# Weak interactions in metal complexes of amino acids with a phosphorylated side chain. Conversion of aromatic ring stacking to electrostatic bonding by tyrosine phosphorylation

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**Abstract**

Ternary Cu(II) complexes with the phosphoesters of amino acids (A = *O*-phosphoserine (Pser) or *O*-phosphotyrosine (Ptyr)) and basic amino acids (B = arginine (Arg) or lysine (Lys)), Cu(L-A)(L-B), have been studied by absorption and circular dichroism (CD) spectral, pH titration and X-ray diffraction methods. CD spectral magnitude anomaly in the d–d region at neutral pH was interpreted as due to the electrostatic interactions between the side chain phosphoester group of A and the protonated group of B. The stability constants for the ternary complexes were determined at 25 °C and $I = 0.1$ and 1.0 M($KNO_3$), and the stability enhancement due to the ligand–ligand interactions was evaluated by calculating the equilibrium constant $K$ for the following hypothetical equilibrium from the overall stability constant of each ternary species: Cu(L-A)(L-B′) + Cu(L-A′)(L-B) $\overset{K}{\rightleftharpoons}$ Cu(L-A)(L-B) + Cu(L-A′)(L-B′), where A′ refers to serine or tyrosine (Tyr) and B′ to alanine and the interactions are possible only in Cu(L-A)(L-B). The ionic strength dependence of the log $K$ values ($> 0$) indicated that Cu(L-A)(L-B) is stabilized by electrostatic interactions. X-ray crystal structure analysis of [Cu(bpy)(L-Tyr)$ClO_4$]·$2H_2O$ (bpy = 2,2′-bipyridine) revealed that the non-phosphorylated complex involves aromatic ring stacking between the Tyr phenol and coordinated bpy rings with the average spacing of 3.35 Å in a tetrahedrally distorted square-pyramidal structure with the two nitrogen atoms of bpy and the nitrogen and oxygen atoms of L-Tyr at the equatorial positions and an oxygen atom of the perchlorate ion at the axial position. The results show that the stacked Tyr phenol ring is phosphorylated to be involved in the electrostatic interactions with the positively charged side chain of Arg or Lys, which may be a possible mechanism of the conformational change due to Tyr phosphorylation in proteins.

## Introduction

Protein phosphorylation is recently recognized as an important step in information transfer and control of various biological processes such as enzyme activity [1, 2]. Phosphorylation takes place at the OH groups of serine (Ser), threonine (Thr) and tyrosine (Tyr) in proteins, most frequently on seryl and threonyl residues [2]. Tyr phosphorylation which occurs less frequently plays a specific role in the growth of cell and tumor [3]. In spite of various important biological reactions connected with protein phosphorylation, little is known about the effects and mechanisms by which relevant functions are regulated. Evidently phosphorylation introduces a negatively charged phosphoester group into a network of hydrogen bonding and hydrophobic interactions such as aromatic ring stacking and thus triggers conformational changes. Recent X-ray structural studies have revealed that Ser phosphorylation of the active and less active forms of an allosteric enzyme muscle phosphorylase induces a conformational transition due to electrostatic or hydrogen bonds involving the phosphate moiety and histidine and arginine (Arg) residues [4, 5].

A classical example of electrostatic interactions that play a vital role in enzyme activity is that revealed for a zinc enzyme carboxypeptidase A. In its complex with a substrate, glycyl-L-tyrosine, it fixes the substrate by electrostatic interactions between the carboxylate group of the substrate and the arginine guanidinium group of the enzyme [6]. A phosphate group can interact with the guanidinium group of Arg, and an X-ray structural study has been reported for the arginine phosphate [7] and guanidinium phosphate salts [8]. However, information on the mode and energy of such interactions in solution is lacking, and in view of the importance of calcium and magnesium ions in the trigger actions of phosphorylation and possible involvement of transition metal ions in the reactions following phosphorylation [9], studies on intermolecular interactions

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through a phosphoester group around a metal ion may give clues to understanding structural changes due to phosphorylation and roles of the metal ion involved.

