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Copper(II) Complexes of Glycyl-L-histidine, Glycyl-L-histidylglycine, and Glycylglycyl-L-histidine in Aqueous Solution

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The complex formation of glycyl-L-histidine, glycyl-L-histidylglycine, and glycylglycyl-L-histidine with copper (II) ion in aqueous solutions containing equimolar amounts of copper(II) and the respective ligand was investigated by potentiometric and visible spectrophotometric methods. The presence of the following copper (II) complex species was assumed: $CuLH^{2+}$, CuX, and $Cu_nY_n^{n-}$ for glycyl-L-histidine-copper(II) and glycyl-L-histidylglycine-copper(II) systems, and $CuLH^{2+}$, CuY^-, and CuZ^{2-} for glycylglycyl-L-histidine-copper(II) system, where LH is the neutral species of the ligand and $LH=XH_2=YH_3=ZH_4$. The formation and ionization constants were obtained graphically, and the distribution of copper(II) among the complex species was calculated for each system. The results indicate that copper(II) ions are bound to the imidazole and carboxyl (or carbonyl) groups in $CuLH^{2+}$ and to the amino, imidazole, and deprotonated amide groups in CuX, CuY^-, and CuZ^{2-}. The spectral blue shift accompanying a deprotonation in the region from $a=4$ to 5 in the glycyl-L-histidine and glycyl-L-histidylglycine copper(II) systems indicates the formation of a polymer complex $Cu_nY_n^{n-}$ in which the imidazole ring acts as a bridge to connect copper atoms, where a represents moles of KOH added per completely protonated ligand.

In previous papers,[1,2] we discussed the complex species formed in the reaction of L-histidylglycine and L-histidylglycylglycine with copper(II) ion in an aqueous solution. Since the histidyl residue in the peptides plays an important role in their complex formation with metal ions, the position of the histidyl residue in the peptide molecule may cause remarkable differences in the modes of their complex formation. In an attempt to obtain further information on the interaction of the histidyl residue in the peptides with metal ion, the complex formation of glycyl-L-histidine, glycyl-L-histidylglycine, and glycylglycyl-L-histidine (abbreviated as GH, GHG, and GGH hereafter) with copper(II) ion was studied in aqueous solutions containing equimolar amounts of copper(II) and the ligand by potentiometric and spectrophotometric methods.

The copper(II) complexes of GH and GGH in aqueous solution have been investigated by various physico-chemical methods, and the binding sites of copper(II) have been deduced.[3-7] The structure of the neutral 1 : 1 GH-copper(II) complex was elucidated by X-ray diffraction analysis.[8] Recently, copper(II) complex of GHG was also studied in both aqueous solution and crystalline state.[9-11] However, the complex formation of these peptides with copper(II) ion in aqueous solution has not been elucidated completely, and there remain problems on the quantitative interpretation of the experimental results, the complex species formed in the reaction, and their structures.

Experimental

Materials. The preparation of the peptides were described previously.[12] Other reagents were commercial products.

Methods. The potentiometric and visible absorption spectral measurements were carried out as described previously.[1,12]

Results

Potentiometric Titration of GH–copper(II) and GHG–copper(II) Systems. The titration curves for the solutions containing $CuCl_2$ and GH or GHG (molar ratio 1 : 1) are shown in Fig. 1, together with those for the ligands in the absence of copper(II). Each curve shows the onset of pH fall at a point near $a=1$ and an extensive overlap of the ionization, giving a strong pH inflection at $a=4$, where a represents the number of moles of KOH added per completely protonated ligand. The same titration curve has been reported by Martin and Edsall,[3] and Bryce *et al.*[4] for the GH–Cu(II) system. From these titration behaviors, coordination is presumed to occur initially at the imidazole group with subsequent release of the protons from the amino and the peptide amide groups. Accordingly, the following equilibria are assumed in the region between $a=1$ and $a=4$,

