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Structural Studies of Cyclo(L-histidyl-L-histidyl) and Its Metal Complexes

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The crystal structure of cyclo(L-histidyl-L-histidyl) was determined by X-ray diffraction techniques, and was refined to a final R index of 0.066 for 680 reflections. This showed that one imidazolyl group of this compound faces the piperazine ring(folded) whereas the other is kept away from the ring(unfolded). A calculation using the α-β coupling-constant data of this peptide obtained by an ^1H NMR measurement shows that the ratio of a folded conformation to an unfolded one is 49:51 in D_2O. Also, NMR investigations of zinc(II) and silver(I) complexes with this peptide were carried out in D_2O.

Cyclic dipeptides(3,6-disubstituted 2,5-piperazinedione) are the simplest cyclic peptides of biological interest. The cyclic character of 2,5-piperazinedione simplifies the interpretation of NMR and other spectroscopic data and greatly limits the number of the preferred conformations. Numerous cyclic dipeptides have been studied in solution using NMR measurements.[1] Some of them have been studied the solid state by X-ray structural analyses.[2]

The most interesting result from the studies of cyclic dipeptides is that molecules containing an aromatic side chain (such as phenylalanyl, tyrosyl, and histidyl residues) tend to have a folded conformation with the aromatic ring facing the piperazine ring.[1a] An interaction between the aromatic ring and the piperazine ring (probably a dipole-induced dipole one) appears to have a great influence on the overall conformation of cyclic dipeptides.[2a]

We have mainly engaged in a study[3] of the metal complexes of cyclic dipeptides, including a histidyl residue in the solid state and in solution using X-ray diffraction methods and NMR techniques, respectively. These peptides are good model compounds for metalloproteins.

In a series of studies of the metal complexes of cyclic dipeptides, we suggested that the conformation of the cyclic dipeptide greatly affects the formation and conformation of the metal complex.[3]

This paper describes the X-ray crystallographic structure of cyclo(L-histidyl-L-histidyl)(abbreviated to CHH)·$5/2H_2O$ with two imidazole rings on the same side of the piperazine ring. Also, the conformations of CHH and its metal complexes in an aqueous solution are discussed using NMR (^1H and ^{13}C) and the molecular-weight data.

Experimental

CHH and its silver(I) complexes were prepared according to methods reported earlier.[3a]

Preparations of Zinc(II) Complexes. A mixture of CHH (0.70 mmol) and zinc(II) nitrate (0.35 mmol) was completely dissolved in hot water (70 mdm^3). After standing at room temperature for a few days, a formed precipitate was filtered off. Mp 240—260° dec., Yield; 95%. Found: C, 36.87; H, 4.11, N; 24.65%. Calcd for Zn $(C_{12}H_{14}N_6O_2)_2(NO_3)_2\cdot 5/2H_2O$: C, 36.81; H, 4.25; N, 25.04%. Another complex was similarly obtained by the above procedure. Mp 209—222° dec., Yield; 85%. Found; C, 33.04; H, 3.65; N, 19.08%. Calcd for Zn-$(C_{12}H_{14}N_6O_2)_2(ClO_4)_2\cdot 7/2H_2O$: C, 32.91; H, 4.03; N, 19.19%.

X-Ray Crystallographic Procedure. A crystal (0.4×0.2×0.04 mm) grown from slow evaporation of an aqueous solution was used for data collection. Crystal data: $C_{12}H_{14}N_6O_2\cdot 5/2H_2O$ for CHH·$5/2H_2O$: M=319.3, orthorhombic, space group $P2_12_12$. On the basis of 20 reflections, the following unit-cell parameters were obtained: a=19.91(1), b=12.66(1), c=6.163(3) Å, V(for Z=4)=1553(2) Å^3, D_c=1.56 gcm^{-3}, and μ(Mo $K\alpha$)=1.31 cm^{-1}.

All unique diffraction maxima with $2°\leq 2\theta \leq 48°$ were recorded in the ω-scan mode using a computer-controlled four-circle diffractometer and graphite monochromated Mo $K\alpha$(0.7107 Å) X-rays. Of the 1448 reflections surveyed, 680 (47%) were judged observed ($I>3\sigma(I)$) after correction for Lorenz, polarization and background effects. No absorption correction was applied.

