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# Complexation of cobalt ion with copolypeptide containing tryptophan treated with nitric acid

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## Summary

Tryptophan within copoly(*N*-hydroxyethyl L-glutamine-*co*-L-tryptophan) treated with nitric acid (EGT-N) dissociates negatively above pH 10.5 by the electron-attractive effect of nitro groups introduced into the indole ring. The interaction between EGT-N and various metal ions was investigated using UV/VIS, ESCA, and ICP measurement. EGT-N was found to selectively form a complex with cobalt ions above pH 10.5. The formation of the cobalt ion complex of EGT-N was confirmed to be caused by the cooperative coordination of the tryptophan-introduced nitro groups and the OH groups of hydroxyethyl L-glutamine.

## Introduction

Aromatic nitro compounds are used for pigments because of their large absorption coefficient and are important as reaction intermediates in products such as dyes and medicaments. Kenneth et al. (1) revealed that the nitro group of various fluorinated aromatic dinitro compounds is useful for N-terminal determination of polypeptides. Previous studies have also reported that tyrosine residue among enzymes can be determined by nitration using tetranitromethane (2), and that the $pK_a$ value of nitrated tyrosine residue, which undergoes a color change upon dissociation, shifts from 10.1 to 7.2 (3). Aromatic nitro compounds are used as indicators in both the qualitative and quantitative analysis of metal ions. Vandeal et al. (4) reported that 1-nitro-2-naphthol forms stable complexes with cobalt ions, because the OH group capable of becoming a ligand resides adjacent to the nitro group. However, few reports exist on compounds that form complexes selectively with specific metal ions, and research on polymers that exhibit such behavior is rarer still.

In our previous studies (5) (6), we observed that when L-tryptophan (Trp) is treated with nitric acid, Trp forms a tricyclic structure in which two nitro groups are introduced into the indole ring. Furthermore, we observed that Trp shows a reversible, pH-dependent color change between yellow (below pH 10.5) and red (above pH 12.5). This color change is due to the dissociation of the indole ring, which is caused by the strong electron attraction of the

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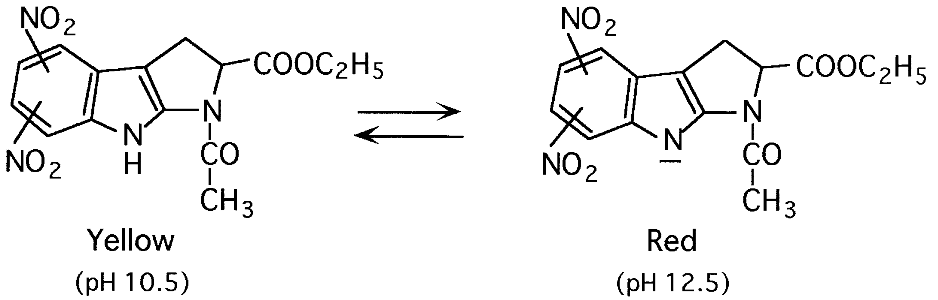
nitro groups (Scheme 1). Moreover, it was found that colored hydrogel can be prepared by treating a copolypeptide that contains Trp with nitric acid. The dissociation of the indole ring caused the physical properties of the membrane, the degree of swelling, and solute permeability to vary drastically.



Scheme 1 Dissociation form of *N*-acetyl Trp ethylester treated with nitric acid.

The present paper describes our spectroscopic investigation of the interaction between various metal ions and copolypeptides that are negatively charged above pH 10.5. The purpose of the present study is to prepare a novel material capable of interacting selectively with a specific metal ion.

## Experimental

### *Materials*

The starting copolymer (GT), composed of γ-benzyl L-glutamate (γ-BLG) and Trp, was synthesized by the *N*-carboxyanhydride (NCA) method, as described in our previous papers (5) (6). The molecular weight of GT was determined to be 376,000 by viscosity measurement in dimethylformamide (7), and the preparative data is listed in Table 1. All the solvents used in synthesis were distilled twice.

