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Studies on the Mixed-Ligand Complexes. III.[1,2] Mixed-Ligand Complexes between Alloxan and Glycine with Bivalent Metals

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The syntheses of the mixed-ligand complexes between alloxan and glycine with bivalent metals (Cu, Ni, Co, Zn, Pb, and Cd) were made by mixing them in the ratios of 1:1:1 at pH 3 and by allowing to stand at a room temperature for about a week. The objective products deposited gradually. The separation from their parent complexes, *i.e.*, bis-glycinato metal complexes, were made by the differences of solubility in H_2O. By decomposing the mixed-ligand complexes in 1N HCl, the constituents were identified by thin-layer chromatography with employing microcrystalline cellulose powder as an adsorbent and BuOH:AcOH:H_2O (4:1:2) as a solvent mixture. The discussions as to the binding sites with the ligands were made from infrared (IR) spectra and the structures were postulated of the alloxan-metal-glycine and alloxan-Cu-glycylglycine complexes.

Since the discovery of producing a metabolic state resembling to diabetes by injection of alloxan in animals was reported by Dunn,[4] many investigations have been made to examine the role of alloxan.[5-10] Siligson,[11] studying the metabolic products of alloxan in plasma, pointed out that, since nearly 90% of alloxan administered transformed to alloxanate, it should play a main role in an increase of blood sugar, instead of alloxan. However, Foye, *et al.*[12] reported the isolation of alloxan-metal chelates whose combining ratios were 1:1 for Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+}. The administration of the Cu and Ni chelates produced blood sugar increase for much longer period of time than in the case of alloxan alone. As these alloxan-metal chelates have a possibility to form mixed-ligand complexes with other ligand, glycine, one of the most simple amino acid, was employed as a second ligand to synthesize the mixed-ligand complexes of alloxan with bivalent metals. Also it was attempted to investigate the stabilization of alloxan and to get some clues for the interaction of alloxan-metal complexes with some enzymes or proteins in the living cells.

Result and Discussion

Alloxan is not stable in neutral and alkaline solution and the syntheses of the mixed-ligand complexes with bivalent metals (Cu, Ni, Co, Zn, Pb, and Cd) were attempted at pH 3 in the solution of alloxan, glycine, and metal chlorides in the ratio of 1:1:1. The resultant solutions were allowed to stand at a room temperature for about a week, then the precipitates

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[2] A part of this paper was presented at the 24th Annual Meeting of Japan Chemical Society, April 1971.

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were deposited gradually. They were taken by filtration and dried over silica gel in a desiccator. As shown in Table I elemental analyses showed the inclusion of more than one water molecules, it is difficult to distinguish them from alloxanate-metal-glycine complexes by elemental analyses. In order to determine whether the obtained complexes are alloxan-metal-glycine or alloxanate-metal-glycine, water molecules were driven off by heating at 130° *in vacuo* for 5 hours. Elemental analyses of the anhydrous samples coincided well with the theoretical values as shown in Table I.