The structure was solved by MULTAN.[4a] All hydrogen atoms were located on difference electron density syntheses. Full matrix least-squares refinements of positional and anisotropic thermal parameters for non-hydrogen atoms and of positional parameters only for hydrogen atoms (to which isotropic temperature factors 1.0 Å^2 greater than those of the atoms bonded to them were assigned) led to a convergence at R=0.066 (R_w=0.054).[4b] Neutral atomic-scattering factors of Cromer and Waber[5] were used for all atoms.[6]

Other Measurements. The ^1H and ^{13}C NMR data were obtained in D_2O at 50°C using sodium 3-trimethylsilyl-1-propanesulfonate as an internal reference. The molecular weights of the zinc(II) complexes were measured in H_2O at 56°C using urea as a standard material. JEOL FX-100 (for ^1H and ^{13}C NMR spectra), PS-100 (for ^1H NMR spectra), and Knauer Vapor Pressure Osmometer (for molecular weight) apparatus were used for the measurements of the samples.

Results and Discussion

Crystal and Molecular Structures of CHH·$5/2H_2O$. The standard peptide notations and bond distances are shown for CHH in Fig. 1. All bond distances and angles agree well with the values for other related compounds.[2] A stereoscopic view of the molecular packing in a unit cell is shown in Fig. 2.[4c] There are nine protons (four from CHH and five from water molecules) available for hydrogen bonding, all of

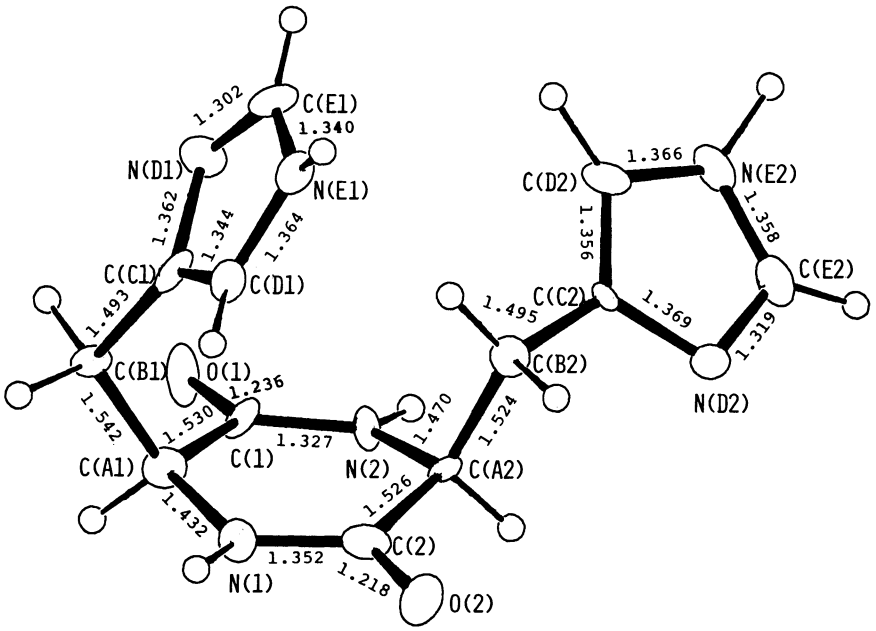

Fig. 1. Peptide notations and bond distances in cyclo(L-histidyl-L-histidyl). Standard deviations are 0.011—0.016 Å for all bond distances.

