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Physico-chemical and Metal-binding Properties of Thiolhistidine

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The macroscopic and microscopic proton dissociation constants and thermodynamic parameters for the proton dissociations of thiolhistidine (TH) have been determined in order to characterize the physico-chemical properties of this ligand. The proton dissociation profile for TH as a function of pH was compared with that for 2-mercaptohistamine. The stability constants of metal complexes of TH with divalent metal ions such as Mn, Fe, Co, Zn, Cd, Hg and Pb have been determined by a potentiometric titration method and the coordination mode of TH-metal complexes were thereby characterized. It was concluded that the coordination with Zn, Cd, Hg and Pb ions occurs through the thiolate and amino nitrogen groups in the ligand.

Introduction

Thiolhistidine (2-mercaptohistidine, TH) which contains a mercaptoimidazole, an amino and a carboxyl group, is an important analogue of ergothioneine (2-mercaptohistidine trimethyl betaine, Erg), which exists naturally as the betaine of thiolhistidine. Considerable interest in TH was aroused due to the finding that its isolation from natural sources had never been achieved, certain proteins, nevertheless, exhibited a positive mercaptoimidazole colour reaction [1].

Hanlon reported the interaction of Erg with a number of transition metal ions and also the inhibitory effect of Erg on metalloenzymes such as polyphenol oxidases [2]. Further, the inhibition of urocanase by Cu(II) was prevented if a metal-binding agent such mercatoimidazole derivatives as Erg or TH was added [3]. The interaction between Erg and transition metal ions has already been reported [2, 4] and was concluded by the comparison with the data of 2-mercaptohistamine (2-MH) metal complexes that the coordination of Erg with such metal ions as Cu(II), Zn(II) and Ni(II) occurs through the thiolate group [5].

The phisico-chemical investigation on Erg has been reported [6], however, much less work has been done on the physico-chemical and metal-binding properties on TH [7].

This paper reports the physico-chemical properties such as macroscopic and microscopic proton dissociation constants and thermodynamic parameters for the proton dissociation of TH and the stability constants of TH–metal complexes comparing with the data of related compounds.

Experimental

Materials

Thiolhistidine (TH) was obtained from ICN Pharmaceutical Inc. and was used after recrystallization from water. The solutions of $MnCl_2$, $FeSO_4$, $CoCl_2$, $Zn(NO_3)_2$, $CdCl_2$, $HgCl_2$ and $Pb(NO_3)_2$ were prepared from guaranteed grade reagents, and were standardized complexometrically with EDTA. Carbonate-free, potassium hydroxide solution was prepared by the procedure described by Armstrong [8] and was standardized by titration with potassium hydrogen phtalate. Deionized water was used throughout the experiments. All other reagents used in the experiments were guaranteed grade reagents.

Measurement of Absorption Spectra

The absorption spectra of aqueous solutions were measured in the ultra-violet region with a Shimadzu UV-200S recording Spectrometer with a quartz flow-cell (light path, 10 mm) under an atmosphere of nitrogen and the temperature of the solution was maintained at 25 ± 0.1 °C. The spectra were measured for ligand concentration of 0.1 m*M*.

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TABLE I. Macroscopic Acid Dissociation Constants and Thermodynamic Parameters of Thiolhistidine and 2-Mercaptohistamine (25 °C, μ = 0.1 $NaClO_4$).

	pka_1	pka_2	ΔG_1^*	ΔG_2^*	ΔH_1^*	ΔH_2^*	ΔS_1^{**}	ΔS_2^{**}
Thiolhistidine[a]	8.59 ± 0.02	11.77 ± 0.04	11.7	16 1	9.8	13.0	−6.4	−10.2
2-Mercaptohistamine[b]	9.12 ± 0 02	11.62 ± 0.03	12.3	15.5	9.5	7.4	−9.7	−28.2
Ergothioneine[b]	10.44 ± 0.04		14.2		11 6		−8 7	

[a]The proton dissociation constant for carboxyl group of thiolhistidine is omitted. [b]Ref 5 *kcal mol^{-1}. **cal mol^{-1} deg^{-1}.

