Amino acid recognition of pyridine bis(oxazoline)–copper(II) complex in aqueous solvent

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Abstract—Enantioselective recognition of amino acids has been studied with C_2-symmetric chiral pyridine bis(oxazoline)–copper(II) complexes at physiological pH condition. UV–visible titration revealed strong binding of submillimolar dissociation constant between pyridine bis(oxazoline)–copper(II) complex and amino acids in aqueous solution. Moderate selectivity of up to 2:1 between D- and L-amino acids was achieved. The enantiomers were baseline resolved by capillary electrophoresis, using the bis(L-lysine)–copper(II) complex as a chiral selector. © 2003 Elsevier Science Ltd. All rights reserved.

Molecular recognition of amino acids and peptides is a challenging goal because they are vital components of proteins which play major roles in living organisms. While many receptors with fairly good residue-selectivity and stereoselectivity for amino acid derivatives have been intensively studied in nonpolar solvents,[1] receptors working in polar and aqueous solvents are less developed.[2] As a receptor operating in water, we chose a Cu(II) complex to utilize the water-soluble properties of metalloreceptors.[3] Herein we report a high affinity of simple chiral hosts for underivatized amino acids and their moderate enantioselectivity in aqueous solvents. The chiral hosts are known for their enantioselective catalytic activities in Diels–Alder[4] and Friedel–Craft reactions.[5]

The ligand, pyridine bis(oxazoline) ($\mathbf{L}^{pybox}$) was synthesized according to the modified procedure from the literature.[6] Conversion of 2,6-pyridine dicarboxylic acid to 2,6-pyridine dicarbonyl dichloride with 2 equiv. of oxalyl chloride under catalytic DMF, coupling with amino alcohol in the presence of TEA and activation of the resulting alcohol with methanesulfonyl chloride, followed by cyclization under basic conditions afforded the desired product, $\mathbf{L}^{pybox}$ in high yield over three steps (Fig. 1).[7]

Ligand, $\mathbf{L}^{pybox}$ in dichloromethane was mixed with 1 equiv. of cupric nitrate in aqueous methanol according

to a similar literature procedure.[4] The light green aqueous solution turned to deep blue-green ($\varepsilon_{281\ nm} = 4525$ M^{-1} cm^{-1}, $\varepsilon_{310\ nm} = 3062$ M^{-1} cm^{-1}) in aqueous HEPES buffer (pH 7.4 with 10 mM HEPES, MeOH:H$_2$O = 1:1, v/v) during the metal–ligand complexation. Additional evidence for the formation of [CuL$^{pybox-Ph(R)}$](NO$_3$)$_2$ came from electrospray ionization (ESI) mass analysis (positive, HCO$_2$H/MeOH: observed 477.5 ([CuL$^{pybox-Ph(R)}$·HCO$_2$]$^+$, 15%), observed 401.5 ([L$^{pybox-Ph(R)}$·CH$_3$OH·H]$^+$, 100%), observed 370.5 ([L$^{pybox-Ph(R)}$·H]$^+$, 52%)).

The metal–ligand complex, [CuL$^{pybox-Ph(R)}$](NO$_3$)$_2$ in aqueous solvent was assumed to be a square pyramidal structure similar to [CuL$^{pybox-iPr(R)}$(H$_2$O)$_2$].[8] Upon adding amino acid (**AA**) to aqueous solution of [CuL$^{pybox-Ph(R)}$](NO$_3$)$_2$, bidentate ligand **AA** plausibly replaces the two water ligands to afford [CuL$^{pybox-iPr(R)}$-(**AA**)]$^+$ as shown in Figure 2.[3c]

Complexation between the **pybox**–copper complex and amino acids was investigated at physiological conditions (pH 7.4) with HEPES buffer. When adding under-
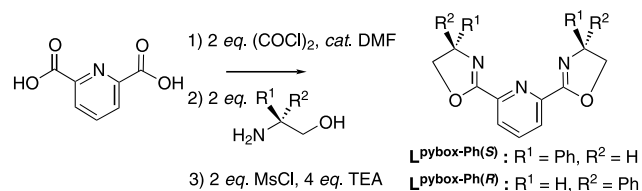

Figure 1. Synthetic scheme of $\mathbf{L}^{pybox}$.

