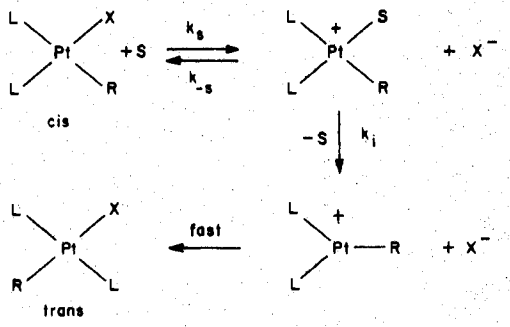
Scheme III



mational change was found to be very high for Pt(II) and Pd(II) complexes.[10] Calculations have also shown that this cis–trans isomerization mechanism is symmetry forbidden for MX_2L_2 complexes.[11] However, the square-planar configuration of *cis*-$PtXR(PEt_3)_2$ may be so distorted as a result of steric strain that these calculations are no longer applicable. Although here again, it is rather difficult to understand why the parent complex would not also be capable of undergoing the transition to a tetrahedral conformation. Furthermore, for R = phenyl steric strain is not so acute, and yet from the activation parameters the mechanism for isomerization appears to be the same as for R = mesityl. It is difficult to predict either the sign or the magnitude of ΔV^* for such a process.

As the k_i values are of the order of 10^{-3} s^{-1} the most severe criticism of this mechanism is that conformational reactions are generally in the milli- and microsecond time range. If the k_i step is indeed in this time range, the rate-determining step must then be the uptake of Br^- by *trans*-PtR(methanol)-$(PEt_3)_2^+$ to form the trans product. However, the latter is known to be an associative process and does not therefore comply with a ΔV^* value of +7.7 cm^3 mol^{-1}. Moreover, if this were true, the substitution reaction 2 must yield mixed cis–trans products, and the solvolysis reaction must be faster than the subsequent Br^- uptake, both of which are not the case. It must therefore be accepted that the k_i path is the rate-determining step.

(d) A Dissociative Mechanism. This mechanism is probably the most likely since it can best explain the ΔV^* value of +7.7 cm^3 mol^{-1}. In Scheme III the transition state of the isomerization step is visualized as having a trigonal-planar configuration which can rapidly take up a halide ion to form the trans product. The relative weakness of the Pt–$HOCH_3$ bond may allow the normally energetically unfavorable three-coordinate transition state to compete with the five-coordinate "solvolysis" transition state.

Since a three-coordinate d^8 high-spin complex has not yet been isolated or detected as a reaction intermediate and because its existence violates Tolman's 16–18 electron rule,[12] more work needs to be done to clearly establish that isomerization results from the dissociative release of a methanol molecule from the *cis*-$PtR(MeOH)(PEt_3)_2^+$ intermediate.

It is of interest to note that for the isomerization reaction

$$trans\text{-}PtCl_2L_2 \underset{k_r}{\overset{k_f}{\rightleftharpoons}} cis\text{-}PtCl_2L_2$$

in $CDCl_3$, where L = Et_2SO and *n*-Pr_2SO,[13] a two-step process was postulated. This NMR kinetic study leads the authors[13] to propose reaction schemes similar to Schemes II and III.

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Registry No. *cis*-PtBr(phenyl)$(PEt_3)_2$, 15702-94-0; *cis*-PtBr(mesityl)$(PEt_3)_2$, 22289-37-8.

(10) Tobe, M. L. "Inorganic Reaction Mechanisms"; Nelson: London, 1972; p 90.
(11) Eaton, D. R. *J. Am. Chem. Soc.* **1968**, *90*, 4272.
(12) Tolman, C. A. *Chem. Soc. Rev.* **1972**, *1*, 337.
(13) Price, J. H.; Birk, J. P.; Wayland, B. B. *Inorg. Chem.* **1978**, *17*, 2245.

Contribution from the Institute of Chemistry, College of General Education, Osaka University, Toyonaka, Osaka 560, Japan

Interaction of Copper(II) and Nickel(II) with L-Histidine and Glycylglycyl-L-histidine as an Albumin Model

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The ternary system composed of Cu(II) (M), L-histidine (A), and glycylglycyl-L-histidine (B) designed to mimic the copper-transport site of serum albumin has been investigated by potentiometric titrations and spectroscopic measurements. A solution equilibrium study (25 °C; I = 0.1 (KNO_3)) indicated that the binary species $CuH_{-2}B$ and CuA_2 are the main species while the ternary species CuAB is the minor one at physiological pH, and $CuH_{-2}B$ is predominant in the higher pH region. This result coincides well with the absorption, CD, and ESR spectral behaviors of the ternary systems. The quite similar absorption and CD spectral behaviors were observed for the corresponding systems with Ni(II) in place of Cu(II). In order to shed light on details of the interaction of Cu(II) with B and A, we similarly investigated the ternary systems containing histamine or glycine in place of A. The biological copper transport mechanism in blood was discussed on the basis of both the above results and the information obtained by studies on exchange reactions of Cu(II) and Ni(II) complexes of B with A, histamine and glycine.

It has been considered that the albumin-bound fraction of serum copper is in a rapid equilibrium with copper in tissues through some specific amino acid complexes and in the intermediate stage copper is bound by albumin and an amino

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acid such as L-histidine (His).[1] The first copper-binding site of most albumins has been considered to be the N-terminal tripeptide moiety, of which the third amino acid residue is histidine. Accordingly, some workers have attempted to disclose the mechanism of copper transport in blood by employing glycylglycyl-L-histidine (GlyGlyHis) as a model for the particular copper binding site of serum albumin. However, as a matter of fact, the understanding of the solution equilibrium of the binary Cu(II)–GlyGlyHis system[2] is still in confusion and that of the equilibrium of the ternary Cu(II)–GlyGlyHis–His system[3] is even more so.