In a ternary system involving a transition metal ion and two ligands with side chains capable of non-covalent interactions, the metal ion assists the interactions by serving as a template which places ligands in an appropriate way and by exerting an electronic effect, and at the same time it transmits information regarding the interactions. From the synthesis of the complexes, spectroscopic data and stability constants, we have concluded that the oppositely charged side chains of acidic and basic amino acids interact with each other electrostatically when they are coordinated in a ternary Cu(II) or Pd(II) complex, such as Cu(L-Asp)(L-Arg) (Asp = aspartate) [10]. Deviation from magnitude additivity of circular dichroism (CD) spectral magnitude in the d–d region was used as an indication of such intramolecular ligand–ligand interactions, and changes in the rotational isomer population calculated from the $^1$H NMR spectra confirmed the existence of such interactions [10e, 10f]. Spectroscopic and calorimetric investigations of the adduct formation in the systems involving platinum DNA intercalators and mononucleotides, such as Pt(phen)(en)$^{2+}$–AMP$^{2-}$ (phen = 1,10-phenanthroline; en = ethylenediamine; AMP$^{2-}$ = adenosine 5′-monophosphate), have revealed the adduct stability enhancement due to contribution of the electrostatic interactions by the phosphate group of AMP$^{2-}$ in addition to that due to aromatic ring stacking [11]. Ternary Cu(II) complexes containing aromatic heterocycles such as phen and aromatic amino acids such as phenylalanine (Phe) and Tyr have been shown to be stabilized by calculating the ring stacking equilibrium constant for a hypothetical equilibrium involving ternary species with and without stacking interactions [12]. X-ray crystal structure analysis of Cu(histamine)(L-Tyr), Cu(phen)(L-Trp) (Trp = tryptophane), etc. revealed that the intramolecular stacking exists in the complex in the solid state, supporting similar interactions in solution [13]. Interestingly phosphorylation of Tyr drastically decreased the stabilization due to the Tyr phenol ring–aromatic heterocycle stacking, which indicated that the stacking interaction was virtually inhibited by introducing a dinegative phosphoester group into the aromatic ring. On the basis of these observations we proposed that phosphorylation of the Tyr residue may be a biological on–off switching of aromatic ring stacking [12a, 14] resulting in a conformational change from the stacked structure to the unstacked structure.

With these points in mind, we determined the solid state structure of Cu(bpy)(L-Tyr) (bpy = 2,2′-bipyridine) by the X-ray diffraction method and studied the non-covalent interactions in Cu(L-A)(L-B), where A refers to *O*-phospho-L-tyrosine (Ptyr) or *O*-phospho-L-serine (Pser) and B to Arg or lysine (Lys), by solution equilibrium and CD spectral methods, from which we conclude the mentioned electrostatic interactions and switching from aromatic ring stacking to electrostatic bonding by Tyr phosphorylation.

## Experimental

### *Reagents*

L-Pser was obtained from Sigma, and other amino acids (AA = alanine (Ala), Arg and Lys) were from Ajinomoto (Takara-Kosan) and from Nacalai Tesque. L-Ptyr was prepared according to the literature [15] and checked by elemental analysis and pH titration. Other reagents used were of the highest grade available.

### *Preparation of [Cu(bpy)(L-Tyr)ClO$_4$]·2H$_2$O*

$Cu(ClO_4)_2 \cdot 6H_2O$ (1.85 g, 5 mmol) and bpy (0.78 g, 5 mmol) were dissolved in 0.2 M HCl (25 ml) by heating, and after cooling a solution of L-Tyr (0.91 g, 5 mmol) in 1 M NaOH (10 ml) was added. The mixture was concentrated *in vacuo* and kept at room temperature. The blue crystals which separated were collected and recrystallized from water. *Anal.* Calc. for $C_{19}H_{22}N_3O_9ClCu$: C, 42.64; H, 4.12; N, 7.85. Found: C, 42.40; H, 4.14; N, 7.94%.

