[Chem. Pharm. Bull. 22(3) 654—657 (1974)]

UDC 547.781'293.04 : 547.35.03

Metal Complexes of Ergothioneine

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(Received June 6, 1973)

The interaction between ergothioneine and divalent metal ions has been investigated by means of potentiometric titration, visible and near-infrared spectra. The order of the relative formation constants of ergothioneine-metal complexes was Cu(II)>Hg(II)>Zn(II)>Cd(II)>Co(II)≧Ni(II). For ergothioneine-cobalt(II) complex, the crystal field parameter (Dq) and Racah interelectron repulsion parameter (B) calculated were 453 and 971 cm^{-1}, respectively. It was indicated that ergothioneine coordinates to cobalt(II) ion through its sulfur atom and forms a distorted tetrahedral complex.

Ergothioneine, the betaine of thiolhistidine, was isolated from the ergot of rye by Tanret in 1909[2] and its structure shown in Fig. 1, was established by Barger and Ewins two years after its discovery.[3] Although ergothioneine distributes to blood cell and most organs of the body,[4] an information on metabolic function of the alkaloid is still insufficient. It is known that ergothioneine is precipitated with $HgCl_2$, $AgNO_3$[2] and Cu_2O.[5] In addition, the compound effectively prevents spermatozoan inactivation caused by *o*-iodosobenzoate and cupric ion.[6] We picked up ergothioneine as one of the biologically interesting complexing agents, and have investigated extensively its complex formation by the spectrophotometric and potentiometric methods. Recently, from a standpoint of medical chemistry, the interaction of ergothioneine with a number of transition metal ions was briefly investigated using a pH titration method.[7] However, the information on the structure and the mode of the coordination has not been obtaind.

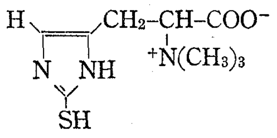

Fig. 1. Structure of Ergothioneine

Experimental

Materials——Ergothioneine was obtained from Sigma Chemical Company. The solution of nickel nitrate, cobalt nitrate, zinc nitrate, cadmium nitrate, mercuric chloride and cupric chloride were prepared from reagent grade materials, and were standardized with EDTA. Carbonate-free sodium hydroxide solution was prepared by using the saturated solution of barium hydroxide and was standardized by the titration with potassium hydrogen phthalate. The buffer mixtures used were: pH 6.0, 0.1 M acetic acid and 0.1 M sodium acetate; pH 8.0—9.0, 0.1 M ammonium chloride and 0.1 M sodium hydroxide; pH 10.0—11.0, 0.05 M borax and 0.05 N sodium carbonate; and pH 11.5, 0.05 M borax and 0.1 N sodium hydroxide. Silica-glass distilled water was used throughout the experiments except the near-infrared spectra measured in heavy water. Heavy water was obtained from E. Merck Company.

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2) *a*) C. Tanret, *J. Pharm. Chim.*, **30**, 145 (1909); *b*) C. Tanret, *C.R. Acad. Sci.*, **149**, 222 (1909).
3) G. Barger and A.J. Ewins, *J. Chem. Soc.*, **99**, 2336 (1911).
4) *a*) G. Hunter, *Biochem. J.*, **22**, 4 (1928); *b*) D.B. Melville and R. Lubschez, *J. Biol. Chem.*, **200**, 275 (1953); *c*) H. Heath, *Biochem. J.*, **54**, 689 (1953); *d*) D.B. Melville, W.H. Horner, and R. Lubschez, *J. Biol. Chem.*, **206**, 221 (1954).
5) S.W. Williamsen and N.U. Meldrum, *Biochem. J.*, **26**, 815 (1932).
6) T. Mann and E. Lenone, *Biochem. J.*, **53**, 140 (1953).
7) D.P. Hanlon, *J. Med. Chem.*, **14**, 1084 (1971).

