# Interaction between Side-Chain Pyridyl Groups of α-Helical Polypeptide and Guest Trp in the Ternary $Cu^{2+}$ Ion Complex

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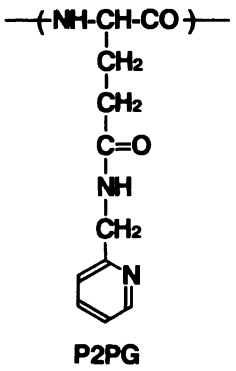
Interaction between the side-chain pyridyl ligands of an α-helical poly($N^{\omega}$-2-pyridylmethyl-L-glutamine) (P2PG) and guest ligands such as tryptamine and D- and L-tryptophan (D- and L-Trp) within ternary $Cu^{2+}$ complexes was investigated by absorption and circular dichroism (CD) spectroscopy in 2,2,2-trifluoroethanol solutions. The host complex species, $Cu^{2+}$-one pyridyl-substituted side-chain (s-2PG) complexes (1:1 $Cu^{2+}$-P2PG, $[Cu^{2+}]/[\text{s-2PG}]>1.0$) and $Cu^{2+}$- two s-2PGs complexes (1:2 $Cu^{2+}$-P2PG, $[Cu^{2+}]/[\text{s-2PG}]<1.0$), were prepared, using $Cu^{2+}$ and P2PG depending on the $Cu^{2+}$ ion concentration. All guest molecules could bind noncovalently to the 1:1 $Cu^{2+}$-P2PG system, having the ligand sites of $Cu^{2+}$ partly unfilled, to yield their ternary $Cu^{2+}$ complexes. The respective ternary complexes had induced optical activities in the CD spectra and a charge-transfer band indicating ligand–ligand stacking interactions involving charge transfer between the coordinated s-2PG and the guest indole moieties around $Cu^{2+}$. The CD spectral intensity was in the order tryptamine < D-Trp, L-Trp, which suggests that the coordination of both amino and carboxyl groups of Trp to $Cu^{2+}$ is important for the regular arrangement of the ligands on the periphery of the α-helix backbone of P2PG. The differences in the CD spectra suggest that the indole rings of D- and L-Trp face in a direction opposite to that of the pyridyl ring of P2PG. D- and L-Trp could also bind the 1:2 $Cu^{2+}$-P2PG host system, whose $Cu^{2+}$ coordination sites are filled with s-2PGs, but the 1:2 $Cu^{2+}$-P2PG did not yield the ternary complexes with tryptamine.

Biological molecular recognition, called "multiple recognition", is achieved by a variety of interactions such as electrostatic interaction, hydrophobic interaction, aromatic ring stacking, hydrogen bonding, and metal complexation. This leads to the specificity of substrate recognition and enzymic activation in biological systems.[1—4]

Many studies of molecular recognition in artificial systems have been done using the "lock and key" and "induced fit" concepts.[5—18] For example, amino acid recognition was achieved by two-point fixation using bifunctional metalloporphyrin receptors.[19] Structural regularities of polypeptides have also been used for molecular recognition. Ihara et al.[20] showed that cyanine dye dimers bound to the ammonium residues in α-helical poly(L-lysine·HBr) could distinguished diamines containing alkyl residues of different length by their CD spectra. Voyer et al.[21] pointed out that the distance between the ligand side chains could be changed by the polypeptide transition from the α-helix to the β-conformation.

We[22] have reported that α-helical poly($N^{\omega}$-2-pyridylmethyl-L-glutamine) (P2PG, Scheme 1) binds monomeric pyridine, with the aid of $Cu^{2+}$ ion without any changes in α-helix structure of P2PG. This ternary $Cu^{2+}$ complex formation could be monitored by the induced CD peaks due to the aromatic rings of the ligands around $Cu^{2+}$ on the periphery of the α-helix.

