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Metal Complexes of D-Glucosamine and Its Derivatives. VIII.[1] Metal Complexes of N-Methyl-D-glucosamine and Its Related Amino Sugars

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N-Methyl-D-glucosamine (I), D-glucosamine (II), D-galactosamine (III), D-mannosamine (IV) and D-talosamine (V) were investigated by pH titration method on their complex formations with metal ions. The pK_a of these amino sugars were found to be I 7.86, II 7.38, III 7.61, IV 7 50, and V 7.98 respectively. Stability constants, log K_1 of seven I-metal complexes were in the order; Cu 4.5>Pb 3.7>Zn 3.2>Ni 3.1>Cd 2.9≫Ca, Mg. Hydrolysis study on I-Cu complex revealed that I-Cu complex was more hydrolyzable to form monohydroxo complex I-Cu (OH), but less dimerized than II-Cu complex. Stability constants, log K_1 of the amino sugar-Cu complexes were measured; II-Cu 4.8, III-Cu 4.6, IV-Cu 5.0 and V-Cu 5.7 respectively. Discussions are given on the complex formation of amino sugar with Cu^{2+} with conformational considerations.

Keywords——metal complex; N-methyl-D-glucosamine; amino sugar; pH titration; pK_a; stability constant; olation; conformation

An amino sugar, N-methyl-D-glucosamine(2-N-methylamino-2-deoxy-D-glucopyranose, I) has attracted much attention on its chemical and physiological properties in relation to those of N-methyl-L-glucosamine which is a partial constituent of an antibiotic, streptomycin.

The complex forming ability of I with metal ions has been suggested by a spectrophotometric investigation[3], but no further detailed study has been reported on metal complexes of I. In this paper, measurements of pKa for I and stability constants of metal complexes of I by pH titration method are described, and results are compared with those of D-glucosamine(II), D-galactosamine(III), D-mannosamine(IV) and D-talosamine(V).

Experimental

Materials and Solutions——N-Methyl-D-glucosamine (2-N-methylamino-2-deoxy-D-glucopyranose, I) and D-glucosamine(2-amino-2-deoxy-D-glucopyranose, II) were the same preparations reported previously.[4] D-Galactosamine(2-amino-2-deoxy-D-galactopyranose, III) was supplied by Dr. Takanashi, and D-mannosamine(2-amino-2-deoxy-D-mannopyranose, IV) and D-talosamine(2-amino-2-deoxy-D-talopyranose, V) were purchased from Nakarai Chemicals, Ltd. All of the amino sugars were in hydrochloride form. Metal salts, $Cu(NO_3)_2$, $Ni(NO_3)_2$, $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Pb(NO_3)_2$, $Mn(NO_3)_2$, $Mg(NO_3)_2$ and $Ca(NO_3)_2$ were used to prepare 10^{-2} M metal ion solutions and the solutions were standardized by ethylenediaminetetraacetic acid (EDTA) titration with metal indicators, respectively. 0.1 M HNO_3 to adjust pH of a sample solution and 0.1 M NaOH as a titrant were prepared, and 1 M $NaNO_3$ was used to maintain constant ionic strength of the sample solution. All reagents used were of analytical grade.

Apparatus——A Metrohm Herisau Multi-Burette E 485 and a Denki Kagaku DKK-1 digital pH meter Model HG-3 were used for titration studies. The pH meter was equipped with a compound-type of a calomel and a glass electrode, Type 6063 and calibrated in advance with standard buffers of pH 4.01 and 6.86 at 25°. A water-jacketed titration vessel was maintained at $30° \pm 0.1°$, and the sample solution in the vessel was stirred with a mechanical stirrer under constant bubbling of nitrogen.

Procedure of pH Titration——Thirty ml of 10^{-3} M solution of I with or without a metal ion was titrated with 0.1 M carbonate-free NaOH by means of the microburette in nitrogen atmosphere, and the ionic strength of the solution was maintained relatively at constant by using a medium of 0.1 M $NaNO_3$.