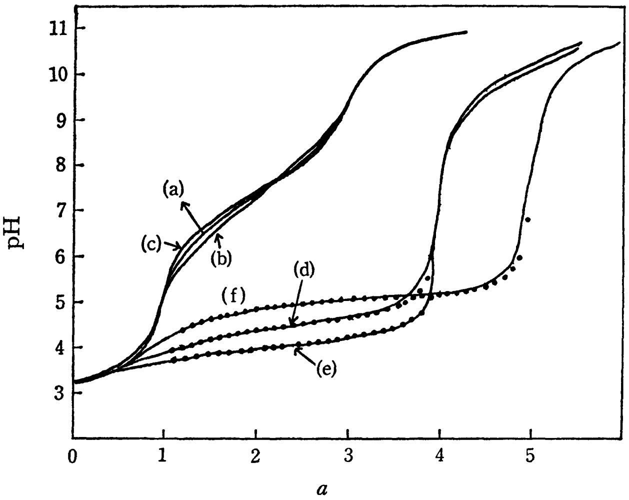


Fig. 1. Titration curves of GH, GHG, and GGH in the absence and presence of $CuCl_2$.
(a) GH+2HCl, (b) GHG+2HCl, (c) GGH+2HCl, (d) GH+2HCl+$CuCl_2$, (e) GHG+2HCl+$CuCl_2$, (f) GGH+2HCl+$CuCl_2$; ligand concentration is 10^{-3} M (at $a=0$).
The lines are experimental curves. Each point was calculated from the equilibrium constant.

TABLE 1. FORMATION AND IONIZATION CONSTANTS FOR COPPER(II)-PEPTIDE SYSTEMS

Ligand	pK_{COOH}[a)]	pK_{Im}[a)]	pK_{NH_3}[a)]	$\log K_1$	pK_{2c}	pK_{3c}	"pK_c"[b)]
GH	2.75	6.85	8.33	5.08	8.40	—	9.65
GHG	3.19	6.63	8.17	4.94	7.10	—	9.55
GGH	2.84	6.99	8.23	5.33	—	14.81	10.69

a) The acid dissociation constants of ligands were reported previouly (Ref. 12). b) The values of "pK_c" are pH values at the half-neutralization points.

$$LH_3^{2+} \rightleftarrows LH_2^+ + H^+$$

$$K_{COOH} = [LH_2^+][H^+]/[LH_3^{2+}] \qquad (1)$$

$$LH_2^+ \rightleftarrows LH + H^+$$

$$K_{Im} = [LH][H^+]/]LH_2^+] \qquad (2)$$

$$Cu^{2+} + LH \rightleftarrows CuLH^{2+}$$

$$K_1 = [CuLH^{2+}]/[Cu^{2+}][H^+] \qquad (3)$$

$$CuLH^{2+} \rightleftarrows CuX + 2H^+$$

$$K_{2c} = [CuX][H^+]^2/[CuLH^2] \qquad (4)$$

where LH is the neutral species of the ligand and $LH = XH_2$. For the sake of electroneutrality we have

$$2[Cu^{2+}] + 2[CuLH^{2+}] + 2[LH_3^{2+}] + [LH_2^+] + [K^+] + [H^+] = [OH^-] + [Cl^-]. \qquad (5)$$

The total concentrations of the ligand and the copper(II) ion are given as follows.

$$[Cu]_T = [Cu^{2+}] + [CuLH^{2+}] + CuX] \qquad (6)$$

$$[L]_T = [LH_3^{2+}] + [LH_2^+] + [LH] + [CuLH^{2+}] + [CuX] \qquad (7)$$

From Eq. (1)—(7), the following relationship between K_1 and K_{2c} is derived.

$$K_1 = (ABC + 2\alpha B^2 + ABDK_{2c})/(\gamma C^2 + 2\gamma CDK_{2c} + \gamma D^2 K_{2c}^2) \qquad (8)$$

where A, B, C, D, α, β, and γ are defined as follows.

$$A = 2\alpha + \beta$$

$$B = \alpha\gamma - A[Cu]_T$$

$$C = 2\alpha[Cu]_T - \alpha\gamma$$

$$D = -\alpha\gamma/[H^+]^2$$

$$\alpha = 1 + [H^+]/K_{Im} + [H^+]^2/K_{COOH}K_{Im}$$

$$\beta = [H^+]/K_{Im} + 2[H^+]^2/K_{COOH}K_{Im}$$

$$\gamma = [OH^-] + [Cl^-] - [H^+] - [K^+]$$

The acid dissociation constants, K_{COOH} and K_{Im}, have been reported.[12)]