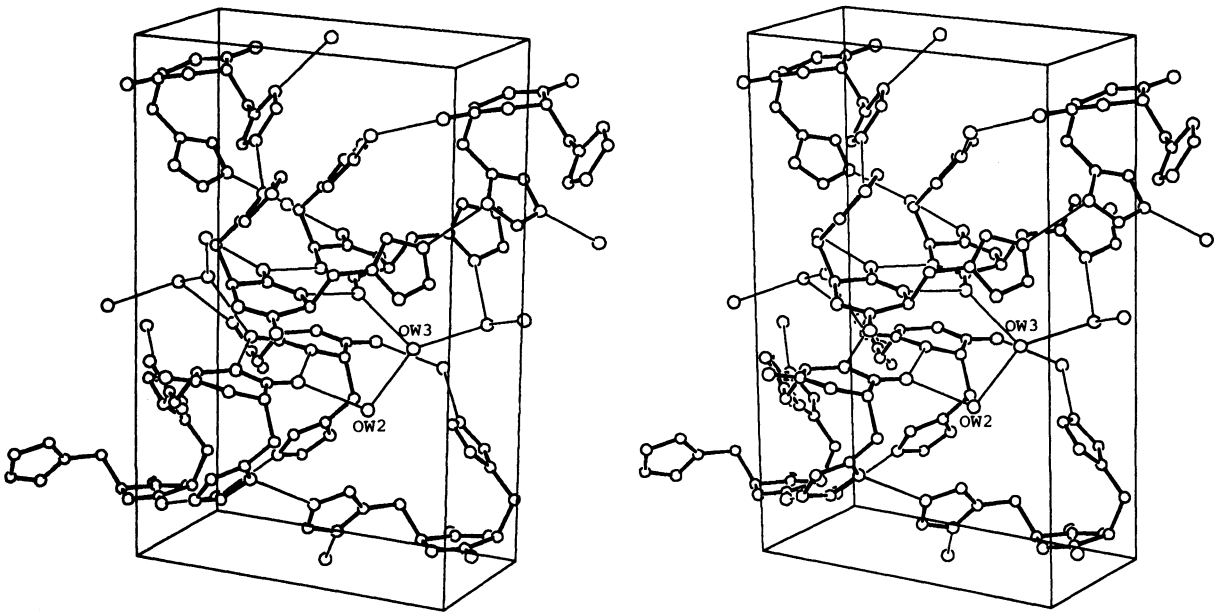

Fig. 2. Stereopicture of the molecular packing in cyclo(L-histidyl-L-histidyl). Thin lines indicate hydrogen bonding.

which are involved in intermolecular hydrogen bonds. Hydrogen bonds of the N-H···O type bind translationally equivalent piperazine rings in ribbons parallel to the c axis. Pairs of ribbons lie face-to-face across a two-fold axis such that one ribbon is connected to another ribbon through a O(1)···OW2···OW3···O(2) hydrogen-bonding scheme.

N(1), C(1), N(2), and C(2) atoms of the piperazine ring are coplanar within 0.03(1) Å with C(Al) and C (A2) deviate from this plane by 0.16(1) and 0.19(1) Å, respectively. The interplanar angles between the plane defined by N(1), C(1), N(2), and C(2) and the planes defined by C(1), C(Al), and N(1) and defined by C(2), C(A2), and N(2) are 12(1) and 13(1)°, respectively. Thus, the piperazine ring has a 'flagpole boat' conformation with cis α-carbon substituents quasi-axial. Both imidazole rings are planar within 0.02(1) Å, making angles of 56.8(5) and 46.6(5)° with the piperazine ring, respectively. Selected dihedral angles are given in Table 1. One imidazole ring is folded back over the piperazine ring with another imidazole ring kept away from the piperazine ring. This conformation is commonly found in 2,5-piperazinediones with aromatic residues.[2] The folded conformation of CHH with a 'flagpole boat' piperazine ring makes the intimate contacts between the imidazole ring and the piperazine ring possible and is compared with cyclo(glycyl-L-tyrosyl)[2a] and cyclo(L-leucyl-L-histidyl).[2e]

The short intramolecular contacts between the atom, five or more bonds apart are for N(1)···C(D1)= 3.26(1), C(1)···N(D1)=3.52(1), C(B2)···N(D1)= 3.87(1), C(B2)···N(E1)=3.74(1), C(B2)···C(D1)= 3.68(2), C(B2)···C(E1)=3.87(2), and C(2)···C(D1)=3.54(1) Å.

NMR of CHH and its Metal Complexes in D_2O. Table 2 shows β-proton chemical shifts, α-β coupling constants, and side-chain conformations for CHH and its metal complexes in D_2O. There are three possible conformations of CHH in solution. These conformations are described as follows: a folded-unfolded conformation (the same conformation in solid state), a folded-folded conformation (both imidazole rings hang over the piperazine ring) and an unfolded-unfolded (both imidazole rings are kept away from the piperazine ring). The results (folded/unfolded=49/51) for CHH shown in Table 2 appear to be consistent with a folded-unfolded conformation of CHH, but do not exclude the remaining two conformations when these two conformational isomers are present in an almost equal amount. According to the molecular structure of solid CHH, a folded-folded conformation is unlikely since the rotation of the unfolded imidazole ring around the C(A2)-C(B2) bond into a folded position produces unusual short contacts between the two imidazole rings. Cyclic dipeptides with aromatic rings usually prefer a folded conformation, but not an unfolded one in solution, as known from NMR studies.[1a] Thus, the major conformers of CHH in solution is a folded-unfolded one, which is similar to that of the solid state.