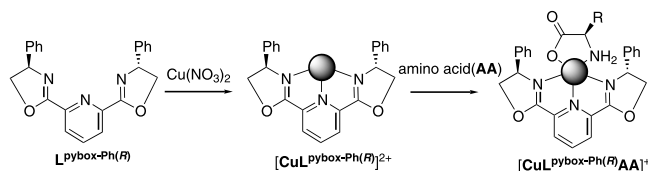* Corresponding author. Tel.: +82-2-880-6682; fax: +82-2-889-1568; e-mail: jihong@plaza.snu.ac.kr

Figure 2. Schematic drawing of stepwise metal–ligand and its amino acid complexation.

ivatized amino acid such as L-phenylalanine (L-Phe) to $[CuL^{pybox-Ph(R)}]^{2+}$, absorbance at $\lambda_{281 \text{ nm}}$ increases, whereas absorbance at $\lambda_{310 \text{ nm}}$ decreases. Therefore, an isosbestic point is observed at $\lambda_{298 \text{ nm}}$ (Fig. 3). This isosbestic point is also observed in the case of interactions with other amino acids (Ala, Val, Asn, etc.). The evident isosbestic point is regarded as the 1:1 host–guest equilibrium between the host ($[CuL^{pybox-Ph(R)}]^{2+}$ and the amino acid guests. The apparent dissociation constant (K_d) was determined by fitting the UV–vis data to the nonlinear regression method. The binding constants of the host, $[CuL^{pybox-Ph(R)}]^{2+}$, with several amino acids are summarized in Table 1. The dissociation constants between the host and amino acids are in the range of submillimolar concentrations: $K_d = 10^{-4} \sim 10^{-5}$ M. The host with (R,R) configuration, $[CuL^{pybox-Ph(R)}]^{2+}$, shows a favorable affinity toward D-amino acids. This enantioselectivity seems to come from the steric hindrance between one phenyl group of the host and the α substituents of amino acids (Fig. 4). When the α substituents of the amino acids are nonpolar, more favorable binding affinities between the host and guests are observed as in the case of guests with hydrophobic side chains (entries 1–6). It is also interesting that guests with hydrophilic side chains show stronger binding affinity to the host when the guests have shorter side chains: Asp, Asn > Glu, Gln (entry 7, 8
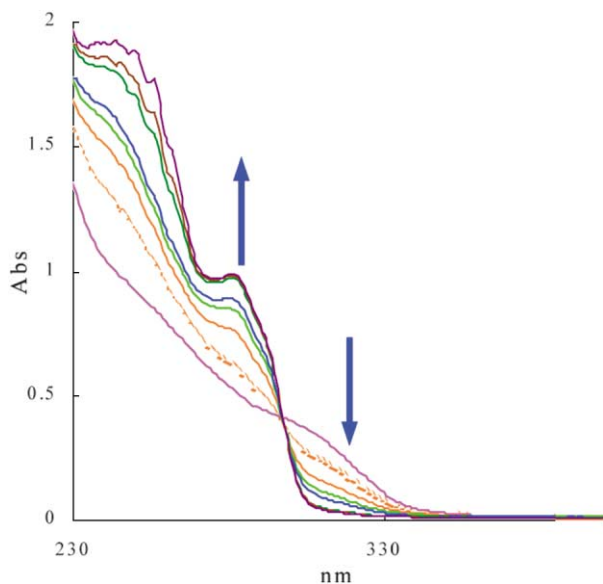
Table 1. Dissociation constants (K_d) between $[CuL^{pybox-Ph(R)}]$-$(NO_3)_2$ and amino acids[a]