The values of K_1 and K_{2c} were evaluated by the graphical method of Schwarzenbach *et al.*[13)] The results for the GH–copper(II) system are shown in Fig. 2. The equilibrium constants obtained are listed in Table 1. From the K_1 and K_{2c} values, the titration curves were recalculated and compared with those obtained experimentally in Fig. 1. In the region between $a=4$ and $a=5$, an additional deprotonation is observed. The apparent ionization constants "pK_c'", which are the pH values at the half-neutralization point, are 9.65 and 9.55 for the GH–copper(II) and the GHG–copper(II) systems, respectively. The distribution of copper(II) among the complex species at various pH values was calculated. The results for the GH–copper(II) system are shown in Fig. 3.

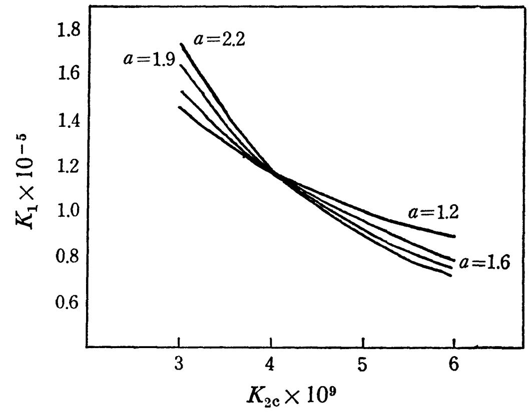


Fig. 2. Graphical calculation of K_1 and K_{2c} for GH-Cu(II) system.

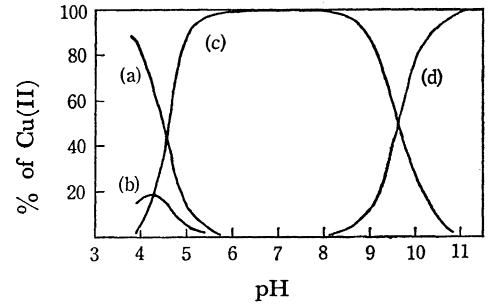


Fig. 3. Distribution of Cu(II) species for the 1 : 1 GH-Cu(II) system;
(a) Cu^{2+}, (b) $CuLH^{2+}$, (c) CuX, (d) $Cu_nY_n^{n-}$.

GGH-copper(II) System. The titration curve o the 1 : 1 GGH-copper(II) system is given in Fig. 1. The same curve has been reported by Bryce *et al.*[4)] The titration behavior in the GGH–copper(II) system is similar to that of the GH–copper(II) system, in the region between $a=1$ and $a=4$, but a remarkable pH inflection is observed at $a=5$. This indicates the deprotonation of the two amide groups of the peptide bonds. Accordingly, the following equilibria are assumed, with Eqs. (1) and (2), where $LH = YH_3$.

$$Cu^{2+} + LH \rightleftarrows CuLH^{2+}$$

$$K_1 = [CuLH^{2+}]/[Cu^{2+}][LH] \qquad (9)$$

$$CuLH^{2+} \rightleftarrows CuY^- + 3H^+$$

$$K_{3c} = [CuY^-][H^+]^3/[CuLH^{2+}] \qquad (10)$$

The values of K_1 and K_{3c} were calculated by the same method as in the case of GH–copper(II) system. The results are shown in Fig. 4 and Table 1. An additional deprotonation is observed in the region between $a=5$ and $a=6$, giving the apparent ionization constants 10.69. The value is a little higher than the values of

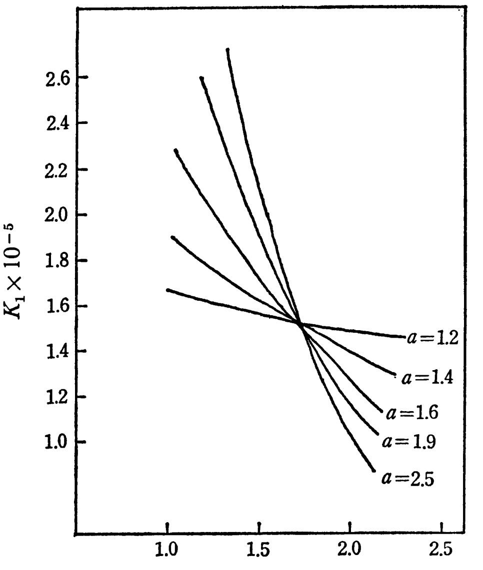


Fig. 4. Graphical calculation of K_1 and K_{3c} for GGH-Cu(II) system.