The ^1H NMR data in Table 2 indicates that the ratio of a folded form to an unfolded one is 8:92 for an $Ag(CHH)^+$ ion. Furthermore, the extremely low solubility of silver(I) complexes with CHH in protic and aprotic solvents suggests the preference of a polymeric form.

In addition, Table 2 shows that the chemical shift (2.7—3.1 ppm) of β-protons of a zinc(II) complex with CHH almost does not change from those (2.39 and 2.86 ppm) of CHH in spite of the broad and unresolved signals. Therefore, the side chain conformations of a zinc(II) complex are essentially identified with those of CHH. The broad and unresolved signal may be attributed to the rapid rotations about α-β carbon single bonds and the exchangeable coordination of nitrogen atoms [N(D1) or N(E1) and N(D2) or N(E2)] of imidazolyl groups to Zn^{2+} ions.

Table 3 shows the data of ^1H and ^{13}C NMR for CHH and its zinc(II) complex. It is suggested from Table 3

Table 1. Dihedral angles in cyclo(L-histidyl-L-histidyl)

Dihedral angle	Notation*	φ/°
C(2)-N(1)-C(A1)-C(1)	ϕ_1	17(1)
N(1)-C(A1)-C(1)-N(2)	ψ_1	−11(1)
C(A1)-C(1)-N(2)-C(A2)	ω_1	−8(1)
C(1)-N(2)-C(A2)-C(2)	ϕ_2	19(1)
N(2)-C(A2)-C(2)-N(1)	ψ_2	−12(1)
C(A2)-C(2)-N(1)-C(A1)	ω_2	−5(1)
N(1)-C(A1)-C(B1)-C(C1)	χ_1^1	58(1)
C(1)-C(A1)-C(B1)-C(C1)		−71(1)
C(A1)-C(B1)-C(C1)-N(D1)	χ_1^2	102(1)
C(A1)-C(B1)-C(C1)-C(D1)		−80(1)
N(2)-C(A2)-C(B2)-C(C2)	χ_2^1	−66(1)
C(2)-C(A2)-C(B2)-C(C2)		170(1)
C(A2)-C(B2)-C(C2)-N(D2)	χ_2^2	−63(1)
C(A2)-C(B2)-C(C2)-C(D2)		118(1)

*See Ref. 7.

Table 2. β-Proton chemical shifts, α-β coupling constants, and side chain conformation for cyclo(L-histidyl-L-histidyl) and its metal complexes in D_2O at 50°C

Compound	β-proton chemical shifts δ(Δδ[a])(ppm) high	low	α-β coupling constants Hz $J_{\alpha h\beta}$	$J_{\alpha l\beta}$	side chain conformations[b] F	U_I	U_{II}
CHH	2.39[c]	2.86[c]	6.6	4.2	49	15	36
$Zn(CHH)_2^{2+}$[d]	2.7—3.1[e]						
$Ag(CHH)^+$[f]	3.25[c] (0.86)	3.32[c] (0.46)	7.7	7.7	8	46	46

a) Δδ=δ[silver(I) complex ion]−δ(free ligand). b) Expressed in %; for notations and calculctions see Ref. 1. c) β-Methylene[C(B1) or C(B2)]and α-methine[C(A1) or C(A2)] protons of histidyl residues exhibit an ABX spin system, in which A and B are the β-protons and X is the α-proton. e) Broad and unresolved signals. f) $[Ag(CHH)(PF_6)]_n$ was used for the measurement.

Table 3. ^1H and ^{13}C chemical shifts(ppm) of CHH and $Zn(CHH)_2^{2+}$ in D_2O at 50°C

Compound	Chemical shift, δ(Δδ[a]) ^1H[b] HCAn	HCDn	HCEn	^{13}C[b] C(n)	C(An)	C(Bn)	C(Cn)	C(Dn)	C(En)
CHH	4.26	6.89	7.71	171.4	57.2	33.7	134.3	120.8	138.8
$Zn(CHH)_2^{2+}$[c]	4.35	6.90	7.97	171.5	57.0	32.1	131.6	122.9	139.1
	(0.09)	(0.01)	(0.26)	(0.1)	(−0.2)	(−1.6)	(−2.7)	(2.1)	(0.3)

a) Δδ=δ[zinc(II) complex ion]−δ(free ligand) b) n=1 and 2. c) $Zn(CHH)_2(NO_3)_2\cdot 5/2H_2O$ was used for the measurement.