### *pH titration*

pH titrations were carried out at 25 ± 0.05 °C and $I$ = 0.1 or 1.0 M ($KNO_3$) under a nitrogen atmosphere. pH values were measured with a Beckman pHI-71 or an Orion 901 pH meter equipped with a Beckman 39419 double junction reference electrode and a Beckman 39314 or an Orion 91-01-00 glass electrode. NBS standard buffers (4.008, 7.143, 9.180 at 25 °C) were used for calibration of the pH meters. The samples with the Cu(II):ligand A:ligand B ratios of 1:1:1, 1:2:0, 1:0:2 and 1:0:4 with the Cu(II) concentration of 1.5–3 mM were titrated at least twice for checking the reproducibility. For the systems containing Cu(II) and L-A the pH meter readings above 7 were observed to decrease gradually with time probably because of hydrolysis of the phosphoester group of A [16], so that measurements at pH > 7 were not considered for the calculation. The titration data were subjected to the least-squares treatment by using SUPERQUAD [17] to give the stability constants $\beta_{pqrs}$ for the following equation (eqn. (1); charges are omitted for simplicity):

$$pCu + q\text{L-A} + r\text{L-B} + sH \overset{\beta_{pqrs}}{\rightleftharpoons} Cu_p(\text{L-A})_q(\text{L-B})_rH_s \quad (1)$$

$$\beta_{pqrs} = \frac{[Cu_p(\text{L-A})_q(\text{L-B})_rH_s]}{[Cu]^p[\text{L-A}]^q[\text{L-B}]^r[H]^s}$$

where A and B refer to free amino acids except Arg which denotes its monoprotonated form. Conversion of pH meter readings to hydrogen ion concentrations were made by the conversion factors $10^{-pH}/[H^+]=0.855$ ($I=0.1$ M) and 0.783 ($I=1.0$ M), and $pKw'=13.96$ ($I=0.1$ and 1.0 M). These values are closer to the upper limits of the values reported in a recent paper [18].

*Spectra*

CD spectra in the visible region were measured in a 5-cm path-length cell by a JASCO J-500C spectropolarimeter. Absorption spectra in the visible region were obtained with a Shimadzu UV-3100PC and a Hitachi 330 recording spectrophotometer. ESR spectra at 77 K were measured with a JEOL JES-RE1X ESR spectrometer. Samples were prepared by mixing the stock solutions of Cu(II) and ligands, and the pH values were adjusted with aq. NaOH, the concentrations being 1–3 mM with respect to Cu(II).

*X-ray structure determination of [Cu(bpy)(L-Tyr)ClO$_4$]·2H$_2$O*

Single crystals suitable for X-ray diffraction were obtained as [Cu(bpy)(L-Tyr)ClO$_4$]·2H$_2$O from an aqueous solution. A blue needle-like crystal with dimensions of 0.20×0.20×0.20 mm was used for collection of intensity data. Diffraction data were collected at 296 K with a Rigaku AFC-5R four-circle diffractometer using a graphite-monochromated Cu K$\alpha$ radiation ($\lambda=1.54178$ Å). Crystal data and experimental details associated with data collection are given in Table 1. Data collection uniquely defined the triclinic space group $P1$. The intensities of three standard reflections measured every 56 reflections showed no significant variations. Intensity data were collected by the $\omega$–$2\theta$ scan technique in the range $1<2\theta<120°$. Lorentz and polarization corrections were applied, but absorption correction was not. For the determination and refinements of the structure, 1752 independent reflections with $|F_o|>3\sigma(F_o)$ were used. The structure was solved by the heavy atom method and refined by the block-diagonal least-squares method including anisotropic thermal parameters for non-hydrogen atoms. The hydrogen atoms were included and refined as isotropic in the last cycle; their positions were located in the calculated positions. The final $R$ and $R_w$ values were 0.052 and 0.070, respectively. The final difference Fourier maps did not show any significant feature, with most of the largest peaks occurring around the copper and chlorine atoms. Atomic scattering factors and anomalous dispersion terms were taken from the International Tables for X-Ray Crystallography [19]. All calculations were carried out on a FACOM M780 computer at the Data Processing Center of Kyoto University by using the program system KPPXRAY [20].