Entry	Guest	K_d (M)
1	L-Ala	$5.98(\pm0.69)\times10^{-4}$
2	D-Ala	$3.12(\pm0.42)\times10^{-4}$
3	L-Val	$4.02(\pm0.89)\times10^{-4}$
4	D-Val	$2.02(\pm0.43)\times10^{-4}$
5	L-Phe	$1.95(\pm0.30)\times10^{-4}$
6	D-Phe	$1.10(\pm0.08)\times10^{-4}$
7	L-Asn	$2.27(\pm0.59)\times10^{-4}$
8	D-Asn	$9.98(\pm1.81)\times10^{-5}$
9	L-Gln	$6.21(\pm2.04)\times10^{-4}$
10	D-Gln	$5.00(\pm3.16)\times10^{-4}$
11	L-Asp	$1.54(\pm0.26)\times10^{-4}$
12	L-Glu	$3.41(\pm0.51)\times10^{-4}$

[a] UV–vis titration at 298 K, $[CuL^{pybox-Ph(R)}](NO_3)_2 = 0.10$ mM, [amino acid]$_o$ = 10–15 mM. Absorbance changes at $\lambda_{281 \text{ nm}}$ were monitored after each addition of guests to determine dissociation constants.

versus 9, 10 and 11 versus 12). It is surprising that this simple host without a hydrophobic cavity shows sub-millimolar dissociation constants toward polar amino acids even in aqueous solvent.[3c] In order to understand why the polar amino acids are strongly recognized by the **pybox**–copper complex, thermodynamic driving forces were examined by isothermal microcalorimetric (ITC) analysis during the host–guest binding. The host–guest complexation is enthalpically driven due to favorable metal–ligand interaction:[9] $\Delta H = -10.56(\pm0.18)$ kcal/mol, $T\Delta S = -5.14(\pm1.27)$ kcal/mol and $\Delta H = -12.99(\pm0.71)$ kcal/mol, $T\Delta S = -7.48$ kcal/mol at 30°C for L-Ala and D-Ala, respectively (Fig. 5).

Even though chiral discrimination of amino acids by **pybox**–copper complex was not large, we were able to resolve the enantiomers by capillary electrophoresis (CE). In order to directly detect the analytes and suppress the background UV–vis absorbance, we employed the selector as a nonchromophoric $[Cu(L-\textbf{Lys})_2]$ and the analyte as a chromophoric $\textbf{L}^{pybox-Ph(R)}/\textbf{L}^{pybox-Ph(S)}$.[10] Ligand L-lysine of the chiral selector, $[Cu(L-\textbf{Lys})_2]$, is assumed to be exchanged with $\textbf{L}^{pybox}$ during the electrophoresis. The exchange product, $[Cu(L-\textbf{Lys})(\textbf{L}^{pybox})]$ is thought to be less cationic than the selector, $[Cu(L-\textbf{Lys})_2]$ at pH 7.4, which implies tighter binding as the copper complex moves faster in normal applied voltage

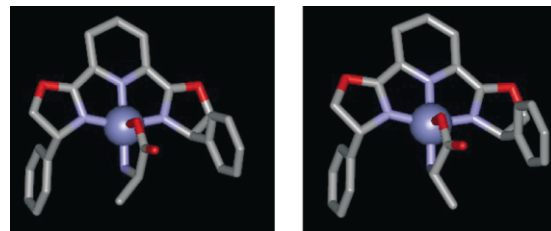**Figure 3.** UV–vis spectral change upon addition of L-Phe to a solution of $[CuL^{pybox-Ph(R)}](NO_3)_2$ in aqueous HEPES buffer (pH 7.4, 10 mM HEPES in MeOH/H$_2$O, 1:1, v/v).

Figure 4. Modeling structures between host and alanine guests: (left) calculated structure for $[CuL(R)]/(D)$-Ala (27.9 kcal/mol), (right) calculated structure for $[CuL(R)]/(L)$-Ala (28.2 kcal/mol).

