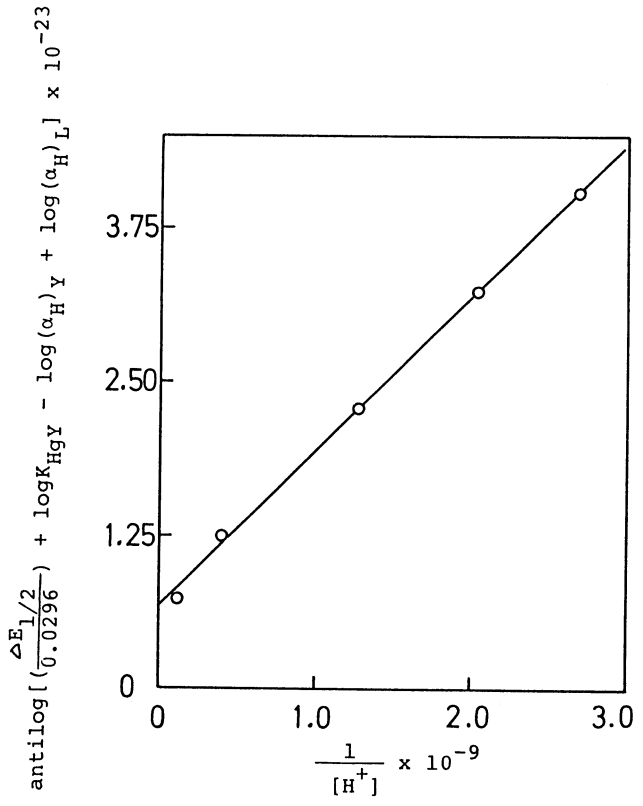

Fig. 5. Plots of antilog $[(\Delta E_{1/2}/0.0296)+\log K_{HgY}-\log(\alpha_H)_Y+\log(\alpha_H)_L)$ against $1/[H^+]$.
[monooxo[16]aneN$_5$]=0.40 mM, Tris. or borate buffer 0.060 M, 9.75>pH>8.00, I=0.20 M, 25 °C.

K_{HgL}(=$[HgL^{2+}]/[Hg^{2+}][L]$) and $K_{HgH_{-1}L}$ values were determined to be 6.7×10^{22} and $1.2_6\times10^{14}$ respectively.

At pH's lower than 7.0 the anodic wave due to the uncomplexed dioxo macrocyclic polyamine merges into the mercury oxidation wave (background) completely. For this reason, it is impossible to study the complexation reaction at pH's lower than 7.0 by employing the polarographic technique. Two 16-membered macrocyclic dioxotetramines, L^3 and L^4, gave no anodic wave, suggesting that these dioxotetramines do not form stable mercury(II) complexes under the present experimental conditions.

As shown by the $K_{HgH_{-1}L}$ values in Table 2, $HgH_{-1}L^+$ complex of L^1 is more stable than that of L^2. This may suggest that in the complexation with Hg(II) ion L^1 can afford one more amine nitrogen atom than L^2. The $K_{HgH_{-1}L}$ values also show that the mercury(II) complex of L^8 is less stable than that of L^2. This probably suggests that the 18-membered macrocyclic ring is too large to place firmly nitrogen donors including deprotonated amide nitrogen atom for the stable Hg(II)-nitrogen bond formation. All the above findings may imply that in the macromonocyclic dioxopolyamine system, the presence of more than three amine nitrogen donors and a sufficiently large macrocyclic cavity which fits the mercury(II) ion size are essential for the formation of stable $HgH_{-1}L^+$ complexes.

The dioxo[16]aneN$_5$ system with extra-planar benzyl and pyridyl substituents (L^6 and L^7) also yielded stable $HgH_{-1}L^+$ complexes (Table 2). The appended pyridyl donor stabilizes the mercury(II) complexes of dioxo ligands. The affinity enhancement found may be accounted for by the additional coordination of the pyridyl nitrogen donor to the mercury(II) ion.