This paper describes solution equilibria and spectral behaviors of the binary Cu(II)–GlyGlyHis and the ternary Cu(II)–GlyGlyHis–His systems. The systems with Ni(II) instead of Cu(II) exhibit spectral properties similar to those of the Cu(II)-containing systems, affording useful information to make clear details of copper transport in blood. Ligand-substitution reactions of His for GlyGlyHis in the coordination sphere of Cu(II) or Ni(II) are also described in view of the true character of the ternary copper(II)–albumin–histidine complex produced in the copper-transport process.

Experimental Section

Materials. GlyGlyHis was purchased from the Protein Research Foundation, and His, histamine (Hm), and glycine (Gly) were purchased from Nakarai Chemicals, Ltd., being dried in vacuo over P_4O_{10} before use. Histidine hydrochloride hydrate was purchased form Ajinomoto Chemical Co. and used without purification. All other reagents used were of the highest grade commercially available. Water was distilled and deionized.

Potentiometric Titrations. Potentiometric titration data (25 ± 0.05 °C; I = 0.1 (KNO_3)) were collected with the use of an Orion Research 801A digital pH meter as reported previously,[4] and the formation constants were derived by the computer program SCOGS least-squares approach.[5] The apparent ion product of water and the activity coefficient of the H^+ ion ($f_{H'}$) were determined by titrating 0.1 M KNO_3 and 0.01 M HNO_3, respectively, with 0.01 M potassium hydroxide under the same conditions. The values $pK_{w'}$ = 13.90 and $f_{H'}$ = 0.852 were used in the calculations.

Spectral Measurements. Absorption spectra were recorded in a 1-cm path length quartz cell in the range 250–800 nm with a Union Giken SM-401 high-sensitivity recording spectrophotometer at room temperature, and CD spectra were measured in a quartz cell having either a 1- or a 2-cm path length in 0.1 M KNO_3 over the range 300–800 nm with a JASCO MOE-1 spectropolarimeter. The pHs were adjusted with dilute NaOH solution. Electron spin resonance (ESR) spectra of Cu(II)-containing systems were measured at room temperature by using a JEOL JES-ME-2X instrument. Proton magnetic resonance spectra of Ni(II)-containing systems were measured at 35 °C by using a JEOL JNM-MH-100 instrument at 100 MHz with sodium 3-(trimethylsilyl)propionate-d_4 (TSP) as an internal standard. For the Ni(II)-containing systems all measurements were carried out under O_2-free conditions to avoid the effect of O_2 dissolved in water.[6]

Ligand-Substitution Reactions. A reaction of 1:1 Cu(II)–GlyGlyHis (2×10^{-3} M) with excess His was investigated at pHs 7.5, 8.5, and 10.0 at 25 °C by following the decrease of the peak at 525 nm arising from the binary GlyGlyHis complex with a Union Giken RA-1100 stopped-flow spectrophotometer. For a slower reaction of the 1:1 Ni(II)–GlyGlyHis (2×10^{-3} M) system, two times as much His was employed, and the reaction was followed by measuring the decrease of the peak at 425 nm with a Hitachi 323 spectrophotometer at 35 °C under O_2-free conditions. Since the pH of the solutions was unchanged within experimental error after each reaction, no buffer was used in particular to keep the pH constant in the ligand-substitution reactions. Rate constants were obtained by treating the data according to linear least-squares methods.

Table I. Stability Constant ($\log \beta_{pqrs}$) of the Ternary Complex Species $M_pH_qA_rB_s$[a]

species				$\log \beta_{pqrs}$		
p	q	r	s	A = His	A = Hm	A = Gly
1	1	1	1	22.31 ± 0.05	21.35 ± 0.08	
1	0	1	1	16.78 ± 0.07	16.45 ± 0.05	15.32 ± 0.19

[a] At 25 °C and 0.1 M (KNO_3) ionic strength. M = Cu^{2+}, H = a_H, A = completely deprotonated form of His, Hm, or Gly, and B = completely deprotonated form of GlyGlyHis.

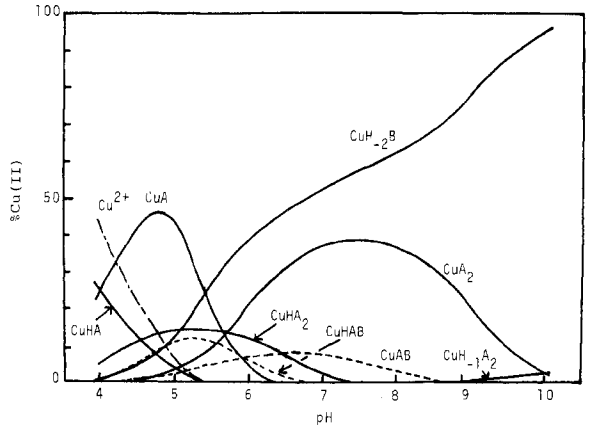


Figure 1. Species distribution in the Cu(II)–His–GlyGlyHis system as a function of pH in 0.1 M KNO_3 at 25 °C. $C_{Cu^{2+}} = C_{His} = C_{GlyGlyHis} = 2 \times 10^{-3}$ M.

Results

Solution Equilibria. Stability of the complexes is represented by the overall formation constants (β_{pqrs}) of the equilibrium reaction

$$pM + qH + rA + sB \overset{\beta_{pqrs}}{\rightleftharpoons} M_pH_qA_rB_s$$

$$\beta_{pqrs} = [M_p(a_H)_qA_rB_s]/[M]^p[a_H]^q[A]^r[B]^s$$

where p, q, r, and s are numbers of M (Cu(II)), H (proton activity), A (His, Hm, or Gly anion), and B (GlyGlyHis anion), respectively (charges are omitted). For the binary 1:1 Cu(II)–GlyGlyHis system, the calculation was satisfactorily converged by taking CuHB, CuB, $CuH_{-1}B$, and $CuH_{-2}B$ into account, and log β for each species was estimated to be 12.24 ± 0.08, 7.59 ± 0.05, 2.53 ± 0.07, and −1.98 ± 0.01, respectively.[7] Formation of the $CuH_{-2}B$ species reached almost 100% at pH 6, and the amounts of other species did not exceed 10% even in a more acidic solution. In accordance with the above, the titration solution appeared reddish even at the early stage of titration, and the color of the solution gradually became intensive with addition of alkali.