Apparatus and Procedure——On the titration vessel, 5 ml of 1.0×10^{-2} M metal ion solution and 5 ml of 2.0×10^{-2} M ergothioneine were added. The titration was carried out with 0.1 N sodium hydroxide solution. All potentiometric measurements were made at 22±0.1°. An auto buret of 5.0 ml was used with calibration up to 0.05 ml. The pH measurements were made with a Hitachi-Horiba pH meter, model F-5, equipped with combination pH electrode. The visible spectra were measured in an aqueous solution of pH range from 6 to 11.5, with a Shimazu recording spectrophotometer, model Double UV-200, and the near-infrared spectra were obtained in heavy water by a Hitachi recording spectrophotometer, model 323.

Calculation——The acid dissociation constant of ergothioneine and the formation constants of its metal complexes were calculated by the method described in the previous paper.[8] The crystal field parameter (Dq) and the Racah interelctronic repulsion parameter (B) were calculated by the adaptation of Lever's method[9] on the transition observed in electronic ligand field spectra.

Result and Discussion

The titration curves of ergothioneine and its metal complexes are presented in Fig. 2. The acid dissociation constant of ergothioneine calculated from the titration data is shown in Table I together with those of histidine[10] and 2-thiolhistidine.[11] The pK value of ergothioneine obtained by the direct pH titration with alkali was 10.5. In general, pK values of sulfhydryl group of aliphatic mercaptans[12] and imidazole[13] are known to be about 10.5 and 7.1 respectively. While, the pK values of imidazole in histidine and 2-thiolhistidine result in 5.97 and $\ll 1.5$ respectively, because in these ligands amino group gives large effect on the dissociation of imidazole group. In carsinone and anserine the amino group is replaced by the peptide bond, therefore, the dissociation constant of imidazole group becomes to normal value, about pK 7.0.[10] From these facts, it can not be simply assigned, although this pK value from the uncharged thiolimidazole group of ergothioneine corresponds to deprotonation of sulfhydryl group of the aliphatic mercaptans. Factly, it is suggested that thiolimidazoles are in the thione form in the uncharged state, but the ionized species exists as a thiolate ion.[14] Further detailed studies are needed to determine the precise dissociation equiribrium of ergothioneine.

Table I. Acid Dissociation Constants of Ergothioneine and Its Related Compounds

	pK_1	pK_2	pK_3
Ergothioneine	<2	10.5	
Histidine[10]	1.78 (COOH)	5.97 (imidazole)	8.97 (NH_3^+)
2-Thiolhistidine[11]	1.84 (COOH)	8.47 (NH_3^+)	11.4 (SH)

As shown in Fig. 2, the titration curves of ergothioneine to metal ion molar ratio 2:1 gave inflections at $a=2$, indicating the formation of 2:1 complexes. In addition, in the titration of 4:1 molar ratio with Hg^{2+}, the inflection was observed at $a=2$. These facts indicate that ergothioneine probably behaves as an unidantate ligand in the complexes. The formation constants of cobalt, nickel, zinc, cadmium, mercury and copper complexes of ergothioneine calculated from titration data are shown in Table II together with those of

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[8] Y. Sugiura, A. Yokoyama, and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **18**, 693 (1970).

[9] A.B.P. Lever, *J. Chem. Educ.*, **45**, 711 (1968).

[10] C.L.A. Schmidt, P.L. Kirk, and W.K. Appleman, *J. Biol. Chem.*, **88**, 285 (1930).

[11] G.M. Richardson, *Biochem. J.*, **27**, 1036 (1933).

[12] A. Albert and E.P. Serjeant, "Ionization Constants of Acid and Basas," Methuen, London, (1962).

[13] C. Tanford and M.L. Wagner, *J. Am. Chem. Soc.*, **75**, 434 (1953).

[14] A. Lawson and H. Morley, *J. Chem. Soc.*, **1956**, 1103.