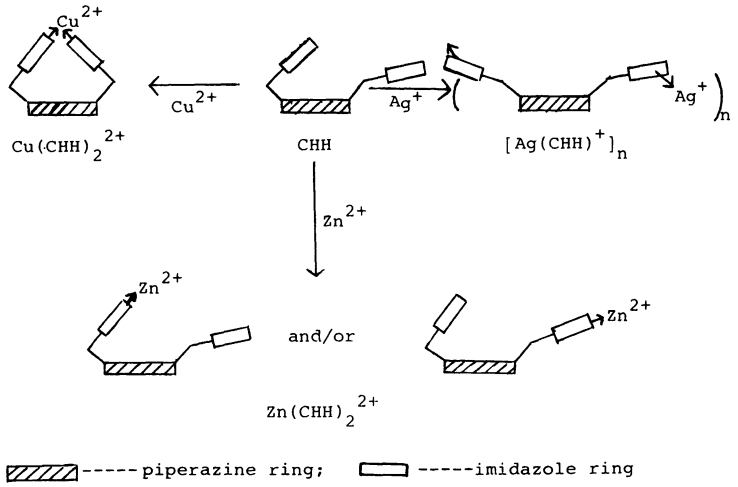

Fig. 3. The predominant conformations of cyclo(L-histidyl-L-histidyl) in complexations of Cu^{2+}, Ag^{+}, and Zn^{2+} ions.

that the imidazole nitrogen atom [N(Dn) or N(En); n=1 or 2] links to the Zn^{2+} ion on the basis of the up- or down-field shifts ($\Delta\delta$=0.26, −1.6, −2.7, and 2.1) of HCEn, C(Bn), C(Cn), and C(Dn) (n=1 and 2) of $Zn(CHH)_2^{2+}$ from CHH, respectively. This distinct shift may be due to the redistribution of electron densities owing to the binding of the imidazole nitrogen atom to the Zn^{2+} ion.[1c] On the other hand, it is indicated that the oxygen atoms of 2,5-piperazinedione do not coordinate to the Zn^{2+} ion on the basis of no distinct shifts ($\Delta\delta$=0.09, 0.1, and −0.2) of HCAn, C(n), and C(An)(n=1 and 2) of $Zn(CHH)_2^{2+}$ from CHH, respectively. Also, the earlier NMR measurements suggested that the imidazole nitrogen atom of CHH coordinates to the Ag^{+} ion, exclusively.[3a]

The molecular weights of zinc(II) complexes were obtained as follows. The perchlorate and nitrate of the $Zn(CHH)_2^{2+}$ ion are a monomeric form, and dissociate only into a complex ion and two anions: $Zn(CHH)_2^{2+}+2ClO_4^-$ (Calcd 813/3=271, Found 304) or $Zn(CHH)_2^{2+}+2NO_3^-$ (Calcd 738/3=246, Found 266). The molecular weight of a silver(I) complex could not be measured because of its extremely low solubility.

X-Ray, molecular weight, ^1H NMR, and CD studies of a copper(II) complex with CHH(reported earlier[3c]) reveal that both of the folded imidazole rings of CHH link to the Cu^{2+} ion, forming a 13-membered chelate rings by the N(E1) and N(E2) atoms. This observation is attributed to the coordination of CHH for the Cu^{2+} ion because of the too short distance(2.76 Å) between N(E1) and N(E2) makes the existence of a folded-folded conformer of free CHH impossible. In the case of the silver(I) complex ion (as described above) CHH prefers two unfolded imidazole rings whose nitrogen [N(Dn) or N(En); n=1 or 2] linearly coordinates to the Ag^{+} ion to give a polymeric material. In the zinc(II) complex ion, CHH has a folded imidazole ring and an unfolded one. However, at this stage, it is difficult to determine which (folded or unfolded) imidazole ring coordinates and, furthermore, which [N(Dn) or N(En); n=1 or 2] nitrogen atom of the imidazole ring links to the Zn^{2+} ion.

From a series of experimental results regarding CHH and its metal complexes, the predominant conformations of CHH in complexations of Cu^{2+}, Ag^{+}, and Zn^{2+} ions are assumed as shown in Fig. 3.

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6) Fractional coordinates (Table 1), bond angles (Table 2), and observed and calculated structure factors (Table 3) are deposited as Document No. 8508 at the Office of the Editor of Bull. Chem. Soc. Jpn.

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