For the ternary Cu(II)–GlyGlyHis–His system, the calculations were accomplished by taking into account two ternary species, CuAB and CuHAB, in addition to the binary species such as $CuH_{-2}B$, $CuH_{-1}B$, CuB, CuHB, CuHA, CuA, Cu-

(1) (a) S.-J. Lau and B. Sarkar, *J. Biol. Chem.*, **246**, 5938 (1971); (b) B. Sarkar and T. P. A. Kruck in "The Biochemistry of Copper", J. Peisach, P. Aisen, and W. Blumberg, Eds., Academic Press, New York, 1966, p 183.
(2) (a) H. Aiba, A. Yokoyama, and H. Tanaka, *Bull. Chem. Soc. Jpn.*, **47**, 1437 (1974); (b) S.-J. Lau, T. P. A. Kruck, and B. Sarkar, *J. Biol. Chem.*, **249**, 5878 (1974); (c) R. P. Agarwal and D. D. Perrin, *J. Chem. Soc., Dalton Trans.*, 53 (1977).
(3) (a) T. P. A. Kruck and B. Sarkar, *Inorg. Chem.*, **14**, 2383 (1975); (b) T. Sakurai and A. Nakahara, *Inorg. Chim. Acta*, **34**, L245 (1979).
(4) T. Sakurai, O. Yamauchi, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, **51**, 3203 (1978).
(5) I. G. Sayce, *Talanta*, **15**, 1397 (1968).
(6) T. Sakurai and A. Nakahara, *Inorg. Chim. Acta*, **34**, L243 (1979).
(7) Prior to the determination of the stability of the binary complexes, pK_a values of the amino and imidazole groups of GlyGlyHis were determined to be 8.01 ± 0.01 and 6.87 ± 0.01, respectively.

Table II. Curve Resolution of Absorption and CD Spectra of the Binary Cu(II)-GlyGlyHis and Ni(II)-GlyGlyHis Systems

system		absorption		CD		assignt
		λ_{max}, nm	ϵ	λ_{max}, nm	$\Delta\epsilon$	
Cu-GlyGlyHis	obsd[a]	525	101	576	−0.22	
				494	0.62	
	resolved	571	36	566	−0.31	$d_{x^2-y^2} \leftarrow d_{xy}$
		522	40			$d_{x^2-y^2} \leftarrow d_{z^2}$
		497	53	494	0.63	$d_{x^2-y^2} \leftarrow d_{yz}, d_{xz}$
Ni-GlyGlyHis	obsd[a]	425	141	479	−1.32	
				410	2.06	
	resolved	473	45	469	−1.85	$d_{x^2-y^2} \leftarrow d_{xy}$
		433	65			$d_{x^2-y^2} \leftarrow d_{z^2}$
		407	85	413	208	$d_{x^2-y^2} \leftarrow d_{yz}, d_{xz}$

[a] 0.1 M (KNO_3) ionic strength; pH 7.5.

H_2A_2, $CuHA_2$, CuA_2, and $CuH_{-1}A_2$ (Table I).[8] A species distribution curve for the equimolar ternary Cu(II)–GlyGlyHis–His system (Figure 1) shows that there exist 38% CuA_2, 56% CuH_2B, and only 6% ternary species CuAB at physiological pH (7.4), whereas in higher pH region the GlyGlyHis-containing binary species $CuH_{-2}B$ exists predominantly. The result contradicts the display by Kruck and Sarkar,[3a] who suggested the predominant formation of ternary species $CuH_{-1}AB$ and $CuH_{-2}AB$ over neutral to alkaline pH regions. One may reach a result similar to that of Kruck and Sarkar by taking into account additional ternary species, $CuH_{-1}AB$ and $CuH_{-2}AB$, other than CuAB and CuHAB.[9] However, that is apparently a fault, since the absorption, CD, and ESR spectra of the ternary system are inconsistent with their conclusion (vide infra). On the other hand, Agarwal and Perrin[2c] have detected no ternary species for the same system containing an equimolar amount of GlyGlyHis and His. To clarify the details of the solution equilibrium for the Hm-containing ternary system, we also performed potentiometric titrations of the Gly- or Hm-containing ternary system. Since the result obtained from a 1:1:1 Cu(II)–GlyGlyHis–Gly or –Hm system shows no significant formation of the ternary species, we had to use two times as much Gly or Hm to derive the stability constants of the Gly- or Hm-containing ternary species. The collected data were treated according to a procedure similar to that described above, and the results thus obtained were listed in Table I.

Absorption and CD Spectra of the Binary Systems Containing GlyGlyHis and Cu(II) or Ni(II). The binary system Cu(II)–GlyGlyHis (1:1) (I = 0.1 (KNO_3)) exhibited almost identical absorption and CD spectra over a wide pH range (Figure 2), offering λ_{max} = 525 nm (ϵ 101) for the absorption spectrum and λ_{max} 574 nm ($\Delta\epsilon$ = −0.22) and λ_{max} = 494 nm ($\Delta\epsilon$ = 0.62) for the CD spectrum. The absorption curve was resolved into three Gaussian curves and the CD curve into two oppositely signed Gaussian curves corresponding to the d–d transitions with the highest and the lowest energy.[10] For the ideal D_{4h} symmetry, the transitions due to $d_{x^2-y^2}(b_{1g}) \leftarrow d_{xy}(b_{2g})$ and $d_{x^2-y^2}(b_{1g}) \leftarrow d_{yz}, d_{xz}(e_g)$ should be optically active, whereas $d_{x^2-y^2}(b_{1g}) \leftarrow d_{z^2}(a_{1g})$ should be optically inactive. Therefore the order of the increasing energy for the 3d orbitals in the copper complex is considered to be $d_{z^2-y^2} > d_{xy} > d_{z^2} > d_{xz} \sim d_{yz}$ (Table II). Such a highly square-planar character of the complex $CuH_{-2}B$ probably arises from the prominently strong ligand field offered by GlyGlyHis.