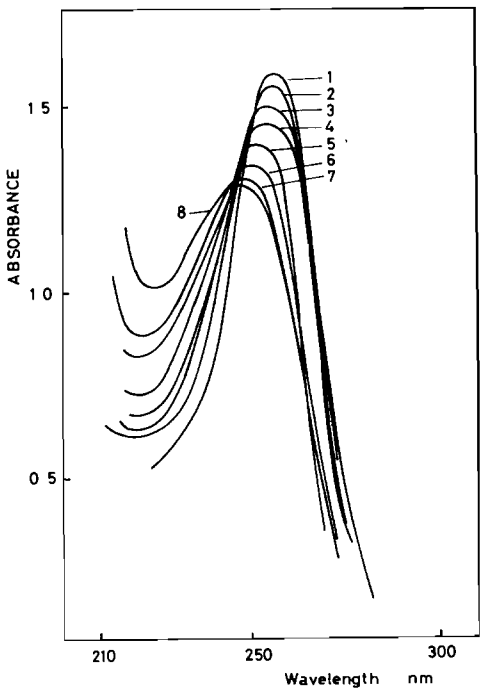


Fig. 1. Absorption spectra of thiolhistidine at various pH. Concentration of TH 1 m*M*. 1: pH 8.08–10.19, 2: pH 10.70, 3: pH 11.10, 4: pH 11.44, 5: pH 11.75, 6. pH 11.94, 7· pH 12.20, 8. pH 12.53.

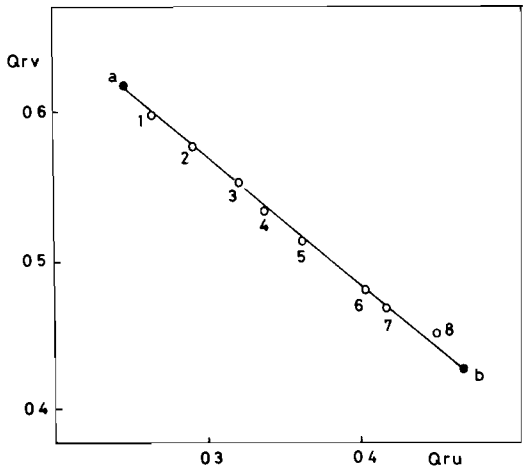


Fig. 2. Qru-Qrv plots of thiolhistidine. a· protonated form of 2-mercaptoimidazole in thiolhistidine. b· deprotonated form of 2-mercaptoimidazole in thiolhistidine. a· pH 8.08, 1 pH 10.70, 2 pH 11.24, 3: pH 11.60, 4. pH 11.75, 5: pH 11.94, 6· pH 12.20, 7: pH 12.39, 8· pH 12.53, b: pH 12.73. U range 220–235 nm, V range 250–265 nm, W range 270–285 nm.

pH Titration

The pH titration was carried out with a Radiometer TTT60 Titrator, equipped with REC61/REA160 Titrigraph and TTA60 Titrator Assembly and associated Autoburette ABU12 using a G2040C glass electrode and a K4040 calomel electrode. The ionic strength was adjusted to 0.1 with sodium perchlorate. The solution was stirred by stirring stick and run by a motor and nitrogen gas was passed slowly into the solution during the titration. In the determination of macroscopic proton dissociation constants, the solution containing 2.5 m*M* of TH was titrated with carbonate-free 0.05 *N* potassium hydroxide. The titrations were done at temperature of 5, 15, 25 and 35 °C (all ±0.1 °C) to calculate the thermodynamic parameters. In the determination of stability constants, the solution containing TH (1 m*M*) and metal ion (0.5 m*M*) was titrated with carbonate-free 0.1 *N* potassium hydroxide at temperature of 25 ± 0.1 °C.