TABLE 1. Crystal data and experimental details

| | |
|---|---|
| Formula | $CuClO_9N_3C_{19}H_{22}$ |
| $M$ | 535.40 |
| Crystal system | triclinic |
| Space group | $P1$ |
| $a$ (Å) | 10.559(2) |
| $b$ (Å) | 9.223(2) |
| $c$ (Å) | 5.847(2) |
| $\alpha$ (°) | 78.11(3) |
| $\beta$ (°) | 105.99(2) |
| $\gamma$ (°) | 92.28(2) |
| $V$ (Å$^3$) | 535.6 |
| $Z$ | 1 |
| $D_{calc}$ (g cm$^{-1}$) | 1.66 |
| $F(000)$ | 275 |
| Crystal size (mm) | 0.20×0.20×0.20 |
| Color | blue |
| $\mu$ (cm$^{-1}$) | 31.02 (Cu K$\alpha$) |
| Diffractometer | Rigaku AFC-5R |
| Scan mode | $\omega$–$2\theta$ |
| $2\theta$ scan range (°) | 1–120 |
| Radiation | Cu K$\alpha$ ($\lambda=1.54178$ Å) |
| Scan interval (°) | $1.1+0.5\tan\theta$ |
| Scan speed in $\omega$ (° min$^{-1}$) | 4 |
| Reflections measured | $\pm h$, $\pm k$, $+l$ |
| No. data collected | 1850 |
| No. data used ($\|F_o\|>3\sigma(F_o)$) | 1752 |
| $R$ | 0.052 |
| $R_w$ | 0.070 |

The final positional and isotropic temperature factors of the non-hydrogen atoms are listed in Table 2.

## Results and discussion

*Absorption, CD and ESR spectra*

The absorption spectra for the Cu(L-A)(L-B) systems* were measured at pH 7.1–10.4 in 0.1 and 1 M NaClO$_4$ and in 50% aqueous methanol (Table 3). They showed a d–d absorption maximum at 618–632 nm with nearly the same $\epsilon$ values. The CD spectra exhibited a negative extremum at 609–632 nm for Cu(L-Pser)(L-B) and at 581–608 nm for Cu(L-Ptyr)(L-B) and a weak positive extremum at 754–785 nm for all the systems. From $^{31}$P NMR spectra Abott and co-workers indicated that the phosphoester moiety coordinates to Cu(II) in the monoprotonated species Cu(Pser)H but not in Cu(Pser)$_2$ [16a, 21]. Since the absorption and CD spectral patterns and ESR spectral parameters (Table 4) for Cu(L-Ala)$_2$, Cu(L-Pser)$_2$ and Cu(L-Pser)(L-B) are very similar to each other, we may conclude the glycine-like mode of

*The abbreviation B is defined to indicate the protonated form with a proton in the side chain when it is used for a system, e.g. Cu(L-A)(L-B).

TABLE 2. Positional and isotropic temperature factors for $[Cu(bpy)(L\text{-}Tyr)ClO_4]\cdot 2H_2O$ with e.s.d.s