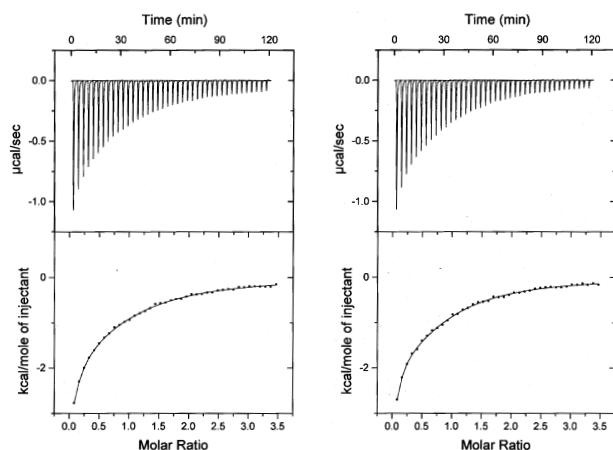**Figure 5.** ITC titrations between [CuL$^{pybox-Ph(R)}$](NO$_3$)$_2$ and amino acids under 10 mM HEPES buffer (pH 7.4) in aqueous methanol (1:1, v/v) at 30°C. (left) L-Ala, (right) D-Ala. $[H]_0 =$ 0.10 mM, $[G]_0 = 4.0$ mM (3 μL×40).

(Fig. 6). It was confirmed that **L**$^{pybox-Ph(R)}$ is the first eluent by feeding on **L**$^{pybox-Ph(R)}$/**L**$^{pybox-Ph(S)}$ = 2 as analytes. ITC titration between host ([CuL$^{pybox-Ph(R)}$]$^{2+}$) and guests (D/L-Lys) corroborated the binding analysis.[11]
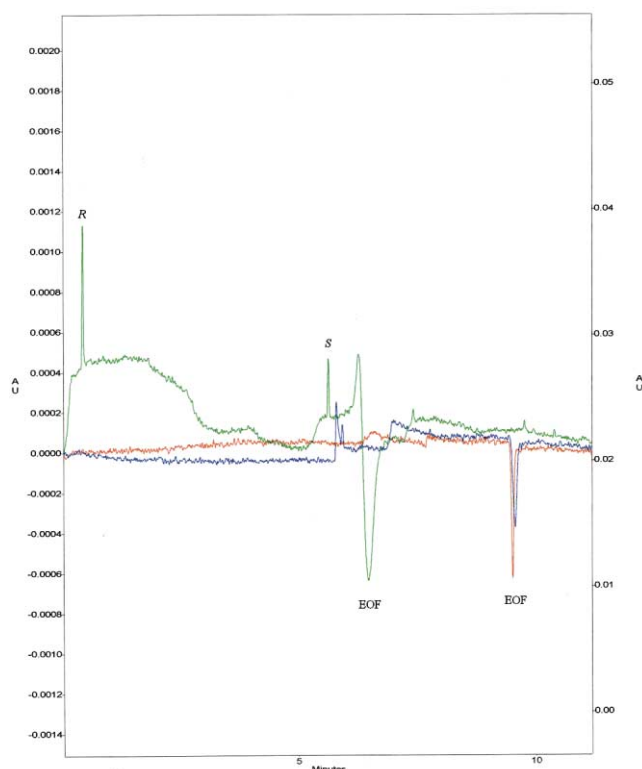

Figure 6. Electropherograms of the chiral separations of **L**$^{pybox-Ph(R)}$/**L**$^{pybox-Ph(S)}$ by CZE (bare fused silica 57 cm×50 μm I.D., applied +23 kV, detection at 280 nm, 6 mM of [Cu(L-**Lys**)$_2$]$^{2+}$ as a chiral selector under 10 mM HEPES buffer (pH 7.4) in aqueous methanol (1:1, v/v) at 23°C. (Red) without a chiral selector, (blue) with a chiral selector in the blank run buffer, (green) with a chiral selector in the selector run buffer.