TABLE I. Elemental Analyses of Alloxan-Metal-Glycine Complexes

Compounds	Formulas	Analyses (%)							
		Calcd.				Found			
		C	H	N	Metal	C	H	N	Metal
Alloxan-Cu-glycine	$(C_4HO_4N_2)Cu(C_2H_4O_2N)\ 3/2\ H_2O$	23.59	2.61	13.78	20.8	23.64	2.73	13.74	20.5
	$(C_4HO_4N_2)Cu(C_2H_4O_2N)$	25.84	1.79	15.07	22.8	26.39	1.84	15.47	22.5
Alloxan-Ni-glycine	$(C_4HO_4N_2)Ni(C_2H_4O_2N)\ 2H_2O$	23.24	2.91	13.55	18.9	22.91	3.06	13.78	18.3
	$(C_4HO_4N_2)Ni(C_2H_4O_2N)$	26.29	1.83	15.34	21.4	26.18	1.91	15.69	20.7
Alloxan-Co-glycine	$(C_4HO_4N_2)Co(C_2H_4O_2N)\ H_2O$	24.74	2.06	14.43	20.2	25.05	2.45	15.03	20.0
	$(C_4HO_4N_2)Co(C_2H_4O_2N)$	26.27	1.82	15.32	21.5	25.82	1.82	15.27	21.3
Alloxan-Zn-glycine	$(C_4HO_4N_2)Zn(C_2H_4O_2N)\ H_2O$	24.96	2.43	14.56	22.7	25.15	2.23	14.76	22.4
	$(C_4HO_4N_2)Zn(C_2H_4O_2N)$	25.79	2.16	15.05	24.2	25.80	2.06	15.25	23.9
Alloxan-Cd-glycine	$(C_4HO_4N_2)Cd(C_2H_4O_2N)\ 2H_2O$	19.80	2.48	11.55	—	20.03	2.40	11.75	—
	$(C_4HO_4N_2)Cd(C_2H_4O_2N)$	21.98	1.58	12.81	—	22.37	2.33	13.20	—
Alloxan-Pb-glycine	$(C_4HO_4N_2)Pb(C_2H_4O_2N)\ 2H_2O$	15.71	1.96	9.16	—	15.83	1.80	9.30	—
	$(C_4HO_4N_2)Pb(C_2H_4O_2N)$	17.05	1.18	9.94	—	17.41	1.14	10.26	—
Alloxan-Cu-glycylglycine	$(C_4HO_4N_2)Cu(C_4H_7O_3N_2)\ 2H_2O$	25.77	3.49	15.07	17.1	25.37	3.47	15.77	16.8

a) Anhydrous samples were prepared by being dried at 130° *in vacuo*. Hydrated samples were prepared by being dried over silica gel in a desiccator.

Some typical thermogravimetric analyses of the hydrated samples are shown in Fig. 1. In the case of copper complex, weight decreases due to dehydration were observed at about 105° and 210°, which corresponded to the liberation of one half of water molecule and the succesive liberation of one water molecule. They were supported by the results of elemental analyses. For nickel and zinc complexes dehydration took place at about 170—175° for coordinated water. For the complexes of cobalt, cadmium and lead, the coordinated water liberated at about 120°, 175°, and 200°, respectively. The chelate formation of alloxan with metals was studied by Patterson and Foye, but the former presumed that alloxan could not form metal complexes and only alloxanate formed complex with cupric ion, whose existence was confirmed by ultraviolet spectrophotometric method. The authors tried to synthesize alloxan-metal complexes according to Foye's method, however, the objective complexes were not isolated and this fact is very interesting when the easiness of synthesizing alloxan-metal-glycine complexes is compared with the difficulty of alloxan-metal chelate formation. In addition to these experiments, thin–layer chromatography was carried out in order to identify the components in these mixed-ligand complexes, which were decomposed in 1 N HCl solution and then developed with a BuOH: AcOH: H_2O (4:1:2) solvent mixture on cellulose as an adsorbent. These results are shown in Table II. In the detection of alloxan, glycine solution was sprayed over a plate and on heating alloxan reacting with glycine showed coloration in red, whereas alloxanate did not show any color change. The mixed-ligand complexes were decomposed in to three spots, each of which corresponded to that of metal, glycine, and alloxan, respectively. These results indicated that the mixed-ligand complexes were obtained undoubtedly. Additional evidence of the alloxan inclusion in the complexes arosed from the

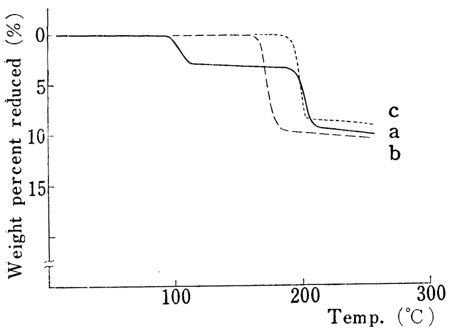

Fig. 1. Thermogravimetric Diagram showing Liberation of Water Molecules

a: alloxan-Cu-glycine, b: alloxan-Ni-glycine, c: alloxan-Pb-glycine

Table II. *Rf* Values of Thin-Layer Chromatography of Alloxan-Metal-Glycine Complexes Decomposed in 1N HCl Solution