In this study, we tried to find whether the $Cu^{2+}$-P2PG complexes are able to serve as a molecular recognition system for the enantiomeric indole ring derivatives. $Cu^{2+}$-pyridyl ligand-tryptophan ternary systems have been well-characterized spectroscopically.[23] We first selected D- and L-tryptophan (D- and L-Trp) as the guest molecules. The ternary complex formation in 2,2,2-trifluoroethanol (TFE) was investigated by absorption and circular dichroism spectroscopy. The ternary complex formation of Trp was compared with that of tryptamine, which is known to be a metabolic product of Trp.




P2PG

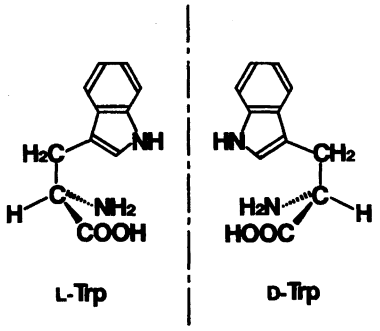
Scheme 1.

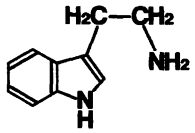
## Experimental

**Materials.** Poly($N^{\omega}$-2-pyridylmethyl-L-glutamine) (P2PG, $2.26\times10^5$) was prepared as previously reported.[22] The starting material, poly(γ-methyl L-glutamate) (PMG) was kindly supplied by Ajinomoto Co., Ltd.

D- And L-tryptophan (D- and L-Trp, Scheme 2), tryptamine (Scheme 2), and $CuCl_2\cdot4H_2O$ were guaranteed reagents from Nacalai Tesque, Co., Ltd. 2,2,2-Trifluoroethanol (TFE, Nacalai Tesque, Co., Ltd.) was of spectroscopic grade.

**Measurements.** Absorption and circular dichroism (CD) spectra of TFE solution of P2PG were obtained with



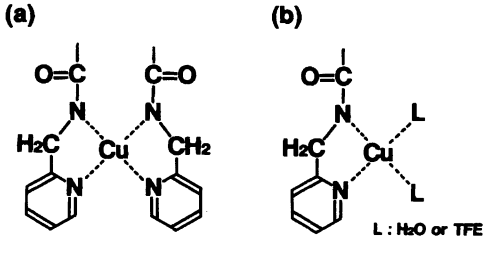

Scheme 2. Second guest molecules.

a spectrophotometer (JASCO, UVIDEC-670) and a spectropolarimeter (JASCO, J-600), respectively. Solutions of $5.0\times10^{-4}$ base molar P2PG in TFE were used.

## Results and Discussion

**$Cu^{2+}$-P2PG Host Systems.** P2PG in TFE forms a unique complex with $Cu^{2+}$ due to the rigidity of the $\alpha$-helix backbone. Two types of complexes, $Cu^{2+}$-one pyridyl-substituted side chain (s-2PG) complexes (1:1 $Cu^{2+}$-P2PG, $[Cu^{2+}]/[s\text{-}2PG]>1.0$) and $Cu^{2+}$-two s-2PGs complexes (1:2 $Cu^{2+}$-P2PG, $[Cu^{2+}]/[s\text{-}2PG]<1.0$), were prepared by addition of $Cu^{2+}$ ion to the TFE solution of P2PG. The coordination structure of 1:1 $Cu^{2+}$-P2PG system was characterized by the d–d band of $Cu^{2+}$ near 680 nm owing to the coordination of two nitrogens of pyridyl and amide groups of s-2PG[22,24] and the charge transfer (deprotonated amide group of s-2PG-$Cu^{2+}$) shoulder band at 330 nm[22,25—27] (Scheme 3). This host system, therefore, has the s-2PS free coordination sites of $Cu^{2+}$ without any side-chain optical activities.