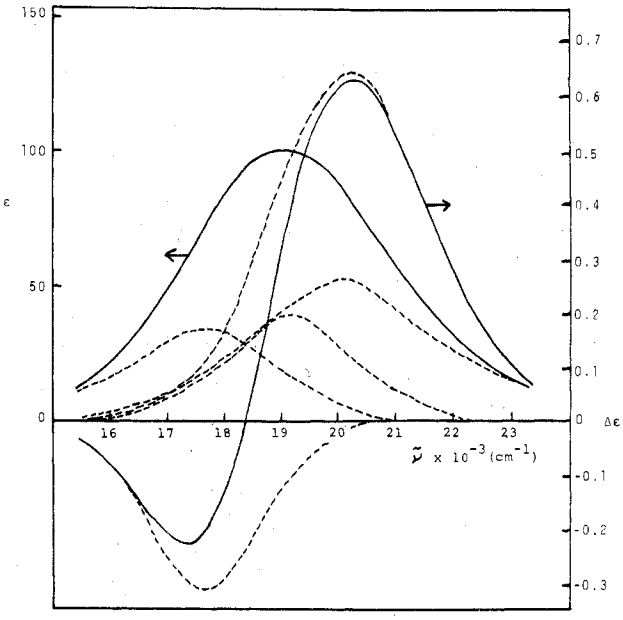


Figure 2. Absorption and CD spectra (solid lines) and their resolved Gaussian curves (broken lines) of the binary Cu(II)–GlyGlyHis system.

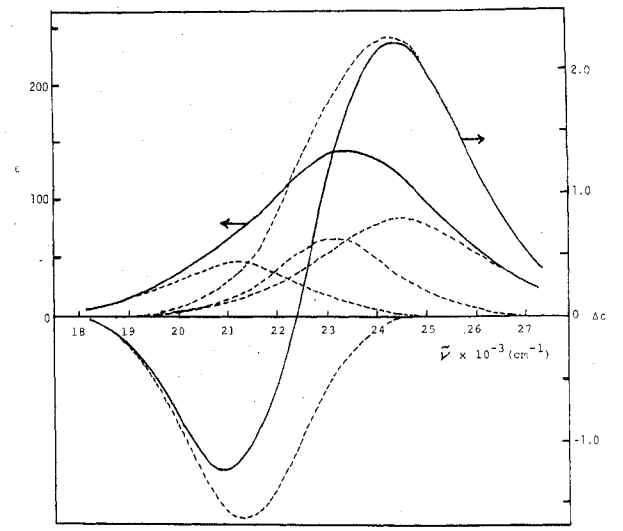


Figure 3. Absorption and CD spectra (solid lines) and their resolved Gaussian curves (broken lines) of the binary Ni(II)–GlyGlyHis system.

Of interest is the fact that the 1:1 Ni(II)–GlyGlyHis system exhibits quite similar absorption and CD spectral behaviors (Figure 3) though the peaks for the nickel(II) complexes shift

(8) The stability constants for the His-containing binary species (appearing in G. Brookes and L. D. Pettit, *J. Chem. Soc., Dalton Trans.*, 1918 (1977)) were used for the computations. log β for the particular species $CuH_{-1}A_2$ was determined to be 7.4. Those of Gly- and Hm-containing species were cited from A. E. Martell and R. M. Smith, "Critical Stability Constants", Vol. I, Plenum Press, New York, 1974.

(9) Concerning these sets of answers, we consider that the coincidence of spectral data and results obtained from a solution equilibrium study is necessary for the proper interpretation of the system. Although we included the $CuH_{-1}AB$ species for the calculation reported in ref 3b, the recent investigation expelled the possibility of that very species.

(10) The computer program for the curve resolution was kindly provided by Dr. T. Komorida of this university, to whom the authors' thanks are due.

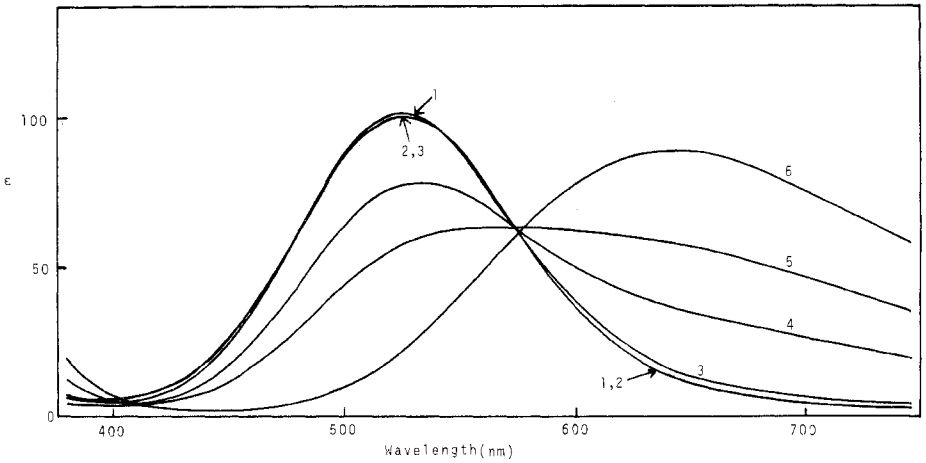


Figure 4. Absorption spectra of the binary and ternary Cu(II) systems: curve 1, Cu(II)–GlyGlyHis (1:1), pH 10.3; curve 2, Cu(II)–GlyGlyHis–His (1:1:1), pH 11.1; curve 3, Cu(II)–GlyGlyHis (1:1), pH 7.5; curve 4, Cu(II)–GlyGlyHis–His (1:1:1), pH 7.5; curve 5, Cu(II)–GlyGlyHis–His (1:1:2), pH 7.5; curve 6, Cu(II)–His (1:2), pH 7.3.

ca. 100 nm to the shorter wavelengths compared with those for Cu(II) complexes to give a more highly square-planar complex $NiH_{-2}B$. Apparently the GlyGlyHis around Ni(II) seems to have not only the same configuration but also very similar conformations as those in the copper complex $CuH_{-2}B$. All spectral data are tabulated in Table II.