Water soluble copolypeptide, treated with nitric acid (EGT-N) was prepared by immersing copoly(*N*-hydroxyethyl L-glutamine-*co*-Trp) (EGT), obtained by the aminolysis of GT with 2-amino-1-ethanol (EA), in nitric acid under an irradiating UV light (wavelength 365 nm, intensity 300 μw/$cm^2$) for 1 hr. The aminolytic reaction was carried out at 58 °C for 72 hr (8). Debenzylation of GT was confirmed by the

Table 1 Preparative data of GT.

| | NCA Feed(mol%) | | copolymer(mol%) | |
|---|---|---|---|---|
| | γ-BLG | L-Trp | γ-BLG | L-Trp |
| GT | 90 | 10 | 88.6 | 11.4* |

| Yield | Mv | [η] |
|---|---|---|
| 97% | 376,000 | 7.05 |

* $^1H$-NMR data.

disappearance of absorption due to the presence of an ester group at 1740 $cm^{-1}$ in the IR spectrum. EGT-N was obtained through dialysis and then lyophilized. Scheme 2 is a schematic diagram of the preparation of EGT-N. Using a method similar to that used to prepare EGT-N, *N*-acetyl Trp ethylester treated with nitric acid (Trp-N) was prepared in order to clarify the composition of the complex.

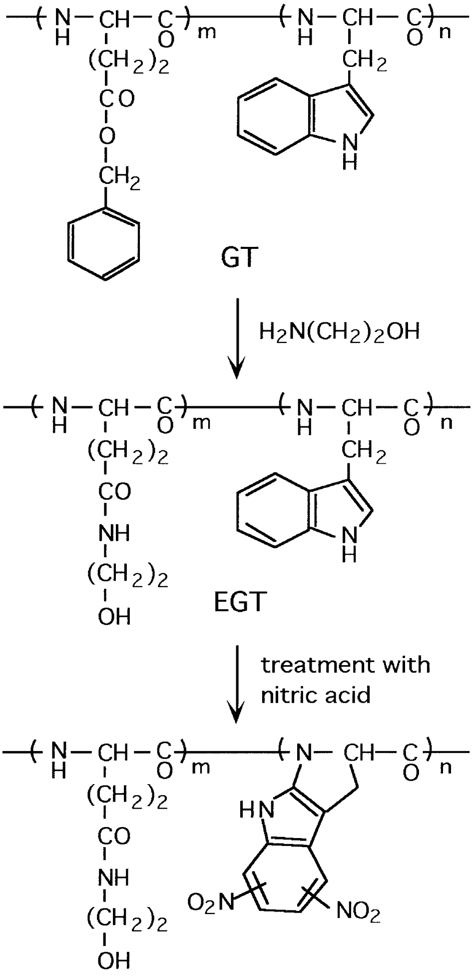
$CuCl_2$•$2H_2O$, $CoCl_2$•$6H_2O$, $ZnCl_2$, $NiCl_2$•$6H_2O$, and $FeCl_2$•$nH_2O$ purchased from Nacalai Tesque, Inc. were guaranteed reagents, and therefore were used in the present study without further purification.

*Measurement*

The interaction of EGT-N and various metal ions was investigated using UV/VIS spectroscopic measurement (Jasco, Model V-520 UV/VIS spectrophotometer). UV/VIS absorption spectra were measured using a quartz cell having a path length of 10 mm. The pH was adjusted with 5M NaOH to assure that changes in concentration were minimized.

Electron spectroscopy for chemical analysis (ESCA) measurement (Surface Science Instruments, Model SSX-100 photoelectron spectrometer) was performed in order to clarify the binding state between EGT-N and metal ions.

In order to determine the composition of the complex between Trp-N and metal ions, inductively coupled plasma (ICP) emission spectrometry (Seiko Instruments Inc., Model SPS 7000A plasma spectrometer) was performed. The complex was dissolved in 0.1 HCl and the metal ion and Trp-N contents were determined using ICP and UV measurement, respectively.



Scheme 2 Schematic diagram of the preparation of EGT-N.

## Results and Discussion

EGT-N indicates the absorption due to the presence of the nitro groups at 370 nm in the

UV spectrum (6). The interaction of EGT-N and metal ions was examined by the variation of the absorption intensity. Figure 1 illustrates the pH dependence of the absorption spectra of EGT-N solutions to which $Co^{2+}$ or $Cu^{2+}$ was added.