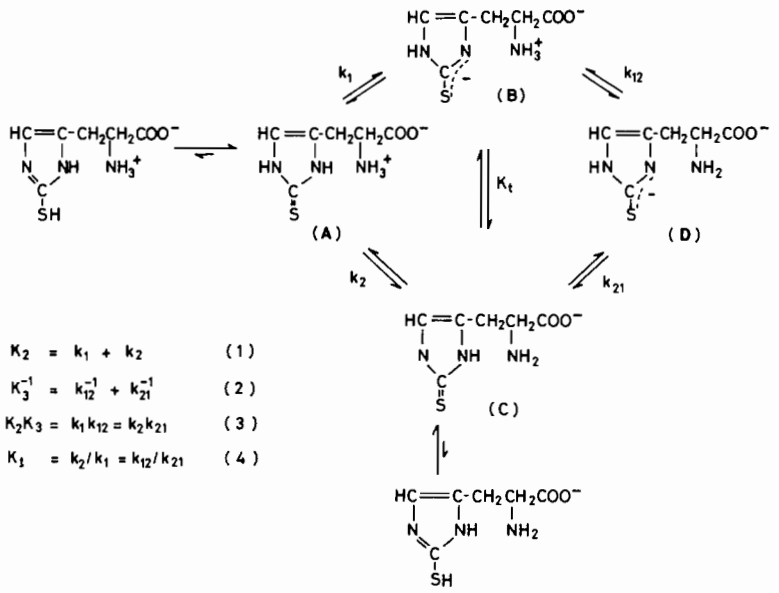
Calculations

Determination of Macroscopic Proton Dissociation Constants of Ligand and Stability Constants of Metal Complex

Macroscopic proton dissociation constants of TH and stability constants of TH–metal complexes were calculated according to reported method [9].

Determination of Microscopic Proton Dissociation Constants

The proton dissociation equilibrium of TH may be expressed as shown in Scheme 1. The microscopic proton dissociation constants of TH were obtained by the following method.



Scheme 1. Scheme for the ionization equilibrium of thiolhistidine.

From the Q_r plots (Fig. 2) obtained from the spectrum shown in Fig. 1, the mole fraction (q_{oh}) of dissociated thiolate anions was calculated for the region between a and b according to the complementary tri-stimulus colorimetry (CTS method) [10]. The M_{oh} value as defined by Edsall *et al.* [11] is given in eqn. (5).

$$M_{oh} = \frac{(H^+)q_{oh}}{1 - q_{oh}} = \frac{k_1(H^+) + k_2k_{21}}{(H^+) + k_2} \quad (5)$$

The eqn. (5) can be rewritten as

$$k_1(H^+) - M_{oh}k_2 + k_2k_{21} - M_{oh}(H^+) = 0 \quad (6)$$

The k_1, k_2 and k_{21} values can be obtained from the three different proton concentrations, and the remaining k_{12} was calculated from the eqns. (1)–(3) in Scheme 1.

Thermodynamic Parameters

The plots of pK_a values against 1/T (T = absolute temperature) were linear or showed slight curvature and the data can be presented by the empirical equations obtained by the method of least-square. The values of free energy, enthalpy and entropy changes for the proton dissociations were calculated from the equation as described in the literatures [12].

Results and Discussion

Proton Dissociation Process

Macroscopic proton dissociation constants and thermodynamic parameters for the proton dissocia-

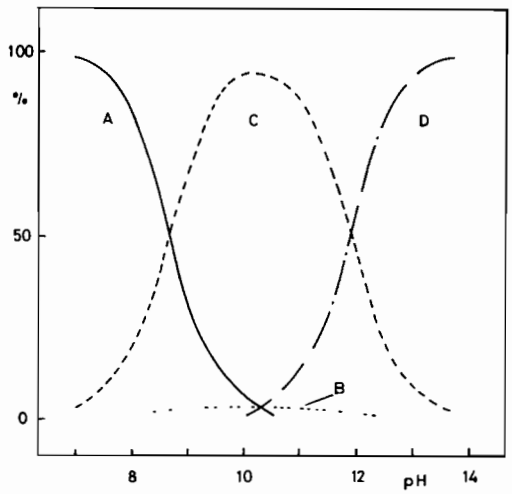


Fig. 3. Relative concentrations of various ionic forms of thiolhistidine.

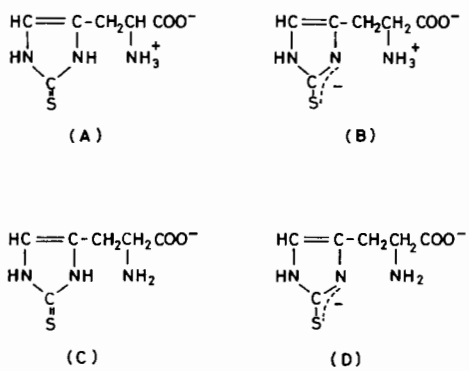


TABLE II. Microscopic Acid Dissociation Constants and Tautomeric Constants (Kt) of Thiolhistidine and 2-Mercaptohistamine (25 °C, μ = 0.1 $NaClO_4$).

	pka_1	pk_2	pk_{12}	pk_{21}	Kt (k_2/k_1)
Thiolhistidine	10.20 ± 0.03	8.68 ± 0.05	10.38 ± 0.03	11.90 ± 0 03	33.1
2-Mercaptohistamine	10.33 ± 0.09	9.49 ± 0.05	10.90 ± 0 08	11.76 ± 0 05	6.9