In conclusion, [CuL$^{pybox-Ph(R)}$] shows strong affinities to amino acids in the range of sub-millimolar concentration ($K_d = 10^{-4} \sim 10^{-5}$ M) even in aqueous solution. Enantioselectivity was moderate up to $K_d(L)/K_d(D) = 2$ with favorable affinities of the *R*-host to D-amino acids. CE enables enantiomers of **L**$^{pybox-Ph(R)}$/**L**$^{pybox-Ph(S)}$ to be baseline separated using [Cu(L-**Lys**)$_2$] as a chiral selector. Binding of [CuL$^{pybox(R)}$] to amino acids was driven by enthalpy. This leads to higher binding constants even in aqueous solution.

Acknowledgements

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7. Typical synthetic procedure for **L**$^{pybox-Ph(S)}$ is as follows: To a white suspension of 340 mg (2.00 mmol) of 2,6-pyridinedicarboxylic acid in 10 mL of dichloromethane were added 2.2 mL of oxalyl chloride (2 M in DCM) and catalytic DMF, which was stirred at rt for additional 8 h to afford a clear yellow solution. All volatiles were evaporated under reduced pressure and dried in vacuo to produce a white 2,6-pyridinedicarbonyl dichloride in

quantative yield. To a solution of 604 mg (4.4 mmol) of (*R*)-2-phenyl glycinol and 2 equiv. (0.7 mL, 5 mmol) of TEA in 5 mL of dichloromethane was dropwise added a solution of 2,6-pyridinedicarbonyl dichloride in 5 mL of dichloromethane at 0°C under nitrogen. Stirring was continued at rt for additional 3 h. After all volatiles were removed under reduced pressure, the white solid of the bisalcohol was redissolved in 10 mL of CH_2Cl_2 and 4 equiv. of TEA (1.3 mL, 9.3 mmol) was added. To the clear solution was dropwise added 2 equiv. of methanesulfonyl chloride (340 μL, 4.39 mmol) at 0°C under nitrogen. Resulting solution was stirred for additional 24 h under nitrogen. All volatiles are removed under reduced pressure. Column chromatography in silica gel (EtOAc:Hex = 2:1, $R_f = 0.18$) afforded the desired product as a white solid in a 70% yield over three steps. Further purification was carried out by recrystallization in $MeOH/CH_2Cl_2$. All the NMR spectroscopic and optical properties are in accordance with the reported values.[6a,b] Mass (ESI+, MeOH): *m/z* 370.5 ([M+H], 100%).

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9. Host–guest complexation was driven by both enthalpy and entropy with some guests with hydrophilic side chains such as Gln and Asn, but the major driving force was the enthalpy: $\Delta H = -3.52$ kcal/mol, $T\Delta S = 0.83$ kcal/mol for $[CuL^{pybox-Ph(R)}]^{2+}$/D-Gln, $\Delta H = -5.52$ kcal/mol, $T\Delta S = 0.93$ kcal/mol for $[CuL^{pybox-Ph(R)}]^{2+}$/D-Asn, $\Delta H = -5.01$ kcal/mol, $T\Delta S = 1.35$ kcal/mol for $[CuL^{pybox-Ph(R)}]^{2+}$/L-Asn at 30°C. For ITC data for Lys, see Ref. 11.

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11. To 0.10 mM of $[CuL^{pybox-Ph(R)}](NO_3)_2$ were stepwise added 3 μL×40 times of 4.0 mM of D-Lys or L-Lys in aqueous methanol at 30°C (10 mM HEPES buffer pH 7.4, MeOH/$H_2O = 1/1$). After each addition of guests, the evolved heat was monitored for 180 s to reach equilibration. Nonlinear regression analysis afforded $K_a = 1.61(\pm 0.39) \times 10^4$ M^{-1} ($\Delta H = -4.93(\pm 0.79)$ kcal/mol and $T\Delta S = 0.298$ kcal/mol for L-Lys) and $K_a = 7.37(\pm 0.73) \times 10^3$ M^{-1} ($\Delta H = -6.06(\pm 0.75)$ kcal/mol and $T\Delta S = -0.166$ kcal/mol for D-Lys).