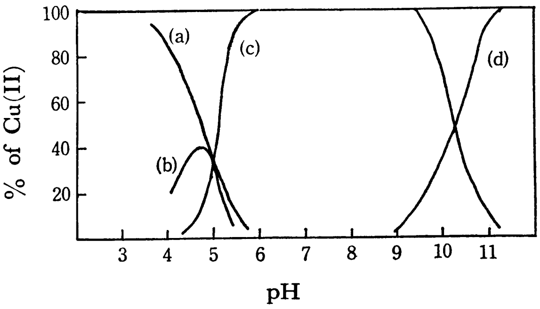


Fig. 5. Distribution of Cu(II) species for the 1 : 1 GGH-Cu(II) system;

(a) Cu^{2+}, (b) $CuLH^{2+}$, (c) CuY^-, (d) CuZ^{2-}.

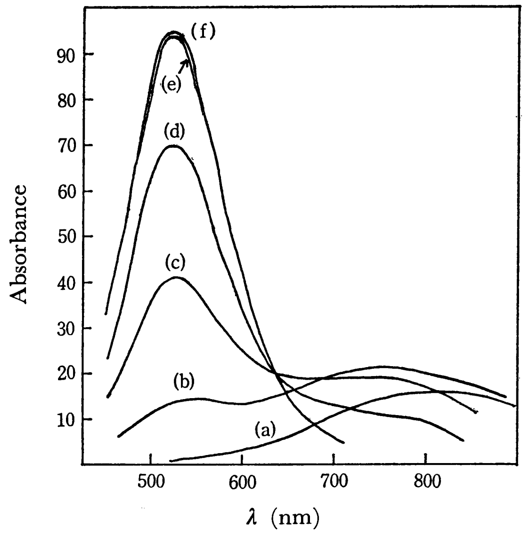


Fig. 6. Visible absorption spectra of the 1 : 1 GGH-Cu(II) system;

(a) GGH+HCl+$CuCl_2$, (b) GGH+$CuCl_2$, (c) GGH +KOH+$CuCl_2$, (d) GGH+2KOH+$CuCl_2$, (e) GGH+3KOH+$CuCl_2$, (f) GGH+4KOH+$CuCl_2$.

Absorbance is expressed per gram atom of copper(II).

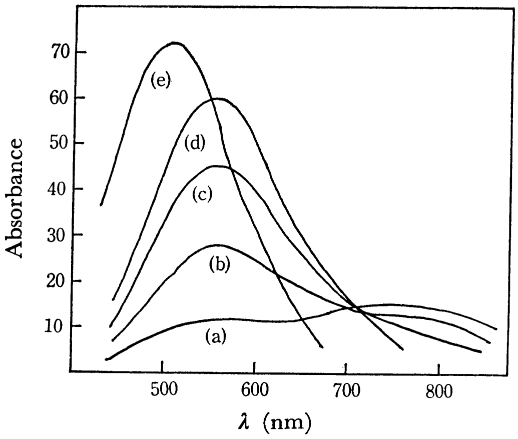


Fig. 7. Visible absorption spectra of the 1 : 1 GHG-Cu(II) system;

(a) GHG+HCl+$CuCl_2$, (b) GHG+$CuCl_2$, (c) GHG +KOH+$CuCl_2$, (d) GHG+2KOH+$CuCl_2$, (e) GHG+3KOH+$CuCl_2$.

Absorbance is expressed per gram atom of copper(II).

TABLE 2. SPECTRAL CHARACTERISTICS OF Cu(II)-PEPTIDE COMPLEXES

Ligand	a Value	λ_{max} (mμ)	ε ($M^{-1}cm^{-1}$)	Main species
GH	4	600	60	CuX
	5	557	70	$Cu_nY_n^{n-}$
GHG	4	602	60	CuX
	5	556	72	$Cu_nY_n^{n-}$
GGH	5	525	94	CuY^-
	6	525	95	CuZ^{2-}
GG[a)]	2	638	87	CuX
	3	641	83	$CuL(OH)^-$

a) GG=glycylglycine, and in this case a value represents moles of KOH added per neutral (monoprotonated) glycylglycine.

the GH-copper(II) and the GHG-copper(II) systems. The distribution of copper(II) among the complex species is shown in Fig. 5.