On the other hand, the structure of 1:2 $Cu^{2+}$-P2PG




Scheme 3. Anticipated complex structure formed between $Cu^{2+}$ and s-2PG. (a): 2:1 $Cu^{2+}$–P2PG, (b): 1:1 $Cu^{2+}$–P2PG.

system can be confirmed by the d–d transition band of $Cu^{2+}$ near 580 nm assigned to the coordination of four nitrogens (two s-2PGs) to $Cu^{2+}$ (Scheme 3).[22,24]

The details were described in our previous report.[22]

**$Cu^{2+}$-P2PG-Tryptamine Systems.** Figure 1 shows the absorption spectra before and after the addition of $5.0\times10^{-4}$ mol dm$^{-3}$ tryptamine to the 1:1 $Cu^{2+}$-P2PG system (Scheme 3). In the presence of tryptamine, the parent 680 nm band assigned to d–d transition of $Cu^{2+}$ was blue-shifted to 670 nm. This suggests the replacement of the solvent ligand by the amino group of tryptamine, confirming the formation of a $Cu^{2+}$-P2PG-tryptamine ternary system. On the other hand, the shoulder band near 330 nm assigned to charge transfer from deprotonated amide nitrogen of s-2PG to $Cu^{2+}$ was scarcely changed, indicating that the interaction between $Cu^{2+}$ and P2PG, i.e., the host complex structure, was preserved. It is also noticed that the indole ring of tryptamine caused an increase in the absorption band near 265 nm via the overlapping with the side-chain pyridyl band. Moreover, a strong band was appeared at 215 nm. Yamauchi et al.[23] reported that bipyridine–$Cu^{2+}$–L-Trp ternary complex involved the aromatic stacking structure with charge transfer (CT) from the indole ring to the electron-deficient pyridyl nitrogen. It is important to note, here, that the band at 215 nm in Fig. 1 disappeared upon the addition of dioxane, an effective agent for breaking the aromatic stacking structure. This band may be assigned, therefore, to the charge transfer from the indole ring of tryptamine to the nitrogen of s-2PG around $Cu^{2+}$ in the ternary complexes. In addition, the band at 215 nm may be associated with the higher-ordered CT structure that may be formed by fixing the CT units around the $\alpha$-helical backbone. The CD spectra (Fig. 2) also support the

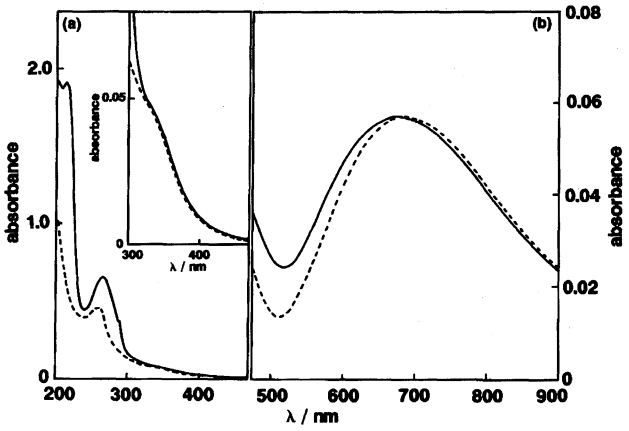



Fig. 1. Absorption spectra of P2PG–$Cu^{2+}$ between the presence and absence of tryptamine in TFE solutions. The light-path lengths of cells were (a) 0.1 cm and (b) 1.0 cm. $[Cu^{2+}]=1.0\times10^{-3}$ mol dm$^{-3}$, (—); [tryptamine]$=5.0\times10^{-4}$ mol dm$^{-3}$, (---); in the absence of tryptamine.

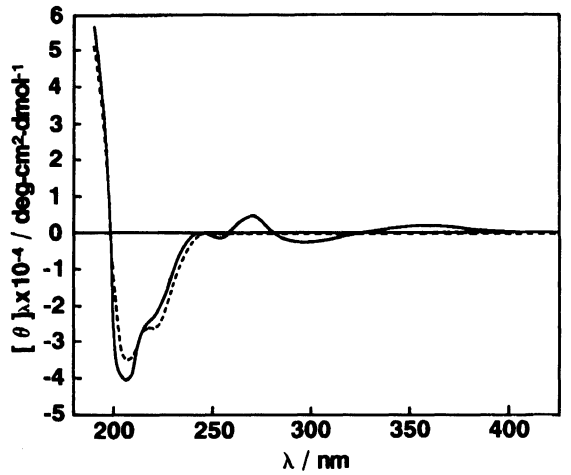



Fig. 2. Circular dichroism spectra of P2PG–$Cu^{2+}$ between the presence and absence of tryptamine in TFE solutions. $[Cu^{2+}]=1.0\times10^{-3}$ mol dm$^{-3}$, (—); [tryptamine]$=5.0\times10^{-4}$ mol dm$^{-3}$, (---); in the absence of tryptamine.