Absorption and CD Spectra of the Ternary Systems Containing GlyGlyHis, His, and Cu(II) or Ni(II). Absorption and CD spectra of the ternary Cu(II)–GlyGlyHis–His system (Figures 4 and 5) unambiguously show the predominant formation of the binary species $CuH_{-2}B$ in the higher pH region, being coincident with the result of the solution equilibrium study represented in Figure 1. However, at neutral pH another peak due to the His-containing binary species CuA_2 appears at 634 nm in the absorption spectrum and at 688 nm in the CD spectrum. On the other hand, the ternary systems containing Hm or Gly instead of His show a predominant formation of $CuH_{-2}B$ even at neutral pH. An equimolar Ni(II)–GlyGlyHis–His system exhibits absorption and CD spectral features similar to those of the corresponding Cu-containing system (Figures 6 and 7). A careful inspection of the figures reveals ca. 50% formation of the yellowish highly square-planar complex $NiH_{-2}B$, which largely contributes to the absorption and CD spectra, and ca. 50% formation of the octahedral green species with a much smaller absorption coefficient ϵ at longer wavelengths.

ESR Spectra. The ESR spectrum for the Cu(II)–GlyGlyHis–His (1:1:1) system at high pH is quite similar to that for the binary system Cu(II)–GlyGlyHis (1:1) which exhibits pseudo-D_{4h} symmetry around the central Cu(II) ion,[11] while the spectrum at neutral pH is considerably complicated because of the presence of the three species $CuH_{-2}B$, CuA_2, and CuAB as revealed by the solution equilibrium study (Figure 8).

Ligand-Substitution Reaction of His, Hm, or Gly for GlyGlyHis. The reaction of $CuH_{-2}B$ with excess His was followed by the stopped-flow technique, and the rate constants for the decrease of the $CuH_{-2}B$ species were obtained at several pHs (Table III). It was disclosed that His is a much more effective ligand than Hm or Gly in the substitution reaction as expected. Of special interest is the fact that the higher the pH value is, the slower the reaction is. For Ni(II)-containing systems the ligand-substitution reactions were followed by the conventional spectroscopy. Also in these cases the ligand-substitution reactions were slower at high pH values as observed for the Cu-containing systems (Table IV).

(11) Y. Sugiura, *Inorg. Chem.*, **17**, 2176 (1978).

Table III. Observed Rate Constants (10^2k_{obsd}) for the Reaction of $CuH_{-2}B$ with His, Hm, or Gly[a]

ligand	$10^2 \times$ [ligand], M	10^2k_{obsd}, s^{-1} pH 7.5	pH 8.5	pH 10.0
His	1	1.29 ± 0.04	0.89 ± 0.02	0.33 ± 0.01
	2	2.93 ± 0.02	2.06 ± 0.01	0.67 ± 0.01
	3	4.56 ± 0.05	3.47 ± 0.02	0.95 ± 0.01
	5	8.10 ± 0.17	6.71 ± 0.04	1.56 ± 0.03
Hm	1	0.09 ± 0.01		0.04 ± 0.01
	2	0.21 ± 0.01		0.08 ± 0.01
	3	0.32 ± 0.01		0.12 ± 0.01
	5	0.76 ± 0.01		0.23 ± 0.01
Gly	5	0.23 ± 0.01		0.17 ± 0.01

[a] $[CuH_{-2}B] = 5 \times 10^{-4}$ M; 25 ± 1 °C; 0.1 M KCl.

Table IV. Observed Rate Constants for the Reaction of $NiH_{-2}B$ with His[a]

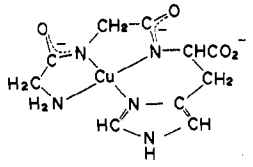
pH	k_{obsd}, s^{-1}	pH	k_{obsd}, s^{-1}
6.9	5.0×10^{-3}	8.7	2.0×10^{-4}
8.5	3.0×10^{-4}	9.1	1.7×10^{-4}

[a] $[NiH_{-2}B] = 5 \times 10^{-3}$ M; [His] = 10×10^{-3} M; 35 °C.

Discussion

Cu–GlyGlyHis Complex. In the binary Cu(II)–GlyGlyHis (1:1) system, the species $CuH_{-2}B$ exists predominantly over acidic to alkaline pH regions, suggesting that the complex $CuH_{-2}B$ is extremely stable. The stability of $CuH_{-2}B$ is estimated to be ca. 10^4 times as high as that of other copper(II)–tripeptide complexes,[12] being almost comparable to that of macrocyclic complexes.[13] The absorption maximum of the present system is observed at 525 nm, corresponding to the intermediate value between the absorption maxima of copper(II) tripeptides (λ_{max} = 550 nm) and copper(II) tetrapeptides (λ_{max} = 510 nm).[14] Tripeptides are generally coordinated around copper(II) through the amino nitrogen, two deprotonated amide nitrogens, and the carboxylate oxygen, whereas tetrapeptides are coordinated through the amino nitrogen and three deprotonated amide nitrogens. In the light of these facts, the structure of $CuH_{-2}B$ is considered to be represented as **1**, which is also suggested by other workers[2] and X-ray crystal analysis of a similar complex, copper(II) glycylglycyl-L-histidine *N*-methylamide.[15]

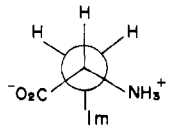
(12) See the work of Martell and Smith cited in ref 8.
(13) M. Kodama and E. Kimura, *J. Chem. Soc., Dalton Trans.*, 325 (1979).
(14) J. M. Tsangaris and R. B. Martin, *J. Am. Chem. Soc.*, **92**, 4255 (1970).