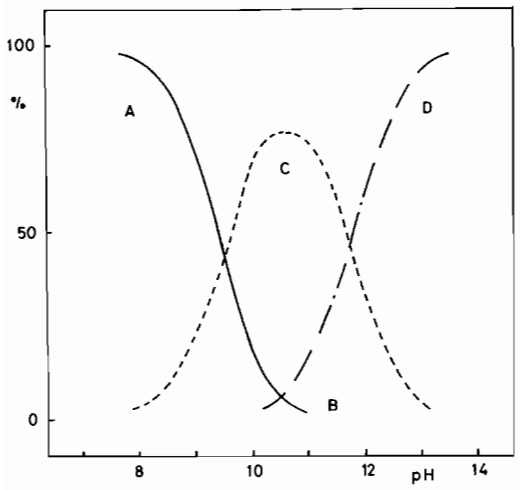
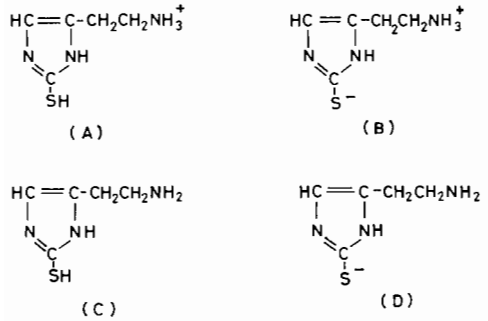


Fig. 4. Relative concentrations of various ionic forms of 2-mercaptohistamine



tions are listed in Table I. The macroscopic proton dissociation constants of TH are in agreement with the previously reported values (pK_{a1} = 8.47, pK_{a2} = 11.4) [7]. The magnitude of ΔH, *i.e.*, 9.8 kcal/mol for pK_{a1} of TH can compare favourably with the values of ΔH = 9 kcal/mol for the proton dissociation from an ammonium group of histidine [13], histamine [6] and 2-mercaptohistamine(2-MH) [6]. The values of ΔH = 13 kcal/mol and ΔS = −10.2 cal/mol deg. for pK_{a2} of TH are similar to those of proton dissociation from mercaptoimidazole moiety of Erg, which has suggested to exist as thione form of mercaptoimidazole group on the basis of thermodynamic considerations [6c]. Therefore, it can be concluded that the pK_{a1} and pK_{a2} of TH are preferentially due to the deprotonation from the α-ammonium and the mercaptoimidazole group, respectively

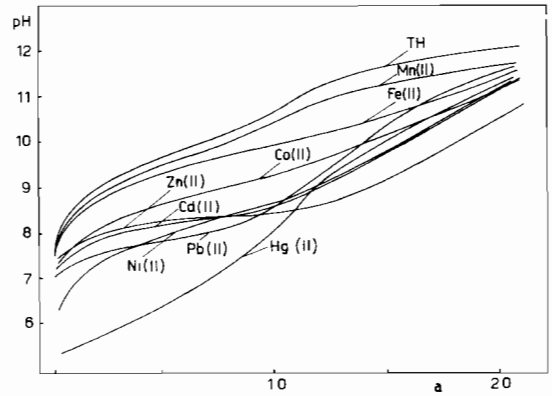


Fig. 5. Titration curves of various divalent metal complexes of thiolhistidine. Concentration of TH. 1 m*M*, TH/metal = 2/1, a. moles of KOH added per mole of the ligand, 25 °C, μ = 0.1 $NaClO_4$

In order to obtain further detail informations on the proton dissociation of TH, the microscopic proton dissociation constants were calculated using the UV absorption spectra by the modified CTS method [10b] as described in the Experimental (Table II). In aqueous solution, neutral molecules and various ionic forms, namely anionic, cationic and zwitterionic may exist in the state of equilibrium. Since the dissociation of the proton from the carboxyl group of TH occurs at low pK_a value (pK_a = 1.87) [7], therefore, this group is fully deprotonized in the studied pH range. The values of tautomeric constant, K_t(= k_2/k_1) were 33.1 and 6.9 in TH and 2-MH. respectively, indicating that both compounds dissociate the protons largely through the process (A)–(C)–(D) in Scheme 1. The pH-dependent relative concentrations of various species of the compound were calculated using the microscopic proton dissociation constants and are shown in Fig. 3 and Fig. 4. In TH, the relative amount of the amino-thione form (C) was reached maximum at pH 10.4. The concentration in this form was about 90% in the pH range of 9.8–10.8, and in this pH range, the ammonium-thiol form (B) exist only 3% or below. On the other

TABLE III. Stability Constants of Divalent Metal Complexes of Thiolhistidine and its Related Compounds (25 °C, μ = 0.1 $NaClO_4$).

