# Note

## A novel amino acid-binding receptor based on weak non-covalent ligand–ligand interactions

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**Abstract**

The Co(III) complex with the $(N)(O)_3$-type tripodal tetradentate ligand, bis-*N*,*N*-carboxymethyl-L-phenylalanine (BCMPA, $H_3$bcmpa), can site-specifically coordinate a bidentate amino acid (AA, Haa) in the *trans*-N configuration rather than the *cis*-N form through the non-covalent weak interligand interactions of hydrogen bondings, steric repulsion, and electrostatic repulsion, on the basis of the $^1$H NMR spectroscopic data and X-ray crystallographic analysis. In addition, the $[Co(bcmpa)(aa)]^-$ complex with *cis*-N form isomerizes to the *trans*-N form, which confirms that the *trans*-N complex is thermodynamically more stable than the *cis*-N one These interactions between the coordination sites of bcmpa and aa ligands are of interest as a molecular recognition model for the enzyme(bcmpa)–substrate(aa) complex.

*Key words:* Crystal structures; Cobalt complexes; Amino acid complexes; Molecular recognition

Biological reactions, characterized by high efficiency and specificity, are achieved by molecular recognition with a combination of weak non-covalent interactions, such as hydrogen bonding, steric repulsion, aromatic ring stacking, electrostatic interaction, hydrophobic interaction, etc., all of which appear at or near to the active site of the enzyme [1]. We have been hitherto interested in the construction of molecular recognition models for enzyme–substrate complex formation through the above weak interactions [2, 3]. In this paper we show that the Co(III) complex with the $(N)(O)_3$ type tripodal tetradentate ligand, bis-*N*,*N*-carboxymethyl-L-phenylalanine (BCMPA, $H_3$bcmpa), can site-specifically coordinate a bidentate amino acid (AA, Haa) ligand; the Co(bcmpa) complex preferentially binds AA in the *trans*-N configuration rather than the *cis*-N form. In addition, the Co(bcmpa) complex with the *cis*-N form isomerizes to the *trans*-N form, which is of interest as a molecular recognition model for the enzyme(bcmpa)–substrate(aa) complex. It is also very interesting that such a simple metal complex with a multidentate ligand demonstrates the coordination-selective recognition of amino acids [4].

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### Experimental

*Preparation of the complexes K[Co(bcmpa)(aa)]*

The complexes, K[Co(bcmpa)(aa)], were prepared according to the following procedure. To 10 ml of a 0.2 M aqueous solution of $K_2[Co(bcmpa)(CO_3)]$ prepared from $K_3[Co(CO_3)_3]$ and BCMPA was added an equimolar amount of amino acid neutralized with KOH, and the resulting mixture was stirred at 50 °C for 4–12 h with adjustment to pH 6–7 by adding 0.1 M HCl. After filtration, the aqueous solution was poured into a QAE Sephadex A-25 column ($Cl^-$ form), which gave two main bands on separation. The primary reddish violet band adsorbed was eluted with an aqueous 0.1 M KCl solution, followed by the secondary faint blue violet band; they were referred to as complex **1** and **2**, respectively. The ratio of the fractions of **1** and **2** was about 6:1, respectively, for all neutral amino acids examined (**a**: L-leucine, **b**: glycine, **c**: L-alanine, **d**: L-phenylalanine, **e**: L-valine) by HPLC measurement at the stage of 30% conversion of the reaction. In the presence of active charcoal that is known to catalyze the chelate ring formation, only the reddish violet band of **1** was formed as the reaction product. Single crystals of **1a** and **2a** suitable for X-ray analysis were obtained by recrystallization from the respective band eluates, when L-leucine was used as the amino acid ligand.

*X-ray structure determinations of 1a and 2a*

*Crystallographic data for complex 1a*: $[KCo(C_{19}H_{24}N_2O_8)]_2 \cdot 7H_2O$, $M = 1139.00$, monoclinic, space group $P2_1$, $a = 14.032(2)$, $b = 13.164(2)$, $c = 14.019(2)$ Å, $\beta = 90.04(1)°$, $V = 2589.6$ Å$^3$, $Z = 2$, $D_c = 1.46$ g cm$^{-3}$, $\mu = 8.79$ cm$^{-1}$, crystal dimensions $0.20 \times 0.20 \times 0.30$ mm. Data were collected at room temperature on an Enraf-


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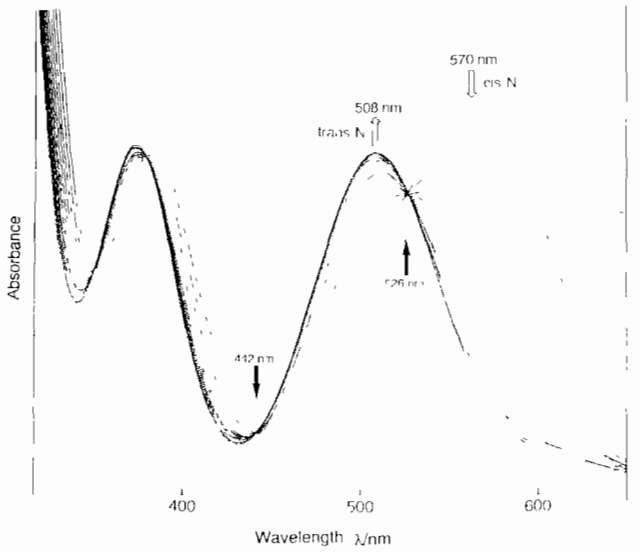