I

Formation of such a stable complex as $CuH_{-2}B$ with a highly square-planar character is reasonably understood from the energy order of the 3d orbitals $x^2 - y^2 > xy > z^2 > yz \sim xz$ as determined by the curve analysis of the absorption and CD spectra (see the Experimental Section). The energy level order of d_{z^2} and d_{xy} here is the reverse of those for copper(II)-tripeptide systems.[14]

The Ni(II)-GlyGlyHis system is interpreted in a similar way though $NiH_{-2}B$ has even stronger planar character than $CuH_{-2}B$ because of the lack of electrons in the $d_{x^2-y^2}$ orbital. In the ^1H NMR spectrum of the Ni(II)-GlyGlyHis system, the coupling constant between α-methine and β-methylene protons in the His residue was observed to be 4 Hz, which is indicative of the coupling between gauche protons.[3b,16] Therefore, the favorable conformation of the His residue can be depicted as **2**. The space-filling model suggests pertinency



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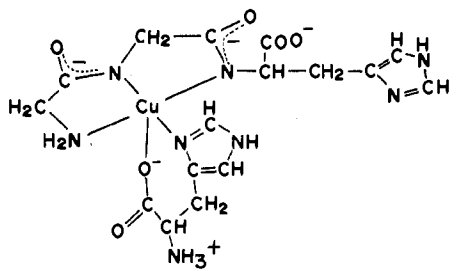
and rigidity of the conformation. The similarity of absorption and CD spectra of Cu(II)- and Ni(II)-GlyGlyHis systems affirms the expectation that GlyGlyHis has similar conformations in both Cu and Ni complexes.

Cu-GlyGlyHis-His Complex. An analysis of the pH titration data of the ternary system gave three sets of answers, which differ from one another in ternary species but not in the binary species involved: the first answer involves CuHAB and CuAB, the second one CuHAB, CuAB, and $CuH_{-1}AB$,[3b] and the third one CuHAB, CuAB, $CuH_{-1}AB$, and $CuH_{-2}AB$.[3a] Among these, the first answer is most probable, and the other two are excluded as improbable from several spectral behaviors as cited under Results. The absorption, CD, and ESR spectra for the ternary systems apparently indicate the predominant formation of the binary species $CuH_{-2}B$ and CuA_2 in neutral solution and only $CuH_{-2}B$ in basic solution (Figures 4, 5, and 8). If the third answer, where Kruck and Sarkar[3a] suggested the predominant formation of $CuH_{-1}AB$ at neutral pH and $CuH_{-2}AB$ at basic pH, were the real one, the spectral properties of $CuH_{-2}AB$ and $CuH_{-1}AB$ would have to be identical with those of $CuH_{-2}B$ in order to explain the mentioned spectral properties. The species $CuH_{-1}AB$, whose structure was figured as **3** by Kruck and Sarkar, should exhibit CD



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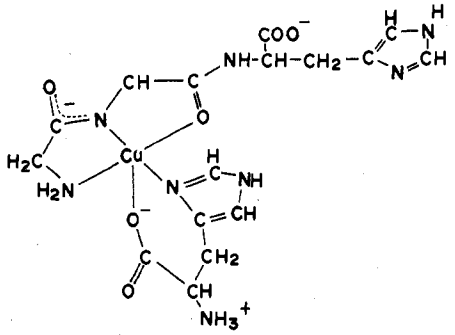
bands of much smaller intensities since the His residue in GlyGlyHis coordinates only through the amide nitrogen. Further, if the ternary species $CuH_{-1}AB$ exhibits an absorption maximum at 540 nm as suggested by them,[3a] the species should contain intermediate g_0 and the nuclear hyperfine structure constant A_0 values,[17] making the ESR spectrum more complicated. Accordingly the third possibility is reasonably excluded. For the same reason, no spectroscopic evidence supports the second answer, for which we suggested the existence of ca. 20% formation of $CuH_{-1}AB$ in the ternary system (1:1:1) in a previous communication.[3b] Inspection of Figure 4 reveals that an approximately isosbestic behavior is observed in the absorption spectra of the ternary systems. Thus each curve of the ternary system can be almost satisfactorily simulated by taking only two species into consideration. The same is also true for the CD and ESR spectra (Figures 5 and 8). This excludes the second answer, supporting the first one in conclusion.

In line with this, all species except $NiH_{-2}B$, which exists in neutral solutions of the Ni(II)-containing ternary systems, were found to have very small absorption coefficients at the longer wavelengths as is evident from Figure 6. The ternary species, even though they might really exist, make no exceptions to the above observation. In the ternary complexes, a tridentate ligand such as His is considered to favor the octahedral structure with much smaller ϵ and $\Delta\epsilon$ values compared with those of the square-planar complexes such as $NiH_{-2}B$. As log β values of the CuAB species are 16.78, 16.45, and 15.32 for His-, Hm-, and Gly-containing ternary systems, respectively, it is evident that the imidazole group increases the stability of the His-containing ternary complex, whose structure might be represented as **4** and **5**, though it seems to be difficult to predict which of the two structures is more probable.



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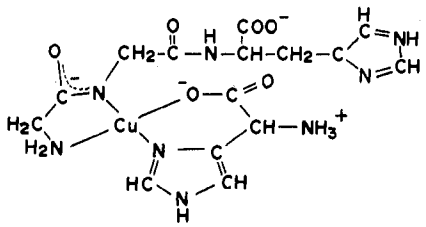

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Biological Significance. GlyGlyHis binds copper(II) in the same manner as the native albumin as reflected in the similar spectroscopic data: λ_{max} = 525 nm (ϵ 101) for the Cu(II)-GlyGlyHis system; λ_{max} = 525 nm (ϵ 101) for the copper-albumin system. Other albumin model compounds, glycylglycyl-L-histidine *N*-methylamide[18] and L-aspartyl-L-alanyl-L-histidine *N*-methylamide,[19] also exhibit similar absorption