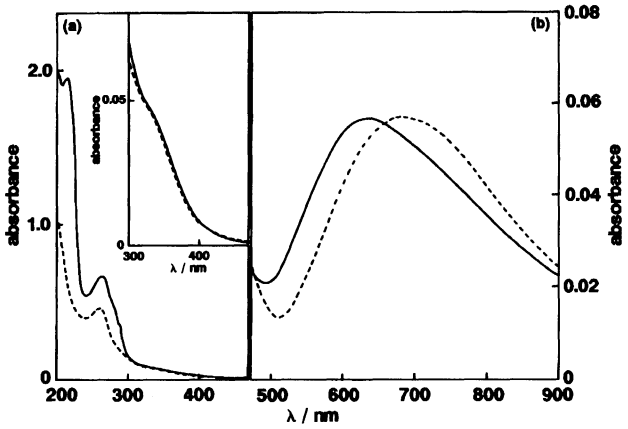



Fig. 3. Absorption spectra of P2PG–$Cu^{2+}$ between the presence and absence of D-Trp in TFE solutions. The light-path lengths of cells were (a) 0.1 cm and (b) 1.0 cm. $[Cu^{2+}]=1.0\times10^{-3}$ mol dm$^{-3}$, (—); [D-Trp]$=5.0\times10^{-4}$ mol dm$^{-3}$, (---); in the absence of D-Trp.

formation of ternary complexes, indicating two asymmetric positive exciton couplets with peaks at 351 and 294 nm and at 270 and 255 nm, respectively, and a peak increase around 210 nm. That is, the optical activities of the side-chain region of P2PG,[28,29] in correspondence to the CT bands at 330 and 215 nm and the pyridyl (s-2PG) and the guest indole bands near 265 nm, could be induced by the ternary complex formation. Similar phenomena had been also observed with P2PG–$Cu^{2+}$–Py ternary complexes.[22]

On the other hand, the addition of tryptamine to the 1:2 $Cu^{2+}$-P2PG system, the $Cu^{2+}$ coordination sites of which are filled with the s-2PG ligands[22] (Scheme 3), did not induce any changes in the absorption spectra of the host complexes. This indicates that tryptamine can hardly be exchanged with one of s-2PGs coordinated to $Cu^{2+}$ in the host complex to yield their ternary system.

**$Cu^{2+}$–P2PG–Trp Systems.** Figure 3 shows the changes in the absorption spectra owing to the addition of $5.0\times10^{-4}$ mol dm$^{-3}$ D-Trp to the 1:1 $Cu^{2+}$–P2PG system. Almost the same spectral changes to those of the $Cu^{2+}$–P2PG–tryptamine system were observed, i.e., a blue-shifted d–d band at 630 nm, a peak increase near 265 nm, and a new intense CT band at 215 nm. Another CT band, a shoulder peak near 330 nm, remains intact in this system. It is confirmed, therefore, (i) the carboxylic group of Trp, in addition to the amino group, can coordinate to $Cu^{2+}$ (larger magnitude of the blue shift for the d–d band compared with that of tryptamine); (ii) the stacking interaction between the aromatic rings of s-2PS and guest Trp involving appreciable charge transfer must have occurred (band appeared at 215 nm); (iii) the original coordinated structure between $Cu^{2+}$ and P2PG is hardly affected by the additional coordination of Trp to $Cu^{2+}$ (consistancy of the shoulder band near 330 nm), thus confirming a stable complexation of the $Cu^{2+}$–P2PG–D-Trp ternary system.[30] L-Trp added gave quite similar absorption spectra to those of D-Trp.