1) Part VII: M. Miyazaki, T. Senshu, and T. Tamura, *Chem. Pharm. Bull.* (Tokyo), **14**, 114 (1966).
2) Location: *Takara-machi, Kanazawa.*
3) M. Ishidate, T. Sakaguchi, K. Taguchi, and S. Kanao, *Anal. Chim. Acta*, **22**, 452 (1960).
4) Z. Tamura, and M. Miyazaki, *Japan Analyst*, **12**, 470 (1963).

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Treatment of Data——Treatment of the data obtained were the same one as reported previously,[5] but computations for pK_a and log K_1 were carried out with a FACOM 230-35 computor at Data Processing Center, Kanazawa University. A programming was carried out in the author's laboratory according to Fortran, using the equations given by Schwarzenbach[6] for pK_a and by Bjerrum[7] for log K_1. During the computations of the constants, 0.78 and 1.89×10^{-14} were used for activity coefficient of H^+ and ionic product of H_2O at 30°, respectively.

Results and Discussion

pK_a of I

An electron donating group, CH_3 at nitrogen atom of I would increase electron density on the nitrogen atom. As expected, I showed to be more basic than II to give a value 7.86 which was about 0.5 pK_a unit higher than that of II 7.38.

Stability Constants of Metal Complexes of I

The titrations of I in the presence of seven metal ions respectively were carried out. The results are shown in Fig. 1. As is seen in Fig. 1, it is found that complex formation of I with Cu^{2+} was the strongest among the complex formations examined, and the magnitude of stability of I-metal complexes was in the order; Cu>Pb>Zn>Ni>Cd≫Ca, Mg. Using the titration data, stability constants log K_1 were calculated and the results are given in Table I. However, log K_1 for I-Ca and I-Mg could not be obtained. Comparison of the stability constants of I-metal complexes with those of II-metal complexes indicates that even the strong I-Cu complex (log K_1 4.5) is slightly less stable than II-Cu complex (log K_1 4.8). Stability constants of the other I-metal complexes were almost the same to those of the corresponding II-metal complexes. As pK_a of I is greater than that of II, more elevated complexing ability with metal ions is expected for I. However, this is not the case for I and the decrease in log K_1 for I-Cu complex might be interpreted as a result of steric effect of N-methyl group at C_2 position of I molecule.

Hydrolysis and Dimerization of I-Cu Complex

As is described in previous paper,[5] hydrolysis and dimerization reaction were remarkable in II-Cu complex. Therefore, these reactions were also examined on I-Cu complex. As can

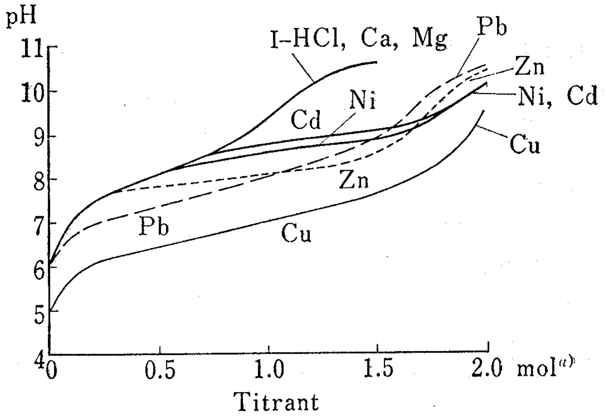

Fig. 1. Titration Curves of N-Methyl-D-glucosamine (I) in the Presence of Respective Metal Ions

Molar ratio of I to metal ion=2:1.
I concentration: 2.00×10^{-3} M.
Metal ion: 1.00×10^{-3} M.
Temperature: 30°±0.1°, μ=0.1 ($NaNO_3$).
Titrant: 0.1M NaOH.
a)=mol of base added per mol of I.

Table I. Stability Constants of Five Metal Complexes of N-Methyl-D-glucosamine (I)

Metal ion	log K_1
Cu^{2+}	4.5
Pb^{2+}	3.7
Zn^{2+}	3.2
Ni^{2+}	3.1
Cd^{2+}	2.9

Molar ratio of I to metal ion=2:1.
I concentration: 2.00×10^{-3} M.
Metal ion: 1.00×10^{-3} M.
Temperature: 30°±0.1°, μ=0.1 ($NaNO_3$).