Absorption Spectra of the Peptide-copper(II) 1 : 1 Solutions. The characteristics of the visible absorption spectra of the GH-copper(II) and GGH-copper(II) complex has been reported,[5,6)] but the relation between the absorption spectra and the pH of the solution has not been fully discussed. The spectra of the GHG-copper(II) complex has not been studied.

The spectra of the GGH-copper(II) and GHG-copper(II) systems are shown in Figs. 6 and 7, where a has the same meaning as those in titration. The spectra of the GH-copper(II) system are similar to those of GHG-copper(II) systems. The absorption characteristics of those spectra are given in Table 2. The spectrum at a=2 in the GGH-copper(II) system shows two absorption maxima at 750 nm and 525 nm. The absorption at 750 nm decreases whereas that at 524 nm increases, with the increase of a. The absorption maximum at 750 nm is presumed to be due to Cu^{2+} and $CuLH^{2+}$, and that at 525 nm to CuY^-. The spectral behavior corresponds reasonably to the equilibrium

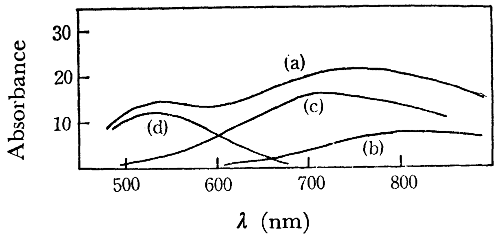


Fig. 8. Resolution of the spectrum at $a=2$ in the 1 : 1 GGH-Cu(II) system;
(a) GGH+Cu(II), (b) Cu^{2+} (45.6%), (c) $CuLH^{2+}$ (38.4%), (d) CuY^- (16.0%).

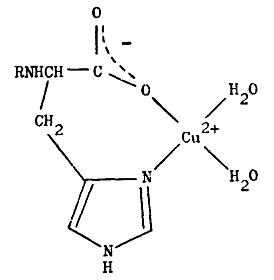
assumed from the titration curve. The spectrum at $a=2$ was resolved into three components corresponding to the three complex species by the use of the equilibrium constants. The results is shown in Fig. 8.

In the GH–copper(II) and GHG–copper(II) systems, the spectral behavior is similar to that in the GGH–copper(II) system up to $a=2$, the neutral complex CuX exhibiting a maximum at ~600 nm. However, the additional deprotonation causes a shift of the maximum to 555 nm.

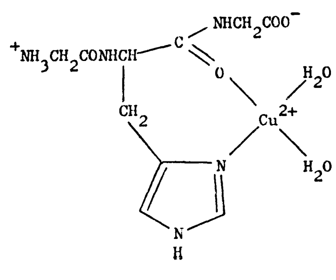
Discussion

The stepwise ionization constants of GH–copper(II) and GGH–copper(II) complexes have been reported.[3,4] However, the formation constants of these complexes have not been calculated because of the complexity of the titration behavior. The assumption in Eqs. (3) and (4) (or (9) and (10)) made the quantitative interpretation of the titration data possible.

In Table 1, the log K_1 values decrease in the order GGH, GH, and GHG, which agrees with that of the basicity of the imidazole group. This suggests the presence of a bond between the imidazole group and copper(II) in the complex $CuLH^{2+}$. The copper(II) ions will also be bound to the carboxyl or the carbonyl groups. The following structures are suggested for the complexes $CuLH^{2+}$.

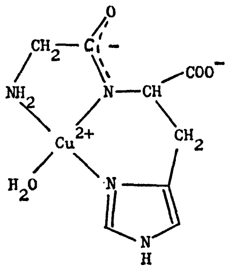


(I) $CuLH^{2+}$
$R={}^+NH_3CH_2CO$: GH-Cu(II)
$R={}^+NH_3CH_2$-$CONHCH_2CO$: GGH-Cu(II)