Ligand: / Metal		Thiolhistidine (TH)	2-Mercaptohistamine[a] (2-MH)	Histidine[b] (His)	Histamine[c] (H)	$\Delta = \log K_1^X/K_1^Y$ X = TH Y = His	X = 2-MH Y = H
Mn(II)	$\log K_1$	4.07 ± 0.11	5.35 ± 0.09	3.24		0.83	
	$\log K_2$	4.47 ± 0.09	4.27 ± 0.20	2.92			
Fe(II)	$\log K_1$	5.80 ± 0.04	5.40 ± 0.13	5.88		−0.08	
	$\log K_2$	5.31 ± 0.04	5.97 ± 0.14	4.55			
Co(II)	$\log K_1$	7.64 ± 0.10	6.14 ± 0.05	6.71	5.03	0.93	1.11
	$\log K_2$	4.79 ± 0.10	6.06 ± 0.08	5.35	3.74		
Ni(II)	$\log K_1$	9.31 ± 0.05	7.48 ± 0.03	8.43	6.78	0.88	0.70
	$\log K_2$	6.37 ± 0.06	5.66 ± 0.08	6.71	4.00		
Zn(II)	$\log K_1$	9.16 ± 0.06	9.17 ± 0.14	6.34	5.40	2.82	3.77
	$\log K_2$	8.04 ± 0.07	6.55 ± 0.12	5.35	4.60		
Cd(II)	$\log K_1$	9.08 ± 0.03	8.09 ± 0.10	5.39	4.83	3.43	3.26
	$\log K_2$	7 92 ± 0.06	6.08 ± 0.06	4.27	3.40		
Hg(II)	$\log K_1$	12.43 ± 0.21	12.45 ± 0 15	7.28	6.01	5.15	6.44
	$\log K_2$	–	–	5.00	3.72		
Pb(II)	$\log K_1$	10.27 ± 0.07	8.66 ± 0.16	5.96		4.31	
	$\log K_2$	–	5.31 ± 0.16				

[a]Ref. 5. [b]Ref. 14. [c]Ref. 15.

hand, in the case of 2-MH, the concentration of the zwitterionic form was about 10% or above in the pH range of 10.5–11.3. Thus, the amino-thione form of TH was major ionic species in proton dissociation process.

Stability Constants

The titration curves of TH in the presence of various metal ions are presented in Fig. 5, and the formation curves are shown in Fig. 6. A direct pH titration of TH with alkali gave the macroscopic proton dissociation constants (pk_{a1} = 8.58 ± 0.02 and pK_{a2} = 11.77 ± 0.04).

The stability constants of TH-metal complexes and its related compounds are shown in Table III. In metals, such as Hg(II), Cd(II) and Pb(II), the formation of 1:1 complex of ligand to metal ion was indicated (Fig. 5). The titration curves of the ligand to the metal ion at a 1:1 molar ratio gave an inflection at a = 1. In addition, in the titration at 1.1 molar ratio an inflection at a = 2 was observed.

The relative stabilities of TH-metal complexes lie in the order, Mn(II) Fe(II) Co(II), Ni(II) Zn(II) (Fig. 7). The stability constants in TH–metal complexes were higher than those in 2-MH–metal complexes owing to the lack of a carboxyl group in 2-MH, which has ability to coordinate to metal ions. The same result was found between histidine [14] and histamine [15]. For Mn(II), Fe(II) and Co(II) ions, the stability constants of TH–metal complexes are similar to those of histidine–metal complexes, suggesting that the coordination behaviour between TH and the metal ions is similar to that of histidine and metals. From the study on C-13 NMR spectrometry [16] and X-ray crystallography [17], it was revealed that in histidine the coordination to metal ions such as Mn(II), Fe(II) and Co(II) occurs through the tertiary imidazole nitrogen and the α-amino nitrogen and the carboxyl group. On the other hand, in Zn(II), Cd(II), Hg(II) and Pb(II) the stability constants of TH–metal complexes are similar to those of penicillamin- (Zn(II); $\log K_1$ = 9.95, Cd(II); $\log K_1$ = 10.92, Hg(II); $\log K_1$ = 16.4, Pb(II); $\log K_1$ = 12.9) [18] and cysteine- (Zn(II); $\log K_1$ = 9.04, Hg(II); $\log K_1$ = 14.21, Pb(II), $\log K_1$ = 11.39) [19] metal complexes. This indicates that the metal ions are bound with the α-amino, sulfur atom of mercaptoimidazole and carboxyl group in TH.