| Atom | $x$ | $y$ | $z$ | $B_{eq}$ |
|---|---|---|---|---|
| Cu | 0 | 0 | 0 | 1.85 |
| Cl | 0.2889(3) | 0.2720(4) | −0.1080(7) | 3.58 |
| C(1) | −0.2747(11) | 0.0561(12) | −0.1269(22) | 2.17 |
| C(2) | −0.4065(12) | 0.0429(15) | −0.2200(25) | 3.41 |
| C(3) | −0.4497(11) | −0.0408(16) | −0.4023(27) | 3.60 |
| C(4) | −0.3602(13) | −0.1009(15) | −0.4873(25) | 3.48 |
| C(5) | −0.2257(12) | −0.0828(13) | −0.3855(22) | 2.61 |
| C(6) | −0.2141(11) | 0.1374(12) | 0.0697(20) | 1.99 |
| C(7) | −0.2923(12) | 0.2206(13) | 0.1562(24) | 2.82 |
| C(8) | −0.2197(13) | 0.2954(13) | 0.3444(23) | 2.87 |
| C(9) | −0.0882(13) | 0.2829(13) | 0.4255(23) | 2.78 |
| C(10) | −0.0194(12) | 0.1982(13) | 0.3313(23) | 2.70 |
| C(11) | 0.1919(10) | −0.1432(12) | −0.1164(20) | 1.97 |
| C(12) | 0.2335(10) | −0.1720(12) | 0.1704(19) | 1.96 |
| C(13) | 0.1921(11) | −0.3298(13) | 0.2663(21) | 2.45 |
| C(14) | 0.0494(11) | −0.3548(12) | 0.1727(21) | 2.35 |
| C(15) | 0.0050(12) | −0.4217(12) | −0.0326(22) | 2.47 |
| C(16) | −0.1286(12) | −0.4451(13) | −0.1268(22) | 2.55 |
| C(17) | −0.2219(11) | −0.4048(12) | −0.0289(23) | 2.46 |
| C(18) | −0.1792(12) | −0.3390(13) | 0.1813(21) | 2.51 |
| C(19) | −0.0451(11) | −0.3165(12) | 0.2726(21) | 2.30 |
| N(1) | −0.1841(8) | −0.0058(10) | −0.2025(16) | 2.01 |
| N(2) | −0.0834(9) | 0.1247(10) | 0.1554(17) | 2.05 |
| N(3) | 0.1713(8) | −0.0561(10) | 0.2464(16) | 1.88 |
| O(1) | 0.0803(7) | −0.0725(9) | −0.2176(13) | 2.16 |
| O(2) | 0.2587(8) | −0.1905(10) | −0.2204(14) | 2.75 |
| O(3) | −0.3491(8) | −0.4257(10) | −0.1254(16) | 3.13 |
| O(4) | 0.2943(15) | 0.2481(20) | −0.3533(26) | 8.56 |
| O(5) | 0.3406(18) | 0.4214(14) | −0.0949(33) | 10.14 |
| O(6) | 0.3711(10) | 0.1564(15) | 0.0651(26) | 7.12 |
| O(7) | 0.1564(9) | 0.2581(11) | −0.0965(20) | 4.51 |
| O(1W) | −0.4859(11) | 0.4409(12) | 0.4767(21) | 5.04 |
| O(2W) | −0.5029(8) | 0.6780(10) | 0.0897(17) | 3.39 |

coordination by Pser in the ternary complexes. This is supported by the ESR spectra showing the superhyperfine structures corresponding to two coordinated nitrogens. It is sterically impossible for Ptyr to form an intramolecular bonding with Cu(II) through the phosphoester group when it is bound in the glycine-like mode.

*CD spectral magnitude anomaly*

A large body of experimental evidence has established that the CD spectral magnitude in the d–d regions for mixed ligand Cu(II) and Pd(II) complexes containing $\alpha$-amino acids is an additive function of the magnitudes for the component amino acid complexes in the absence of ligand–ligand side chain interactions but that it deviates from additivity when there are such interactions [10]. Such magnitude additivity was originally observed for Cu(II)–dipeptide complexes by Tsangaris and Martin [22]. For the systems Cu(L-A)(L-B), we may estimate the magnitude $\Delta\epsilon_{calc}$ by eqn. (2)

$$\Delta\epsilon_{calc} = 1/2(\Delta\epsilon_{Cu(L\text{-}A)_2} + \Delta\epsilon_{Cu(L\text{-}B)_2}) \quad (2)$$