5) Z. Tamura and M. Miyazaki, *Chem. Pharm. Bull.* (Tokyo), 13, 330, 333, 387 (1965).
6) G. Schwarzenbach, *Helv. Chim. Acta*, 33, 9479 (1950).
7) J. Bjerrum, "Metal Ammine Formation in Aqueous Solutions," Hassel and Son, Copenhagen, 1941.

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be seen in Fig. 2, I-Cu complex as well as II-Cu complex shows a linear relationship between [I-Cu]/[H^+] and $T_{OH}+[H^+]-[OH^-]$/[I-Cu]/[H^+]. This result supports that olation reaction will occur to form a dimer complex according to the equation, 2 I-Cu(OH)$\rightleftharpoons$I-Cu$\langle{}^{OH}_{OH}\rangle$Cu-I. By comparing equilibrium constants for hydrolysis of I-Cu complex ($pK_{I-Cu(OH)}$ 6.1, $pK_{I-Cu(OH)_2}$ 10.2) and dimerization (log K_{DI} 2.0) with those of II-Cu complex[5] ($pK_{II-Cu(OH)}$ 7.3, $pK_{II-Cu(OH)_2}$ 10.3) and dimerization (log K_{DII} 4.2), it is noticed that I-Cu complex is more hydrolyzable to form a monohydroxo complex (about 10 times), but be less dimerized (about 1/100 times) than II-Cu complex, while dihydroxylation of the complex seems to occur similarly in both I-Cu and II-Cu complexes.

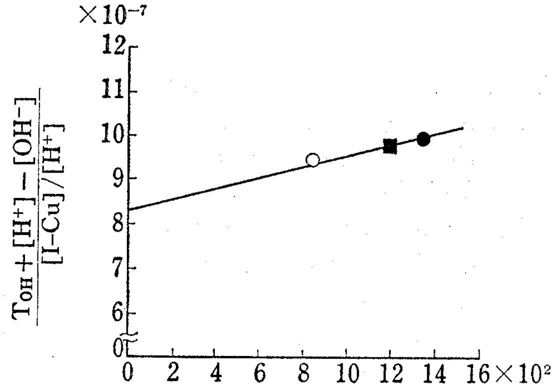

Fig. 2. Relation between $\frac{T_{OH}+[H^+]-[OH^-]}{[I-Cu]/[H^+]}$ and [I-Cu]/[H^+] in 1:1 N-Methyl-D-glucosamine (I)-Copper Complex Systems

Concentrations: ○; 1.99×10^{-3} M, ■; 2.40×10^{-3} M, ●; 3.00×10^{-3} M.

Each plot was the mean value obtained from 3—5 determinations.

TABLE II. pK_a and Stability Constants of Copper Complexes of the Amino Sugars I, II, III, IV and V

Amino sugar	pK_a	Stability constant log K_1
I	7.86	4.5
II	7.38	4.8
III	7.61	4.6
IV	7.50	5.0
V	7.98	5.7

Amino sugar to cupric ion=2:1.
Amino sugar concentration: 1.00×10^{-3} M.
Temperature: 30°±0.1°, μ=0.1 ($NaNO_3$).

pK_a and Stability Constants of Cu Complexes of II, III, IV and V

By using the titration data, pK_a and stability constants of copper complexes of II, III, IV and V were calculated. The results are given in Table II.

Relation between pK_a and Conformation of Amino Sugars

As for pK_a, the examined amino sugars which have an amino group at equatorial position of C_2 in pyranose ring show smaller pK_a than those ones containing an axial amino group at C_2, supposed that the amino sugars hold favorably Cl conformation[8] in solution (Chart 1a). V showed the highest pK_a among the amino sugars investigated. It might be interpreted that a possible hydrogen bonding between C_4-OH and C_2-NH_2 groups has taken place in V to suppress release of H^+ from the protonated amino group. This assumption was obtained from the study with a stereo model. Methylation of amino group at C_2 of II yields an increase in pK_a, but methylation of OH group of II shows rather decrease in pK_a as was reported in the previous paper.[5]

Relation between log K_1 of Cu Complexes and Conformation of Amino Sugars

V-Cu complex has the highest log K_1 5.7 among Cu complexes of the amino sugars examined. In complex formation of the amino sugars with Cu^{2+}, it is assumed that 1,3 diaxial

8) E. Percival, "Comprehensive Biochemistry," Vol. 5, ed. by M. Florkin, and E.H. Stotz, Elsevier Publishing Co., Amsterdam, 1963.