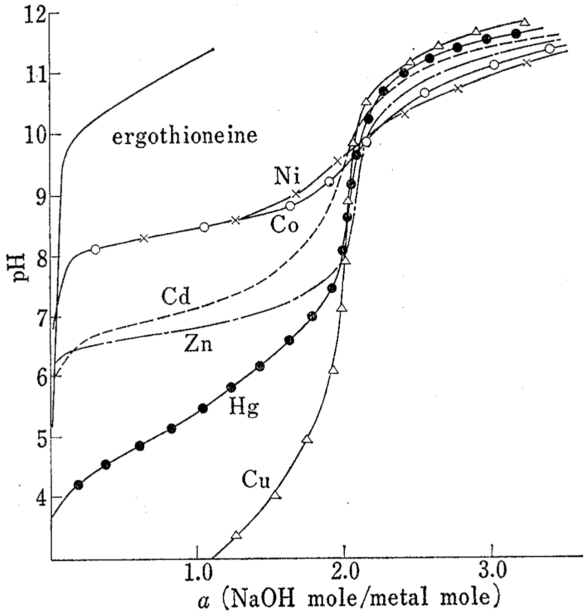

Fig. 2. Titration Curves of Ergothioneine with Metals

ergothioneine (10^{-2}M) to metal ratio=2:1

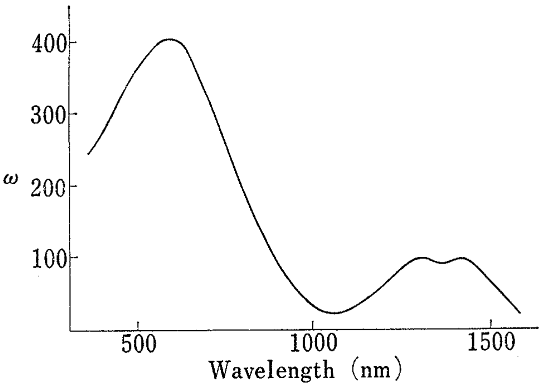

Fig. 3. Visible and Near-infrared Absorption Spectra of Ergothioneine-cobalt (II) Complex at pH 9.3 (in D_2O)

Table II. Formation Constants of Divalent Metal Complexes of Ergothioneine and Its Related Compounds

Metal		Ligand			
		Ergothioneine	6-Mercaptopurine[15)]	N-Acetylhistidine[16)]	Histidine[17)]
Co^{II}	log k_1	4.42	5.44	2.35	6.92
	log k_2	4.42		1.80	5.52
Ni^{II}	log k_1	4.43	5.29	2.85	8.69
	log k_2	4.32		2.20	6.83
Zn^{II}	log k_1	6.06	5.90	2.50	6.63
	log k_2	6.05		2.30	5.63
Cd^{II}	log k_1	5.85		2.70	5.65
	log k_2	5.50		1.95	4.14
Hg^{II}	log k_1	7.93			
	log k_2	6.84			
Cu^{II}	log k_1	10.15		4.35	10.56
	log k_2	8.74		3.40	8.25

the complexes of 6-mercaptopurine,15) N-acetylhistidine16) and histidine.17) The relative stability of ergothioneine-metal complexes decreases in order of Cu(II)>Hg(II)>Zn(II)>Cd(II)>Co(II)≧Ni(II). Zinc forms more stable complexes than nickel. This alteration in the usual stability sequence of the divalent metals has been often observed in the case of thiol ligands, such as β-mercaptopropionic acid and N-acetylpenicillamine.18) Accordingly, it is presumed that the coordination in the metal complexes of ergothioneine occurs through sulfur atom. In addition, the higher stability in the copper and mercury complexes of ergo-

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15) G.E. Cheney, H. Freiser, and Q. Fernando, *J. Am. Chem. Soc.*, **81**, 2611 (1959).

16) R.B. Martin and J.T. Edsall, *J. Am. Chem. Soc.*, **82**, 1107 (1960).

17) R. Leberman and B.R. Rabin, *Trans Faraday Soc.*, **55**, 1660 (1959).