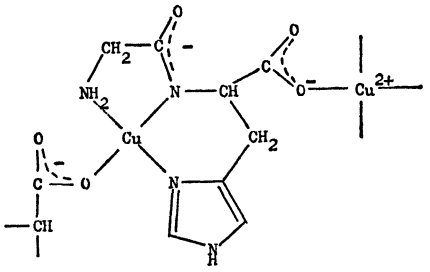


(II) $CuLH^{2+}$, GHG-Cu(II)

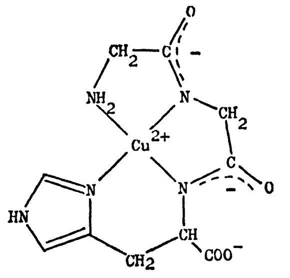
From the titration data it has been suggested that the copper(II) predominantly binds to the three nitrogens of the imidazole, amino, and deprotonated amide groups in the pH region 6—8 in the GH–copper(II) system. In the GH–copper(II) and GHG–copper(II) systems, the liberation of three protons and the remarkable blue shift of the absorption maxima in the region between $a=1$ and $a=4$ indicate that the copper(II) in the neutral complex CuX is bound to the three nitrogen atoms. In aqueous solutions the fourth coordination site of copper(II) is presumed to be mainly occupied by water molecule. The probable structure of CuX can be represented by III. However, the neutral chelate of the GH–copper(II) system precipitates on standing from the solution at $a=4$. Thus in the crystalline state, the fourth coordination site would be occupied by the carboxyl groups of a neighboring molecule to give a polymer Cu_nX_n as shown by IV. The structure of Cu_nX_n has been elucidated by X-ray diffraction analysis.[8] The same structure has been proposed to the GHG–copper(II) complexes.[10,11]



(III) CuX



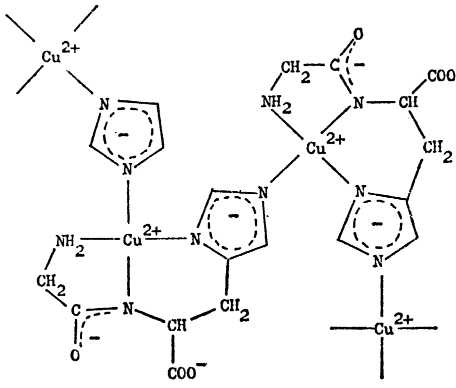
(IV) Cu_nX_n



(V) CuY^-

As in the case of the GH–copper(II) system, it is apparent that the copper(II) in the anionic species CuY^- of the GGH–copper(II) complex is bound to the nitrogen atoms of the amino, imidazole, and two deprotonated amide groups as shown in V.

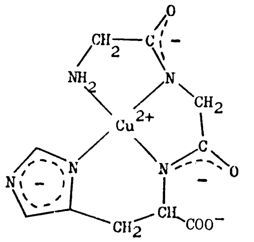
A further spectral blue shift caused by an additional deprotonation at $a=5$ in the GH–copper(II) and GHG–copper(II) systems suggests important changes in the coordination sphere of the copper(II) ion. The following three possibilities have been taken into account for the deprotonation for the GH–copper(II) system: (1) the proton dissociation of the coordinated water molecule at the fourth position,[4,5] (2) the proton dissociation from the imino nitrogen of the imidazole ring,[3] (3) the coordination of the deprotonated imino nitrogen to the fourth position of another copper(II).[6] The dissociation of the water molecule at the fourth position causes some red shift in the glycylglycine–copper(II) system, as indicated in Table 2. Deprotonation of the imino group in the GGH–copper(II) complex CuY^- does not occur below pH 10. The results exclude the possibilities (1) and (2). The most probable assumption is that a deprotonated imino nitrogen of the imidazole ring coordinates to the fourth position of another copper(II) to give a negatively charged polymer complex $Cu_nY_n^{n-}$. The structure of $Cu_nY_n^{n-}$ is shown as VI. The polymer is presumed to be a tetramer from the molecular model, as suggested by Morris and Martin[6] but no evidence was obtained from the quantitative analysis of the titration curve.



(VI)

$Cu_nY_n^{n-}$

Further addition of the base to CuY^- of the GGH–copper(II) system would cause the liberation of a proton from the imino nitrogen to give a dianionic chelate CuZ^{2-} as indicated by VII, where $LH = YH_3 = ZH_4$. The spectral characteristic of CuZ^{2-} is similar to that of CuY^-.



(VII) CuZ^{2-}

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