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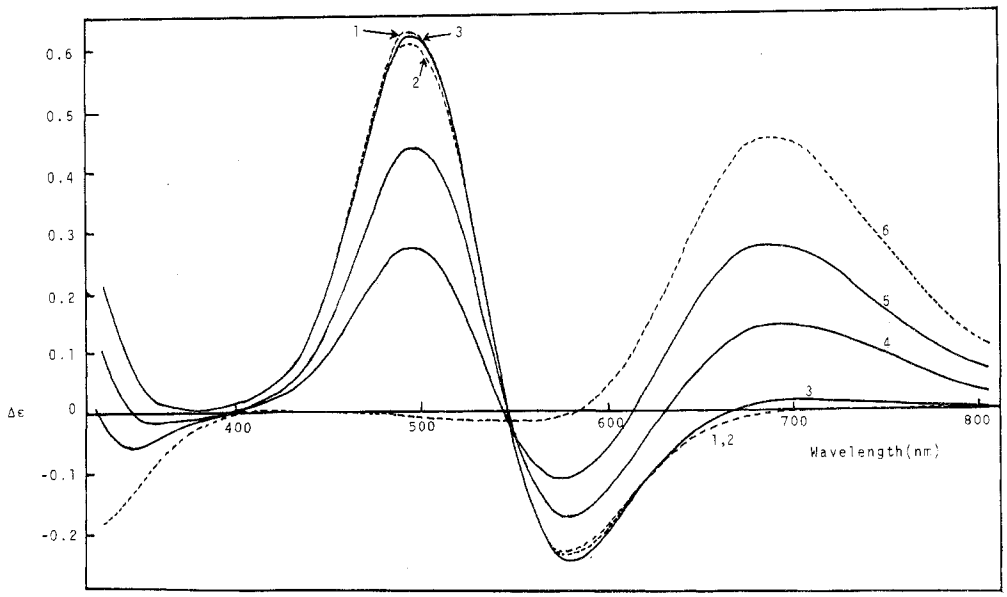

Figure 5. CD spectra of the binary and ternary Cu(II) systems: curve 1, Cu(II)–GlyGlyHis (1:1), pH 10.3; curve 2, Cu(II)–GlyGlyHis (1:1), pH 7.5; curve 3, Cu(II)–GlyGlyHis–His (1:1:1), pH 11.1; curve 4, Cu(II)–GlyGlyHis–His (1:1:1), pH 7.5; curve 5, Cu(II)–GlyGlyHis–His (1:1:2), pH 7.5; curve 6, Cu–His (1:2), pH 7.3.

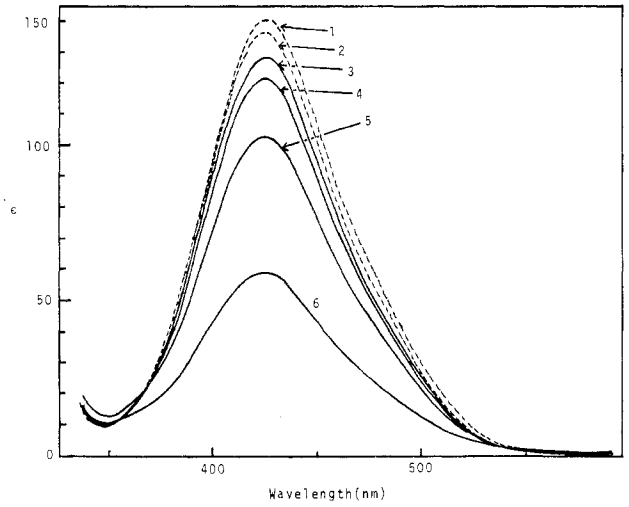

Figure 6. Absorption spectra of the binary and ternary Ni(II) systems: curve 1, Ni(II)–GlyGlyHis (1:1), pH 10.9; curve 2, Ni(II)–GlyGlyHis (1:1), pH 7.5; curve 3, Ni(II)–GlyGlyHis–Gly (1:1:1), pH 7.5; curve 4, Ni(II)–GlyGlyHis–Hm (1:1:1), pH 7.6; curve 5, Ni(II)–GlyGly-His–His (1:1:1), pH 11.4; curve 6, Ni(II)–GlyGlyHis–His (1:1:1), pH 7.4.

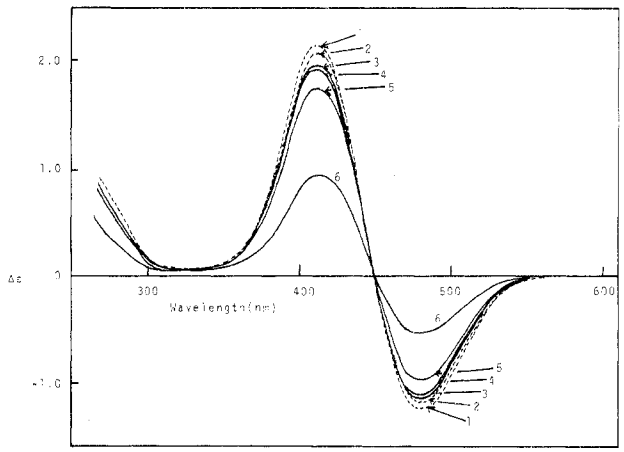

Figure 7. CD spectra of the binary and ternary Ni(II) systems: curve 1, Ni(II)–GlyGlyHis (1:1), pH 10.9; curve 2, Ni(II)–GlyGlyHis (1:1), pH 7.5; curve 3, Ni(II)–GlyGlyHis–Gly (1:1:1), pH 7.5; curve 4, Ni(II)–GlyGlyHis–Hm (1:1:1), pH 7.6; curve 5, Ni(II)–GlyGly-His–His (1:1:1), pH 11.4; curve 6, Ni(II)–GlyGlyHis–His (1:1:1), pH 7.4.

characteristics, indicating that copper-binding abilities of albumin and the model compounds are based on the peculiar mode of coordination at physiological pH. Equilibrium dialyses suggested that the binding ability of these model compounds for copper(II) is comparable with that of the native albumin.[2b,3a]

In spite of the above findings, the mechanism of copper transport in blood has not been thoroughly investigated. Since there exists a considerable amount of albumin (ca. 10^{-4} M) together with amino acids (8.5×10^{-5} M His), the most exchangeable coppers in blood (ca. 10^{-6} M) are considered to be bound almost exclusively to albumin.[1] A computer simulation study[20] has also suggested the predominant binding of copper with albumin in contrast to the expectation of Kruck and Sarkar,[3a] who pointed out the predominant formation of the ternary copper–albumin–histidine complex from the solution equilibrium study. The present study reasonably makes us expect the preponderate binding of Cu only with albumin at physiological pH in accordance with the biological systems.