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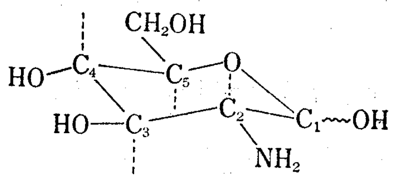
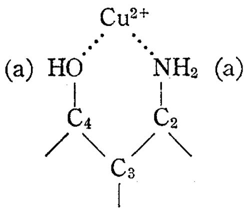


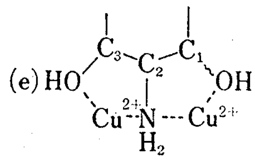
	C_2–NH_2	C_3–OH	C_4–OH
I	e	e	e
II	e	e	e
III	e	e	a
IV	a	e	e
V	a	e	a

a: axial e: equatorial

1a. C 1 conformation of D-amino sugar



1b. 1,3-diaxial effect between C_2–NH_2 and C_4–OH



1c. complex formation between C_2–NH_2 and C_3–OH or C_1–OH

Chart 1. Possible Structural Relations in Amino Sugar-Copper Complex Formation

effect is likely anticipated between C_4-OH and C_2-NH_2 groups as is seen in Chart 1b. As is shown in Chart 1b, a possible six-membered ring without any possible distortion will be formed in V-Cu complex. IV has a similar conformation of C_2-NH_2 group and the residual moiety of the molecule to V except C_4-OH group which has an equatorial conformation. From the results, the equatorial OH group at C_4 may decrease the stability of the complex and thus a depression of about 0.7 log K unit has yielded in IV-Cu complex in comparison with V-Cu complex. Meanwhile, III has an equatorial amino group at C_2 in pyranose ring as well as II, and log K_1 of III-Cu complex is smaller than those of the two amino sugars described above. In both II and III, there are one OH group at C_1 and an equatorial OH at C_3 positions in the molecule (Chart 1c) and it seems that there is an alternative opportunity; which OH group will react with a metal ion and the C_2-NH_2 group of the molecule to form a complex ring. In comparison of log K_1 of III-Cu complex with that of II-Cu complex, it appears that the axial OH group at C_4 of III affects on the complex formation and decrease slightly the stability of III-Cu complex.

Comparison of Cu Complexes of I, Methyl-β-D-glucosaminide (VI) and 3,4,6-Tri-O-methyl-D-glucosamine (VII)

log K_1 of Cu complexes of VI and VII which were reported previously[5] were compared with log K_1 of I-Cu complex in order to see steric effect of methylation in the ligand molecule. Considering from the study using a model, both equatorial C_1-OH and C_3-OH groups appear to be equally apart from the equatorial C_2-NH_2 group in the ligand molecule (Chart 1c). Therefore, the two OH groups will have the same probability to form five-membered ring with Cu^{2+}, respectively. When these OH groups are methylated respectively, the probability of the complex formation will decrease owing to the steric effect caused by the bulky methoxyl group. When I is complexed with Cu^{2+}, decrease in log K_1 of the complex is found and it is considered as a result of the steric effect arisen from I, namely the influence of bulkiness of N-methyl group at C_2 of the ligand. The effect on complex formation seems almost equal in both N-methylation and alternative O-methylation at C_1 or C_3 of the ligand. Considering from the magnitude of log K_1 of Cu complexes of I, VI and VII (4.5, 4.5 and 4.5 respectively), O-methylation at C_4-OH and/or C_6-OH of II appears to have little influence on complex formation.

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