18) *a*) Q. Fernando and H. Freiser, *J. Am. Chem. Soc.*, **80**, 4928 (1958); *b*) Y. Sugiura, A. Yokoyama, and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **18**, 693 (1970).

thioneine would be explained by the fact that these metal ions possesing higher electronegativity form more stable complexes with sulfur donor atom possessing lower electronegativity in comparison with oxygen and nitrogen atoms. In an attempt to discuss the configuration and coordinative properties of ergothioneine-metal complexes, the visible and near-infrared spectra of ergothioneine-cobalt (II) complex were investigated through the comparison with those of other tetrahedral cobalt (II) complexes. Ergothioneine reacts with cobalt (II) ion to form a stable blue complex in alkaline solution. The absorption spectrum of ergothioneine-cobalt (II) complex at pH 9.3 is presented in Fig. 3. The spectral feature is characterized as those of the tetrahedral cobalt (II) complexes from both absorption maxima and extinction coefficient. With reference to the energy-level diagram of cobalt (II) in a tetrahedral field,19) the longer wavelength band in the near-infrared may be assigned to the $^4A_2 \rightarrow {}^4T_1$ (F) transition and the shorter wavelength band in the visible to $^4A_2 \rightarrow {}^4T_1$ (P). Using these assignments, the crystal field parameter (Dq) and the Racah interelectron repulsion parameter (B) of ergothioneine-cobalt (II) complex were calculated and the results are listed in Table III together with data of tetrahedral histidine-, histamine-, imidazole- and N-acetylhistidine-cobalt (II) complexes.20) The Dq values of the regular tetrahedral and octahedral cobalt (II) complexes are known to be 300—400 and 900—1000 cm^{-1}, respectively. The Dq value of ergothioneine-cobalt (II) complex, 453 cm^{-1}, is slightly high compared to that of the former. However, as shown in Table III, this value is lower than those of histidine related compounds-cobalt (II) complexes which have distorted tetrahedral configuration. While, the B value of ergothioneine-cobalt (II) complex, 724 cm^{-1}, corresponds to about 75% of that of free cobalt (II) ion (971 cm^{-1}) and is close to those of the histidine related compounds-cobalt (II) complexes. This indicates that the nephelauxetic effect occurs to the same extent in these cobalt (II) complexes. As shown in Table III, the two transition bands of ergothioneine-cobalt (II) complex exist clearly on the lower energy region than those of other histidine related compounds-cobalt (II) complexes coordinated with nitrogen or oxygen doner atoms. On the basis of these data, it seemed reasonable to assume that ergothioneine coordinates to cobalt (II) ion through its sulfur atom and forms a distorted tetrahedral complex. We have previously observed the effect of ergothioneine on γ-irradiation of metmyoglobin and have suggested an importance of its sulfur atom.21) It is very interesting to investigate a reactivity of thiolimidazole group, especialy sulfur donor atom of ergothioneine.

TABLE III. Spectral Data for Cobalt(II) Complexes

Ligand	$^4A_2 \longrightarrow {}^4T_1(P)$ nm	$^4A_2 \longrightarrow {}^4T_1(P)$ ε	$^4A_2 \longrightarrow {}^4T_1(F)$ nm	$^4A_2 \longrightarrow {}^4T_1(F)$ ε	Dq (cm^{-1})	B (cm^{-1})
Ergothioneine	595	410	1285	125	453	724
Histidine20)	558	320	1115	80	528	738
Histamine20)	572	515	1110	115	532	700
Imidazole20)	569	660	1105	180	535	704
N-Acetylhistidine20)	568	535	1150	130	512	731

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19) L.E. Orgel, *J. Chem. Phys.*, **23**, 1004 (1955).
20) P.J. Marris and R.B. Martin, *J. Am. Chem. Soc.*, **92**, 1543 (1970).
21) N. Motohashi, I. Mori, Y. Sugiura, and H. Tanaka, *Radioisotopes*, **22**, 451 (1973).