Compounds	*Rf* values detected by		
	Dithizone	Ninhydrin	Glycine
Alloxan-Cu-glycine	0.19	0.24	0.39
Alloxan-Ni-glycine	0.16	0.25	0.38
Alloxan-Co-glycine	0.17	0.23	0.41
Alloxan-Zn-glycine	0.31	0.22	0.41
Alloxan-Cd-glycine	0.21	0.24	0.41
Alloxan-Pb-glycine	0.16	0.23	0.40
Alloxan-Cu-glycylglycine	0.20	0.24	0.41
Alloxan	—	—	0.39
Glycine	—	0.23	—

fact that alloxanate-metal-glycine complexes could not be obtained in the similar conditions of synthesis of alloxan-metal-glycine complexes.

In order to speculate the binding sites of the ligands, infrared (IR) absorption spectra were measured in KBr disc method. Because the alloxan-metal chelates were not isolated, the IR spectra of the mixed-ligand complexes were compared with those of alloxan and bisglycinato metal complexes.[13–16] In Fig. 2, the spectra of alloxan-Cu-glycine, alloxan, and bis-glycinato copper are shown. Other IR spectral data are tabulated in Table III.

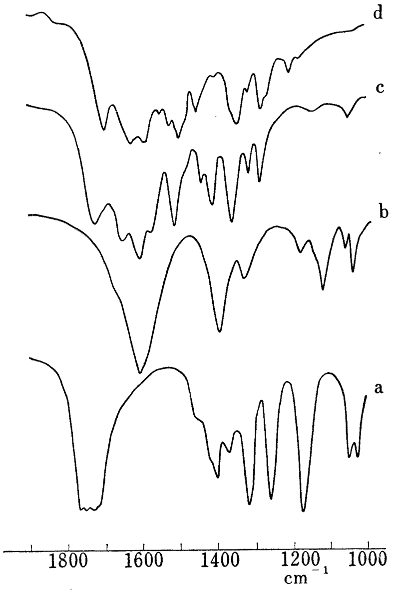

Fig. 2. Infrared Spectra (KBr disc)

a: alloxan, b: $(glycine)_2$-Cu, c: alloxan-Cu-glycine, d: deutero analogue of alloxan-Cu-glycine

In 3000 cm^{-1} region, the band around 3300—3100 cm^{-1} due to the stretching of the amino group in glycinato copper complex[17] disappeared in alloxan-Cu-glycine, in which a broad band was observed around 2950—2780 cm^{-1}, while alloxan a showed broad band around 3260—3000 cm^{-1}, being due to the stretching of the imino and hydroxyl groups. This band in the mixed-ligand complex was due to the stretching of the amino (in glycine) and hydroxyl or imino groups (in alloxan), but the difinite assignments are difficult.

In the frequency region from 1800 cm^{-1} to 1300 cm^{-1}, bis-glycinato copper gave bands at 1608 cm^{-1}, 1593 cm^{-1}, and 1392 cm^{-1} due to the deformation of the amino group, asymmetric and symmetric stretching of the carboxyl group, respectively.[18] While alloxan gave bands at 1775 cm^{-1}, 1755 cm^{-1}, and 1730 cm^{-1} due to the stretching of the carbonyl groups.

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Table III. Main Infrared Frequencies of Alloxan-Metal-Glycine, Alloxan-Cu-Glycylglycine, and Alloxan (cm^{-1})