Mercury(II) forms strong complexes with thiol groups of proteins.[15] Serum albumin readily reacts with mercury(II) ions, forming the dimer, HgL_2, in which Hg(II) is coordinated to the single thiol group

Table 2. Cumulative Formation Constants, $K_{HgH_{-1}L}$, of Hg(II)-Macrocyclic Dioxopolyamine Complexes
I=0.20 M, 25 °C

Ligand	$\log K^{a)}_{HgH_{-1}L}$	$\log K^{a)}_{HgL}$
monooxo[16]aneN$_5$(L^1)	14.10±0.15	22.82±0.22
dioxo[16]aneN$_5$(L^2)	10.14±0.10 (10.34±0.15)$^{b)}$	—
dioxo[16]aneN$_4$(3,3,3,3)(L^3)	—	—
dioxo[16]aneN$_4$(3,2,5,2)(L^4)	—	—
dioxo[16]aneN$_4$S(L^5)	8.40±0.10	—
benzyl-dioxo[16]aneN$_5$(L^6)	10.03±0.10	—
pyr-dioxo[16]aneN$_5$(L^7)	11.46±0.10	—
dioxo[18]aneN$_6$(L^8)	9.64±0.10 (9.81±0.14)$^{b)}$	—

a) At least three separate experiments were conducted for each system. b) Values determined potentiometrically.

of each protein molecule.[16] The Hg(II)-coordination of this group may also offer an explanation for its action as an enzyme inhibitor. We also studied the reaction of mercury(II) ion with dioxo[16]aneN$_4$S (L^5). As illustrated by the $K_{HgH_{-1}L}$ values in Table 2, the incorporation of a sulfur donor into the dioxo 16-membered macrocyclic frame, contrary to our expectation, gave no increase in the stability of Hg(II)-dioxo ligand complex. This trend was also true for the dioxo-free system.[17] The fact that the mercury(II) complex of dioxo[16]aneN$_4$S is thermodynamically less stable than that of dioxo[16]aneN$_5$ may be explained in terms of failure of the sulfur donor incorporated within the 16-membered cyclic frame to form a stable coordination bond with mercury(II) ion because of the cyclic nature of the ligand and of the larger sulfur donor size.

A typically soft metal ion, Cu(I), is reported to bind to the amide nitrogen atom of the peptide with the simultaneous dissociation of its proton.[18] Since the mercury(II) ion can form stronger complexes with peptides than Cu(I) ions, it is reasonable to believe that the mercury(II) ion may form such complexes with peptides. The present finding concerning the Hg(II)-macrocyclic dioxo ligand interaction lends strong support to the above argument. Though great care is required in drawing conclusion about the metal-protein interaction from the studies on the low-molecular weight system, the macrocyclic dioxo polyamines offer a useful model for the study of the metal-peptide interaction and thus, this is the first paper reporting the possibility of forming a mercury(II)-deprotonated amide nitrogen bond.

References

1) M. Kodama and E. Kimura, *J. Chem. Soc., Dalton Trans.*, **1981**, 694.
2) R. Machida, E. Kimura, and M. Kodama, *Inorg. Chem.*, **22**, 2055 (1983).
3) E. Kimura, R. Machida, and M. Kodama, *J. Am. Chem. Soc.*, **106**, 5497 (1984).
4) Unpublished results.
5) I. Tabushi, H. Okino, and Y. Kuroda, *Tetrahedron Lett.*, **1976**, 4439; H. Kato, Doctoral Thesis, Department of Pharmaceutical Sciences, Kushu University (1977).
6) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience, New York (1952), Vol. 1, p. 297.
7) M. Kodama and E. Kimura, *J. Chem. Soc., Dalton Trans.*, **1976**, 2335.
8) M. Kodama and E. Kimura, *J. Chem. Soc., Dalton Trans.*, **1978**, 1081.
9) M. Senda, M. Senda, and I. Tachi, *J. Electrochem. Soc., Jpn.*, **27**, 83 (1959).
10) M. Kodama and A. Kimura, *Bull. Chem. Soc. Jpn.*, **40**, 1639 (1967).
11) C. N. Reilley, W. G. Scribner, and C. Temple, *Anal. Chem.*, **28**, 450 (1956).
12) J. N. Butler, "Ionic Equilibrium," Addison-Wesley, Reading, Massachusetts (1964), p. 437.
13) M. Kodama and E. Kimura, *J. Chem. Soc., Dalton Trans.*, **1979**, 325.
14) J. E. Pure and G. Schwarzenbach, *Helv. Chim. Acta*, **53**, 985 (1950).
15) L. G. Sillen and A. E. Martell, "Stability Constants," 2nd ed., Chem. Soc. Spec. Publ. No. 17 (1964); Suppl. No. 1, Chem. Soc. Spec. Publ. No. 25 (1971).
16) W. L. Hughes, Jr., *Cold Spr. Harb. Symposium Quant. Biol.*, **14**, 79 (1950).
17) M. Kodama, T. Koike, N. Hoshiga, R. Machida, and E. Kimura, *J. Chem. Soc., Dalton Trans.*, **1984**, 673.
18) R. Osterberg, *Eur. J. Biochem.*, **13**, 493 (1970).