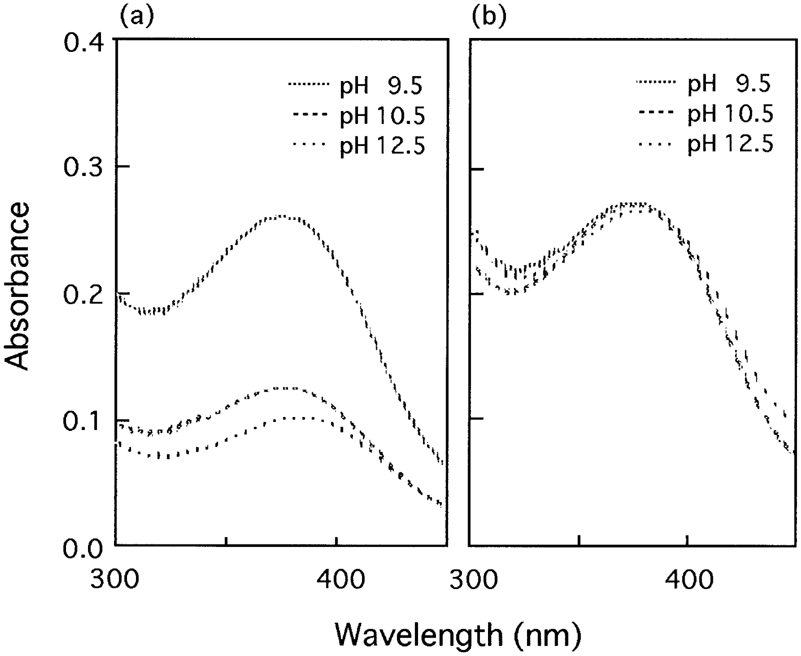


Fig. 1 pH dependence of the absorption spectra of EGT-N in the presence of metal ion, (a) EGT-N and $Co^{2+}$, (b) EGT-N and $Cu^{2+}$.
EGT-N=metal ion=$5\times10^{-4}$mol/l

The absorption spectra of untreated EGT did not vary due to the addition of metal ions. The absorption at 370 nm of EGT-N was decreased by the addition of $Co^{2+}$. In addition, EGT-N was observed to form green precipitates above pH 10.5, at which level Trp residue dissociates negatively. Similarly, Trp-N also precipitated under high-pH conditions upon the addition of $Co^{2+}$. Although $Co^{2+}$ formed precipitates of $Co(OH)_2$ under alkaline pH conditions, EGT-N appeared to interact with $Co^{2+}$, because EGT-N peaks were observed in the IR spectrum of the precipitates. In contrast, when $Cu^{2+}$ was added to EGT-N, variation of the absorption intensity at 370 nm was not observed, and precipitation did not occur above pH 10.5. Although similar trials were conducted for $Fe^{2+}$, $Ni^{2+}$, and $Zn^{2+}$, neither the variation of spectra nor precipitation were observed for these ions. These results suggest that Trp residue within EGT-N interacts with $Co^{2+}$ by the introduction of a nitro group, and that the significant changes, usually attributed to the type of metal ion present, actually results from the formation of a complex between the metal ion and EGT-N. The interaction of EGT-N and metal ions was therefore examined in detail using $Co^{2+}$ and $Cu^{2+}$.

Figure 2 shows the difference spectra of EGT-N when $Co^{2+}$ was added in low concentration, at which EGT-N does not form precipitates. In general, the formation of a metal ion complex is known to cause the UV or VIS spectrum to vary (9) (10). In the difference spectra of EGT-N to which $Co^{2+}$ has been added, a new absorption at 230 nm is observed above pH 10.5. This absorption indicates that Trp residue that contains nitro groups forms a complex with $Co^{2+}$. The spectrum of EGT-N to which $Cu^{2+}$ has been added is consistent with that of untreated EGT, and neither shift in absorption nor new absorption was observed. These results confirm that the Trp residue of EGT-N forms a complex with $Co^{2+}$ selectively.

Figure 3 shows an ESCA measurement of the precipitates of EGT-N and $Co(OH)_2$. In the spectrum of the precipitates, a new peak has appears at 788 eV, in addition to the two peaks due to $Co(OH)_2$ observed at 777 eV and 782 eV. This indicates that $Co^{2+}$, for which the bond state differs from that of $Co(OH)_2$, is present in the precipitates. Moreover, ICP measurement showed that Trp residue in EGT-N and $Co^{2+}$ forms a complex in a composition ratio of 1 to 6.