Alloxan-Cu-glycylglycine	Alloxan-Cu-glycine	Alloxan-Ni-glycine	Alloxan-Co-glycine	Alloxan-Zn-glycine	Alloxan-Pb-glycine	Alloxan-Cd-glycine	Alloxan	Assignment
1705 s	1710 s	1713 s	1720 s	1720 s			1775 s	ν_{CO}
1652 s		1690 s		1690 s	1690 s	1695 s	1755 s	
1620 s	1640 s	1660 s	1640 s	1645 s	1640 s	1640 s	1730 s	
1600 s	1605 s	1597 s	1590 s	1580 s	1583 s	1595 s		ν_{COO^-} (assym.)
1575 s	1570 s	1575 s						
1483 s	1497 s	1485m	1496 s	1500m	1480 s	1485 sh		
	1405m	1405m	1410m	1417m	1395m	1420m		
1400 s	1350 s	1363 s	1345 s	1360 s	1355 s	1380 s		ν_{COO^-} (sym.)
1135w	1140w	1135w	1140w	1140w	1128w	1130w		$\rho_t NH_2$ (?)
1073w	1050w	1055w	1050w	1055w	1060w	1060w		$\rho_w NH_2$ (?)
1023w								

In the mixed-ligand complex of copper, the low shifted band at 1710 cm^{-1}, due to the stretching of the carbonyl groups in alloxan, was found by the bond formation between alloxan and copper ion. And also the band found at 1640 cm^{-1} was assigned as the stretching of the carbonyl group attached directly to copper ion. These low frequency shifts of the carbonyl groups of alloxan were also observed for other mixed-ligand complexes. For example, Ni complex gave bands at 1720 cm^{-1}, 1690 cm^{-1}, and 1645 cm^{-1}, all of which were assigned as the stretching on the carbonyl groups. For copper complex a broad strong band around 1600 cm^{-1} were assigned as the asymmetric stretching of the carboxyl group.

Characteristic bands of the mixed-ligand complexes were found at the frequency region of 1500 cm^{-1} to 1480 cm^{-1}, which were not observed in the bis-glycinato metal complexes and alloxan. These bands were close to the deformation of the ammonium group in glycine, however, deuteration of alloxan-Cu-glycine complex also gave a band at 1490 cm^{-1}, indicating that these bands were not due to the deformation of the ammonium group. As for the symmetric stretching of the carbonyl group, in the copper complex the band at 1350 cm^{-1} was assigned, because the band at 1405 cm^{-1} was disappeared by deuteration, and for other complexes the bands at the region of 1380—1345 cm^{-1} were assigned. These low frequency shifts in the mixed-ligand complexes may arise by the displacement of one glycine molecule in glycinato complexes by alloxan. With these IR spectral data glycine can be regarded as forming the coordinated bond between the carbonyl group and amino group with metal ions in the mixed-ligand complexes. The low frequency shifts of the carbonyl groups in the mixed-ligand complexes indicates that the oxygen atom may be a coordination site, however, the band assignments have not been made for alloxan. In contrast to the difficulty of complex formation between alloxan and metal ions, the facile isolation of the mixed-ligand complexes may support the possibility of the mixed-ligand complex formation between alloxan-metal and enzymes.

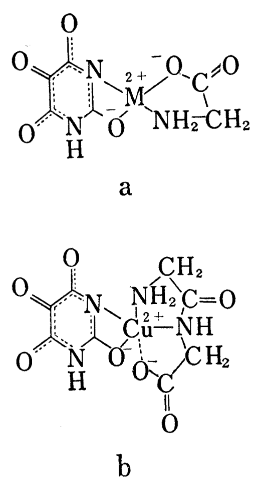

Fig. 3. Proposed Structures for the Mixed-Ligand Complexes

a: alloxan-metal-glycine complexes
b: alloxan-Cu-glycylglycine

The syntheses of the mixed-ligand complexes of Mg^{2+} and Mn^{2+} were also attempted, but the reaction between glycine and alloxan had a priority over that of the mixed-ligand complexes. In adidtion to these alloxan-metal-glycine complexes, the syntheses of the mixed-ligand complexes with glycylglycine instead of glycine were attempted for Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+}, among which only alloxan-Cu-glycylglycine complex was isolated and for other metals the reaction between glycylglycine and alloxan had the priority over their mixed-ligand complex formation. IR spectra and the results of thin-layer chromatography were shown in Table II and III and the components were determined to be alloxan, copper, and glycylglycine. In glycylglycinato copper, the carboxyl group, the terminal amino group and the peptide nitrogen atom are considered to be involved in coordination of copper ion in aqueous solution of higher pH values than 5.5, however, at lower pH values four bands in IR spectrum have been reported to appear in the carbonyl region of 1720—1600 cm^{-1}, where the first complex formed exists as CuL^+ (L=glycylglycine) coordinated through the terminal amino group, the carboxyl group, and the peptide nitrogen atom with copper ion with only displacement of a proton from the terminal amino group.[19,20] With these reported facts a structure of alloxan-Cu-glycylglycine was postulated as shown in Fig. 3, where the carboxyl group is considered to coordinate loosely with copper ion, satisfying the coordination requirements of the metal.