Fig 1. Absorption spectral change during the isomerization from complex **2b** to **1b** in aqueous solution in the presence of active charcoal Successive scans are at 3–6 min intervals

Nonius CAD4 diffractometer using graphite-monochromated Mo K$\alpha$ radiation ($\lambda=0.71073$ Å) by the $\omega$–$2\theta$ scan method ($2\leqslant 2\theta\leqslant 55°$). Of a total of 8145 collected reflections 4399 [$I_o>3\sigma(I_o)$] were unique. A semi-empirical absorption correction based on $\psi$-scans was employed. The structure was solved by use of the program SIR88 [5] and refined by the full-matrix least-squares method to minimize the function $\Sigma_w(|F_o|-|F_c|)^2$ with $w^{-1}=\{\sigma^2(|F_o|)+(0.02|F_o|)^2\}$. The final $R$ and $R_w$ values were 0.060 and 0 072 for 4399, respectively.

Complex **2a**: $KCoC_{19}H_{24}N_2O_8\cdot 5H_2O$, $M=596.51$, orthorhombic, space group $P2_12_12_1$, $a=6.777(1)$, $b=17.962(2)$, $c=21\,785(3)$ Å, $V=2651.7$ Å$^3$, $Z=4$, $D_c=1.49$ g cm$^{-3}$, $\mu=8.60$ cm$^{-1}$, crystal dimensions $0.15\times0.15\times0.30$ mm. Data were collected by the method given above. Of a total of 3110 collected reflections, 2082 [$I_o>3\sigma(I_o)$] were unique. Absorption correction was also applied using the same method. The structure was solved and refined as described above to give final $R$ and $R_w$ values of 0.050 and 0.059 for 2082, respectively.

For both crystals, some peaks less than 0.5 e Å$^{-3}$, which are likely artifacts of the disordered water molecule, appeared on the final differences map, but they were not included in the refinements.

## Results and discussion

The absorption spectra of complexes **1b** and **2b** coordinating glycine are shown in Fig. 1, in which the characteristic absorption peak at 508 nm for **1b** is assignable to a *trans*-N configuration and that at 570 nm for **2b** to a *cis*-N one [6]. Similar spectral changes were also observed in all the other amino acids (**a**, **c**, **d** and **e**) Interestingly, the coordination selectivity for *trans*-N and *cis*-N in the ternary complexes varied in the presence of active charcoal at 50 °C. The absorption band that appeared at 570 nm in the aqueous solution of **2b** changed with time to that at 508 nm with an isosbestic point at 526 nm, which indicates that the isomerization of *cis*-N to *trans*-N proceeds quantitatively. It has been confirmed that the *trans*-N complex **1b** is thermodynamically more stable than the *cis*-N complex **2b**.

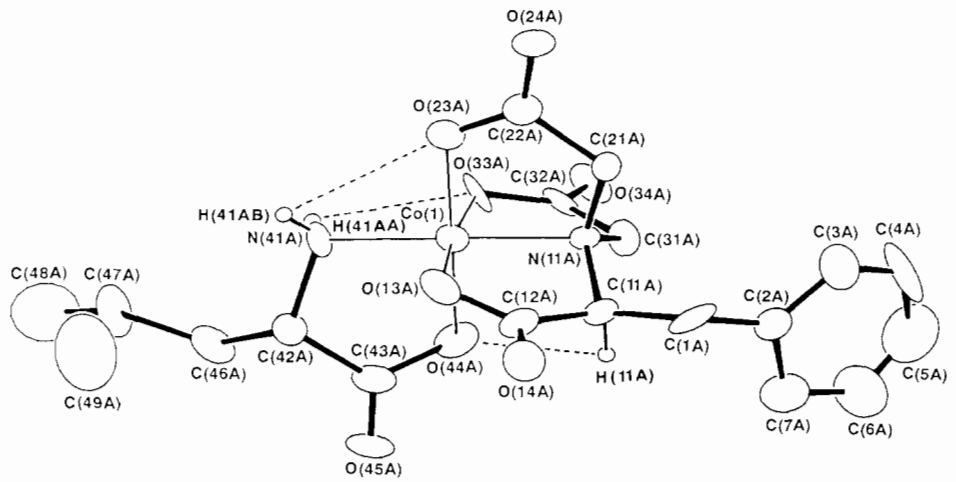


Fig 2 ORTEP drawing of half asymmetric unit of anion parts in the crystal structure of **1a** Hydrogen atoms of amino and $\alpha$-methyne groups are shown as spheres of arbitrary size, and other atoms are represented by ellipsoids corresponding to 30% probability The dotted lines denote hydrogen bonding Important interatomic distances (Å) are as follows, Co(1)–O(13A) 1.885(6), Co(1)–O(23A) 1.895(5), Co(1)–O(33A) 1.887(6), Co(1)–O(44A) 1.884(5), Co(1)–N(11A) 1 938(6), Co(1)–N(41A) 1 929(7), Co(2)–O(13B) 1 879(6), Co(2)–O(23B) 1 895(5), Co(2)–O(33B) 1 900(6), Co(2)–O(44A) 1.889(5), Co(2)–N(11B) 1.950(6), Co(2)–N(41B) 1 919(6).