The reaction of $Cu^{II}GlyGlyHis$ with excess His is faster at lower pH in spite of decreasing population of completely deprotonated His, which is considered to be favorable for the substitution reaction (Table III). The same trend was also observed in the ligand-substitution reactions with Hm or Gly although the observed rates were far from those with His. The rate–pH relationship of the substitution reaction may arise from the degree of protonation of GlyGlyHis around copper(II), since the protonation of GlyGlyHis is an invitation to a weakening of the planar character of the complex $CuH_{-2}B$ and the complex may become susceptible to the attack of His.[21] The displacement of triethylenetetramine from the coordina-

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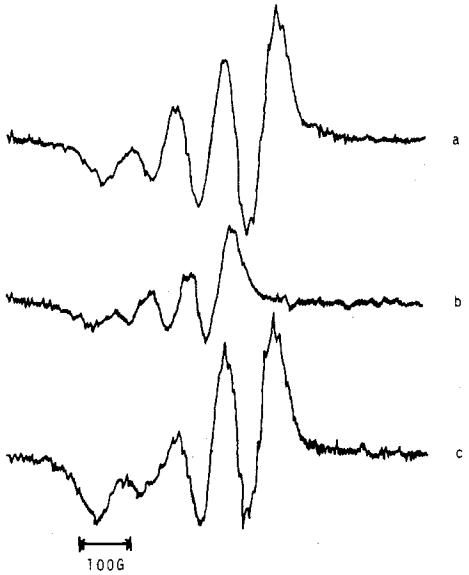

Figure 8. ESR spectra of the binary and ternary Cu(II) systems containing GlyGlyHis and His in neutral pH solutions: curve a, Cu(II)–GlyGlyHis; curve b, Cu(II)–His; curve c, Cu(II)–GlyGlyHis–His.

tion site of the copper(II) complex with His was faster at higher pH than at lower pH.[22] This fact strongly suggests that the completely deprotonated species of His becomes more effective for its nucleophilic attack on the complex $CuH_{-2}B$. A direct attack of His on the highly square-planar complex $CuH_{-2}B$ seems to be difficult since His probably in the first step has to approach the complex from an apical direction. On the other hand, the route involving dissociation of GlyGlyHis does not seem important because the reaction rates were not essentially affected even by the presence of a slight excess of GlyGlyHis, which is expected to ensure the complete complex formation. The ligand-exchange rates were approximately first order with respect to the concentration of His in the presence of 100- to 500-fold excess His, strongly indicating that the step to form the ternary complex of the structures shown as **4** and **5** is a rate-determining one. As is suggested from the foregoing argument, the His molecule may not attack the completely deprotonated copper-binding site of albumin in the ligand displacement but may attack a partially protonated one. Once copper is bound with His, another amino acid may quite easily expel albumin from the coordination sphere to form an amino acid complex of low molecular weight. The resulting amino acid complexes can easily migrate to tissues through biological membranes. As for such amino acid complexes, we intend to point out a high probability of the binary His complex and some ternary complexes containing His and a specific amino acid such as threonine, serine, asparagine, or glutamine with polar side-chain groups which are necessary to form potential intramolecular hydrogen bonding.[23] The equilibrium between Cu(II), serum albumin, and amino acids might be sufficiently fast to realize rapid equilibrium of copper between blood and tissues in living systems, where a statistical factor rather than a structural factor may play a vital role in the formation of intermediary copper–albumin–amino acid complexes.

Acknowledgment. We are grateful to Miss Yoshimi Inagaki for technical assistance. The computations of the stability constants were performed at the Osaka University Computer Center. The financial support of the Takeda Science Foundation and the Ministry of Education of Japan is gratefully acknowledged.

Registry No. $CuH_{-2}B$, 53554-01-1; $NiH_{-2}B$, 60165-85-7; His, 71-00-1; Hm, 51-45-6; Gly, 56-40-6.

(22) The values of $10^2 k_{obsd}$ (s^{-1}) for the reactions of 5×10^{-4} M Cu^{II}trien and 2×10^{-2}, 3×10^{-2}, and 5×10^{-2} M His were 7.67, 17.45, and 52.11 at pH 7.5 and 1074, 2280, and 3301 at pH 10.0, respectively.

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Cyanopentaamminechromium(III). Synthesis, Characterization, and Aquation Kinetics

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The $Cr(NH_3)_5(CN)^{2+}$ cation was synthesized by cyanide anation of $Cr(NH_3)_5(Me_2SO)^{3+}$ in dimethyl sulfoxide (Me_2SO) solution. The complex was isolated as the perchlorate salt and characterized by conductance, an ion-exchange technique, and infrared and electronic spectra. The ligand-field maxima agree with previous theoretical predictions. A vibrational structure on the charge-transfer band is consistent with a metal → ligand transition. The complex phosphoresces in aqueous solution at room temperature. The kinetics of CN^- aquation were studied over a wide range of acidities ((5×10^{-4})–2.0 M $HClO_4$) and ionic strengths (0.5–2.0 M $NaClO_4$). Cyanide release proceeds through two pathways: one is acid independent while the other is acid catalyzed and follows equilibrium protonation of the complex. The latter reaction path ($k_H = 1.5 \times 10^{-2}$ s^{-1} at 25 °C and $\mu = 2.0$) predominates largely over the former ($k_0 < 10^{-7}$ s^{-1} at 25 °C). The activation enthalpy for k_H (17.2 kcal mol^{-1}) is unusually low. Proton uptake by coordinated CN^- occurs to a considerable degree under the experimental conditions ($K = 0.28$ M^{-1} at 25 °C and $\mu = 2.0$). The ionic strength enhances the complex protonation but decreases the rate of acid-assisted aquation.

Introduction

Acidopentaammine complexes of chromium(III) have been extensively studied, especially with regard to their thermal[1,2] and photochemical[3] aquation reactions. Despite the wealth of information available on $Cr(NH_3)_5X^{2+}$ species, with X = halogeno,[1,2] pseudohalogeno,[1,2] carboxylato,[4,5] and oxo-

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