Figure 4 illustrates a possible form of the complex formed between EGT-N and $Co^{2+}$. $Co^{2+}$ forms an octahedral complex and becomes an

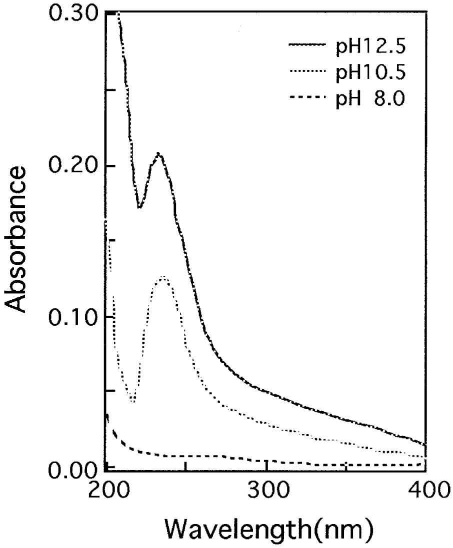


Fig. 2 pH dependence of difference spectra of EGT-N in the presence of $Co^{2+}$: EGT-N=$1\times10^{-3}$mol/l, $Co^{2+}$=$1\times10^{-4}$mol/l

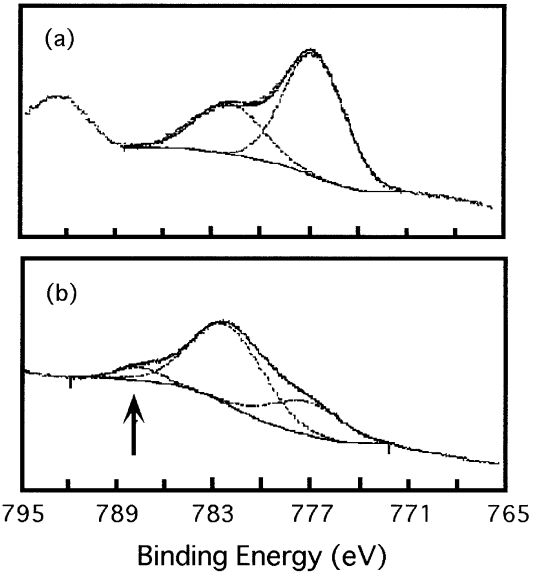


Fig. 3 The results of ESCA measurement, (a) $Co(OH)_2$ and (b) EGT-N and $Co^{2+}$ .

olation with another $Co^{2+}$ across $OH^-$ (11). Trp residue in EGT-N and $Co^{2+}$ appears to form an octahedral complex and EGT-N precipitates due to the cooperative coordination of $Co^{2+}$ with both the Trp residue containing a nitro group and the OH group of the side chain.

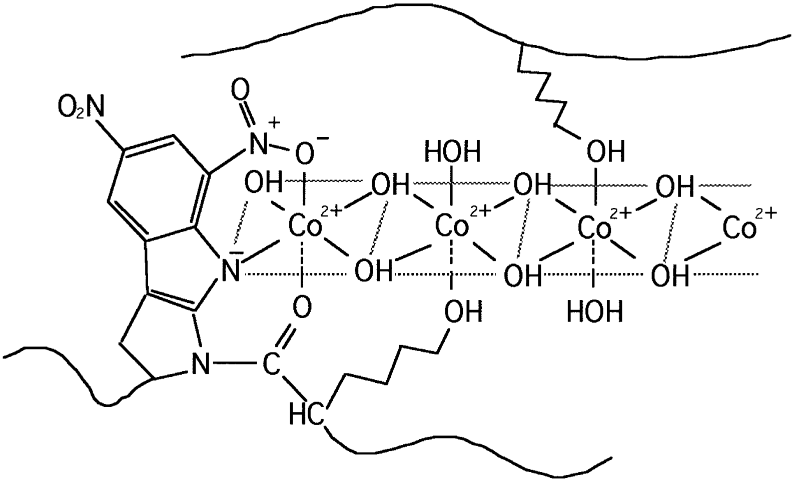


Fig. 4 Illustration of possible form of the complex between EGT-N and $Co^{2+}$.

As previously mentioned, Trp residue containing nitro groups forms a complex with $Co^{2+}$ selectively, even though the ion radius of $Co^{2+}$ is almost equal to that of $Cu^{2+}$. Therefore, EGT-N is expected to have applications in materials used to extract $Co^{2+}$ selectively, e.g. as a membrane or micro-sphere. The mechanism by which EGT-N forms complexes selectively with $Co^{2+}$ is thought to be due to either a difference in the electronic configurations of $Co^{2+}$ and $Cu^{2+}$, or the existence of the Trp residue in EGT-N in a form that allows coordination with $Co^{2+}$. However, the complexation mechanism of EGT-N with $Co^{2+}$ has not yet been clarified; therefore, a more detailed examination is presently being conducted.

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