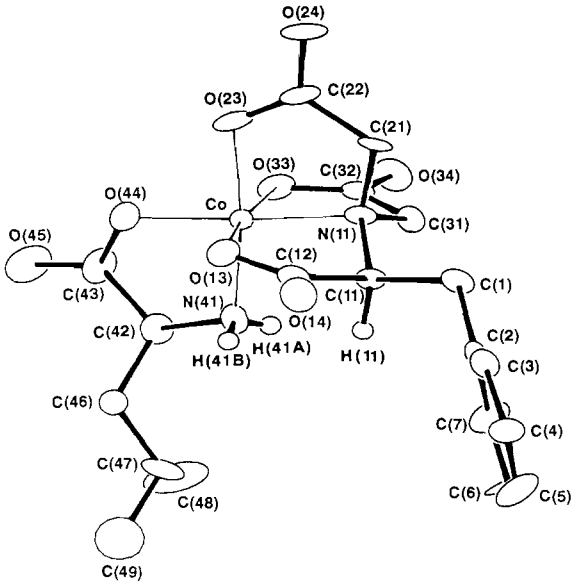


Fig. 3. ORTEP drawing of the anion part in the crystal structure of **2a**. Hydrogen atoms of amino and $\alpha$-methyne groups are shown as spheres of arbitrary size, and other atoms are represented by ellipsoids corresponding to 30% probability Important interatomic distances (Å) are as follows; Co–O(13) 1.916(4), Co–O(23) 1.892(5), Co–O(33) 1 894(4), Co–O(44) 1 885(4), Co–N(11) 1 927(5), Co–N(41) 1.930(5).

In order to elucidate their structural details, the crystal structures of **1a** (Fig. 2) and **2a** (Fig. 3) were analyzed, and shown to be the *trans*-N and *cis*-N configurations, respectively, as expected from their absorption spectral characteristics. These structures include several significant non-covalent interactions between bcmpa and aa. In complex **1a**, some hydrogen bondings were detected between the carboxylate oxygen of bcmpa and the amino group of aa (O(23)···N(41)=2.80, 2.81 Å and O(33)···N(41)= 2.76, 2.76 Å) and between the carboxylate oxygen of aa and the $\alpha$-hydrogen attached to the tertiary carboxymethyl carbon of bcmpa (C(11)·· O(44)=2.83, 2.84 Å). The latter is also supported by the $^1$H NMR spectra; the $\alpha$-proton peak in **1a** was observed at 5.04 ppm (from DSS in $D_2O$, d–d, $J$=10.4 and 5.1 Hz) which was at 0.5 ppm lower field in comparison with that in **2a** (4.58 ppm from DSS in $D_2O$, d–d, $J$=9.5 and 5.2 Hz). Such a hydrogen bonding of C–H···O has also been discussed by Desiraju [7] and Steiner and Saenger [8, 9]. In complex **2a**, on the other hand, no hydrogen bondings were observed in the X-ray structure analysis and $^1$H NMR, but steric and electrostatic repulsions were detected. The former was found between the $\alpha$-proton of bcmpa and amino hydrogens of L-leucine, which is supported by a large N(11)–Co–N(41) angle (99.6(2)°) and elongated C(11)···N(41) distance (3.06 Å). The latter repulsion was observed between electronegative oxygen atoms of carboxylate of bcmpa and aa, which was characterized by the elongated Co–O(13) bond (1.916(4) Å) compared with that of the *trans*-N complex (1.885(6) and 1.879(6) Å) and slightly larger O(44)–Co–O(carboxylate of bcmpa) angles (95.4(2)°, 92.7(2)° and 88.3(2)°, respectively) It is clear that these factors of hydrogen bondings, steric repulsion, and electrostatic repulsion subtly contribute to the preference for the *trans*-N compared to the *cis*-N species.

We found that the *trans*-N complex was preferentially formed as the thermodynamically more stable species through the non-covalent ligand–ligand interactions between the coordination sites of bcmpa and aa ligands. The present findings suggest that the combination of some weak non-covalent interactions makes possible the distinction and recognition of the molecules, even if they are very weak interactions.

## Supplementary material

Tables of atomic coordinates, bond lengths and angles, thermal parameters, and observed and calculated structure factors for the complexes **1a** and **2a** can be obtained from the authors on request.

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