The participation of sulfur atom in the coordination between these metal ions and TH can also indicated from the following results. The differences in the stability constants between TH- and histidine-metal complexes ($\Delta = \log K_1^X/K_1^Y$ in Table III) are larger for Zn(II), Cd(II), Hg(II) and Pb(II) than Mn(II), Fe(II), Co(II) and Ni(II), and the values of Δ

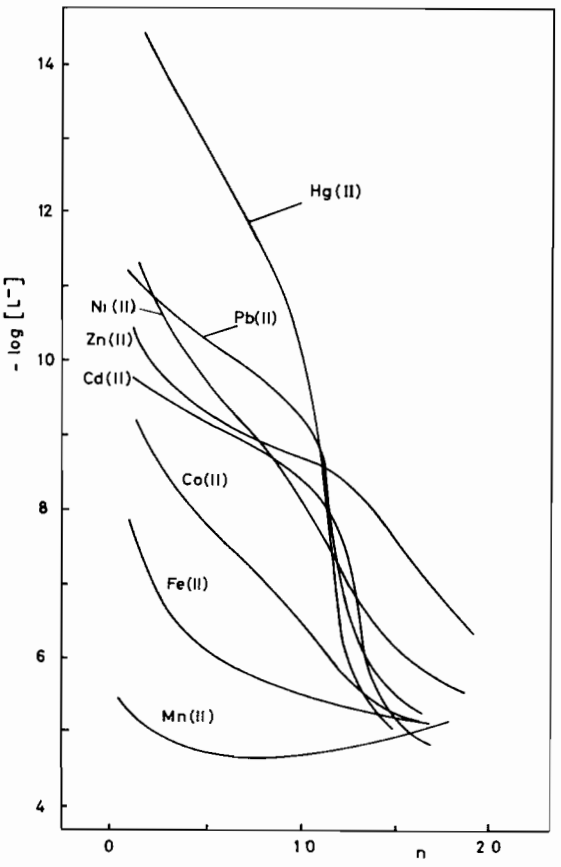


Fig. 6. Formation curves of thiolhistidine–metal complexes

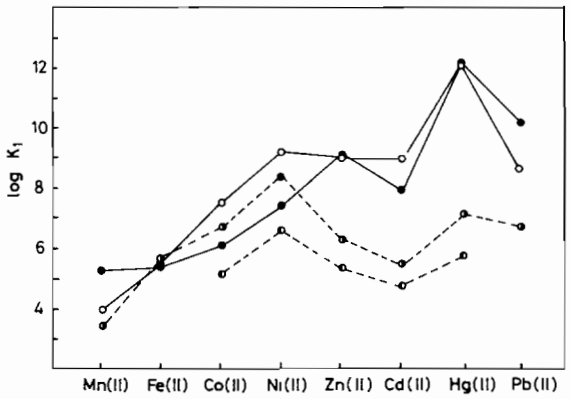


Fig. 7. Plots of first stability constants (log K_1) of divalent metal complexes of thiolhistidine and its related compounds —○—○— Thiolhistidine, —●—●— 2-Mercaptohistamine, —◑—◑— Histidine, —◐—◐— Histamine.

for Zn(II), Cd(II), Hg(II) and Pb(II) are similar to the differences in the stability constants for 2-MH– and histamine–metal complexes. These results indicate that the presence of a strong complex formation between a soft base as sulfur atom and a soft acid such as Zn(II), Cd(II), Hg(II) and Pb(II). Therefore, it may be concluded that in TH and 2-MH the coordinations with Zn(II), Cd(II), Hg(II) and Pb(II) occurs through the sulfur atom and amino nitrogen in the ligand. Thus the high affinity of 2-MH for Hg(II) was proved by the removal experiment of Hg(II) bound to proteins [20]. The interaction of TH and 2-MH with Cu(II) is especially interesting and the results will be published in the future.

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