The CD spectra of $Cu^{2+}$–P2PG–D-Trp system (solid line) are reported in Fig. 4 together with the result for the $Cu^{2+}$–P2PG system (dashed line). The lack of side-chain optical activities ($\lambda>250$ nm) for the $Cu^{2+}$–P2PG system without Trp can be explained considering the absence of the side-chain interactions such as self-stacking of the pyridyl rings. D-Trp induced, as well as tryptamine (Fig. 2), two asymmetric positive exciton couplets centered at 330 and 260 nm, respectively,

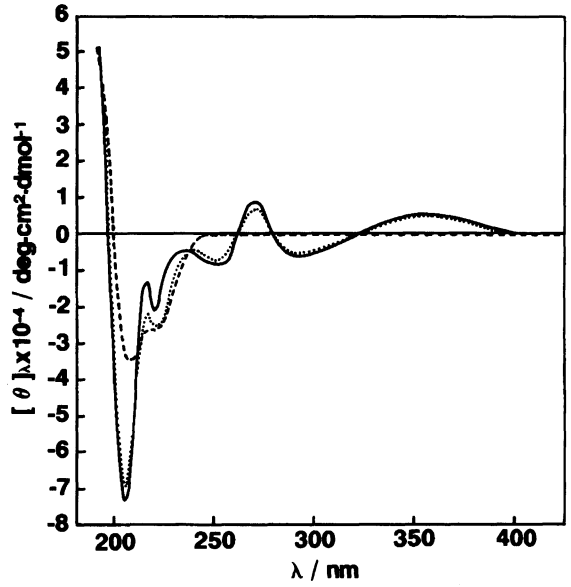



Fig. 4. Circular dichroism spectra of P2PG–$Cu^{2+}$–D-Trp between the presence (····) and absence (—) of 10 vol% dioxane in TFE solutions and (---); P2PG–$Cu^{2+}$ in TFE solution. $[Cu^{2+}]=2.0\times10^{-3}$ mol dm$^{-3}$, [D-Trp]$=2.0\times10^{-4}$ mol dm$^{-3}$.

and a peak enhancement below 250 nm. These signals induced by D-Trp, however, are more intense than those induced by tryptamine, which suggests that increased rigidity of the side-chain conformation of P2PG can be effectively made by the ligand–ligand interactions around $Cu^{2+}$. These enhanced magnitudes for the $Cu^{2+}$–P2PG–D-Trp system were reduced with the addition of a less polar solvent, 10 vol% dioxane (dotted line in Fig. 4), further indicating that the rigidity of the side-chain region is due to the aromatic ring stacking between pyridyl (s-2PG) and indole (D-Trp) rings, accompanying the interaction between these stacking units, which is weakened in hydrophobic environments. These spectral results suggest, therefore, that an ordered structure[31,32] involving an ordered arrangement of the aromatic ring stacking structure on the periphery of the helical backbone must be present in the $Cu^{2+}$–P2PG–D-Trp system.

On the other hand, different optical properties have been found in the $Cu^{2+}$–P2PG–L-Trp system (Fig. 5), while the absorption spectra have no differences between D- and L-Trp ternary systems. Particularly, intensities of the signals below 270 nm, associated with the aromatic ring stacking, strongly depend on the isomer species.[33] It should be pointed out again, here, that Trp in the ternary systems is fixed with two coordinating sites, N(amino group)–$Cu^{2+}$ and O(carboxyl group)–$Cu^{2+}$, and a ligand–ligand aromatic ring stacking, i.e., three-point fixation of Trp, can be achieved by making the ternary complexes. Furthermore, the CD bands of both systems centered at 330 nm, associated with the s-2PG–$Cu^{2+}$ CT structure, agree with each others indicating that the coordinated structure between $Cu^{2+}$ and P2PG within both ternary systems is independent of the guest isomer species. Space-filling models being satisfied with these requirements suggest that in the stacked structure the indole rings of D- and L-Trp are faced oppositely to the pyridyl ring of P2PG (Fig. 6). This conformation would allow for the different optical properties between D- and L-Trp ternary systems.