In glycine and glycylglycine systems, the mixed-ligand complexes of Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} can be regarded to have the similar solubilities, so the following discussions may have meanings. When the formation of the mixed-ligand complexes is speculated from a view point of stability constants, the metal ions which form the mixed-ligand complexes have the higher stability constants for glycine compared with those of the metal ions which were not isolated. The stability constants ($\log \beta_1$) of bisglycinato metal complexes[21a)] are 8.62, 6.18, 5.02, 5.52, 5.53, and 4.80 for Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+}, respectively. While those of Mg^{2+} and Mn^{2+} are 3.44 at the ionic strength of 0. Therefore, if we assume that the tendency of the complex formation between alloxan and glycine is around 5, glycine molecule would react with the metal ions prior to the reaction with alloxan. This consideration was also applied to the mixed-ligand complexes between glycylglycine and alloxan with metal ions. The stability constants ($\log \beta_1$) of glycylglycine with Cu^{2+} is 6.04, whereas those of Ni^{2+}, Co^{2+}, and Zn^{2+} are 4.49, 3.49, and 3.80, respectively.[21b)] Considering these stability constants of the both systems, the metals having greater stability constants than 4.5 seem to form the mixed-ligand complexes.

Experimental

Materials——All the reagents were of analytical grade, Wako Chemicals Co. Ltd., and used without further purification. Thin-layer chromatography was carried out with microcrystalline cellulose.

Apparatus——Infrared spectra were measured by KBr disc method with a Nihon Bunko JASCO, IR-G and the thermal analyses were made with a Shimazu DTA 20 B. Metal analyses of Cu, Ni, Co, and Zn were made with a Hitachi Atomic Absorption Spectrophotometer Type-207 and the samples were prepared by dissolving them in 1N HCl solution and diluted with H_2O.

Syntheses——Syntheses of alloxan-metal-glycine complexes were made by dissolving 3×10^{-3}M of alloxan monohydrate in 10 ml of H_2O, whose pH was brought down at 3, and to this solution 3×10^{-3}M of metal chlorides and 3×10^{-3}M of glycine dissolved in 10 ml of H_2O were added and the pH of the resultant solutions were adjusted at 3 with either 1M NaOH or 1N HCl. The resulting solutions were allowed to stand at a room temperature for a week and the precipitates were deposited gradually. They were taken by filtration and washed with H_2O and EtOH and dried over silica gel in a desiccator. All the mixed-ligand complexes obtained were insoluble in H_2O, EtOH, EtOEt, and other organic solvents.

Syntheses of alloxan-Cu-glycylglycine complex was conducted in the similar procedures to those of alloxan-metal-glycine complexes with employing 3×10^{-3}M of glycylglycine dissolved in 10 ml of H_2O, while

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the resultant solution was allowed to stand for a week and green precipitates were deposited gradually. It was separated by filtration and washed with H_2O, followed by EtOH. It is insoluble in H_2O, EtOH, and EtOEt.

Thin-Layer Chromatography——Thin-layer chromatography was carried out on 0.20 mm thick plates activated at 80° for 40 min. The solvent mixture used was BuOH: AcOH: H_2O (4:1:2).

Detection of the Spots——As to the detection of the components in the decomposition products of the mixed-ligand complexes, the samples were dissolved in 1N HCl and developed with the solvent mixture, then alloxan was detected with glycine, with which alloxanate did not react. Glycine and glycylglycine were detected with ninhydrin and the metals with dithizone. Thin-layer chromatography of the mixed-ligand complexes was not made because of their insolubility in solvents.

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