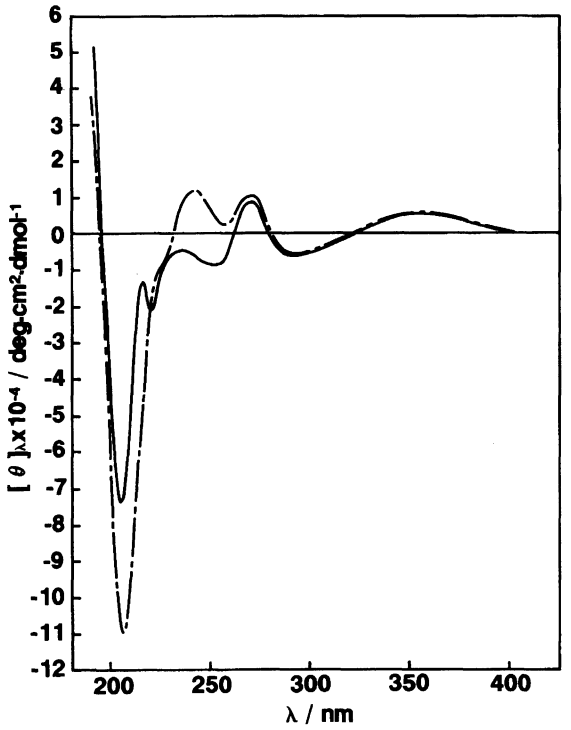



Fig. 5. Circular dichroism spectra of P2PG–$Cu^{2+}$–Trp in TFE solutions. $[Cu^{2+}]=1.0\times10^{-3}$ mol dm$^{-3}$, (—); [D-Trp]$=5.0\times10^{-4}$ mol dm$^{-3}$, (---); [L-Trp]$=5.0\times10^{-4}$ mol dm$^{-3}$.

On the other hand, it was confirmed spectroscopically that added Trps can easily bind to 1:2 $Cu^{2+}$–P2PG system (Scheme 3) resulting from the ligand exchange with one of the s-2PGs in the host complexes to yield their ternary system, whereas tryptamine could not. Interestingly, the stability sequence of the ternary system thus measured by the ternary complexation behaviors accompanying the ligand exchange corresponds well with that of CD spectra magnitude enhancement (tryptamine < D-Trp, L-Trp). It may say, therefore, the three-point fixation described above is also important in the stabilization of the ternary complex system in addition to the distinction of the optical isomers.

## Conclusions

P2PG, an $\alpha$-helical polypeptide containing the side-chain pyridyl groups, was accessible to the indole ring derivatives such as tryptamine, and D- and L-Trp with the aid of $Cu^{2+}$ to produce their ternary systems. Tryptamine is fixed to the 1:1 $Cu^{2+}$–P2PG system with N(amide group)–$Cu^{2+}$ coordination and aromatic ring stacking between side-chain pyridyl and the guest indole rings. In addition to these interactions, O(carboxyl group)–$Cu^{2+}$ coordination is also used for the binding of Trp to the host system resulting in three-pint fixation, the orders of the stability of the ternary systems being tryptamine < D-Trp, L-Trp. It is also confirmed that an ordered structure involving an ordered arrange-

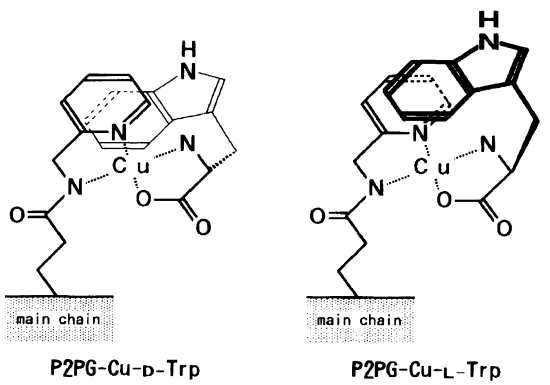



Fig. 6. Schematic picture of anticipated P2PG–$Cu^{2+}$–Trp ternary complexes.

ment of the ligand–ligand aromatic ring stacking on the periphery of the P2PG helical backbone was present, which allowed the spectroscopical (CD) recognition between D- and L-Trp. Furthermore, Trps could bind to 1 : 2 $Cu^{2+}$–P2PG host complexes through the ligand exchange, but tryptamine could not, further indicating a possibility for the separation of such the indole derivatives.

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