where $\Delta\epsilon_{Cu(L\text{-}A)_2}$ and $\Delta\epsilon_{Cu(L\text{-}B)_2}$ are the observed magnitudes for $Cu(L\text{-}A)_2$ and $Cu(L\text{-}B)_2$ at the maximum wavelength of Cu(L-A)(L-B). In the presence of side chain interactions, the experimental values, $\Delta\epsilon_{obs}$, deviate from $\Delta\epsilon_{calc}$ due to restricted side chain motions. Table 3 shows that, while the relative magnitudes $\Delta\epsilon_{obs}/\Delta\epsilon_{calc}$ calculated for the negative peak in the d–d region are close to 1 for Cu(L-Pser)(L-Ala) and Cu(L-Ptyr)(L-Ala), those for the systems with B = Arg and Lys are significantly different from 1. The observed and calculated CD spectra are typically shown in Fig. 1 for Cu(L-Pser)(L-Lys) and Cu(L-Ptyr)(L-Lys). This magnitude anomaly depends on the polarity of the medium; the relative magnitude greatly deviates from additivity in 50% aqueous methanol and in water at lower ionic strengths but is additive in 1 M $NaClO_4$, indicating that the anomaly is due to electrostatic interactions between the negatively charged phosphoester group and the positively charged guanidinium group of Arg or the $\epsilon$-ammonium group of Lys. The observed spectral behavior is consistent with and supported by our previous conclusions on the electrostatic interactions between the side chains of an acidic and a basic amino acid in Cu(II) and Pd(II) complexes [10]. The magnitudes for Cu(L-Pser)(L-Ala) and Cu(L-Ptyr)(L-Ala) in 50% aqueous methanol are slightly different from 1, which may indicate a conformational change due to a possible hydrogen bonding between the apically coordinated water molecule and the phosphoester group of Pser and Ptyr. It should be noted in this connection that Tyr-containing Cu(II) complexes exhibited anomalous magnitudes probably due to close Cu(II)–aromatic ring contact [23]. The fact that Cu(L-Ptyr)(L-Ala) exhibited magnitude additivity implies that there is no such contact in this complex.

The magnitudes for Cu(L-Pser)(L-Lys) calculated by using eqn. (3) are in excellent agreement with the observed values.

$$\Delta\epsilon_{Cu(L\text{-}A)(L\text{-}B)} = \Delta\epsilon_{Cu(L\text{-}A)(DL\text{-}B)} + \Delta\epsilon_{Cu(DL\text{-}A)(L\text{-}B)} \quad (3)$$

This shows that there is no preferential formation of *cis* or *trans* isomers in Cu(L-A)(L-B) and Cu(L-A)(D-B) [10h, 24].

*Stability constants and preferable formation of ternary complexes*

The stability constants log $\beta_{pqrs}$ for the proton–ligand and ternary Cu(II)–ligand complexes are summarized in Table 5. The values for the Cu(II)–L-Pser system at $I = 0.1$ M and 1.0 M ($KNO_3$) are in agreement with the literature values for the DL-form at $I = 0.2$ M ($KNO_3$) [21]. The Cu(II)–L-Ptyr system formed similar species with somewhat lower stability constants. As shown by the species distribution curves for Cu(L-A)(L-Arg)

TABLE 3. Absorption and CD spectra for Cu(L-A)(L-B) systems in various mediums

| Complex | Medium | pH | Absorption | | CD | | CD additivity[a] | | CD additivity[b] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\lambda_{max}$ | $\epsilon$ | $\lambda_{max}$ | $\Delta\epsilon_{obs}$ | $\Delta\Delta\epsilon^c$ | $\Delta\epsilon_{obs}/\Delta\epsilon_{calc}$ | $\Delta\Delta\epsilon$ | $\Delta\epsilon_{obs}/\Delta\epsilon_{calc}$ |
| Cu(L-Ala)$_2$ | 1 M $NaClO_4$ | 9.6 | 620 | 57 | 627 | −0.071 | | | | |
| | | | | | 770 | 0.008 | | | | |
| | 0.1 M $NaClO_4$ | 10.5 | 624 | 56 | 628 | −0.072 | | | | |
| | | | | | 770 | 0.008 | | | | |
| | $H_2O$ | 9.9 | 626 | 55 | 628 | −0.072 | | | | |
| | | | | | 780 | 0.011 | | | | |
| | 50% MeOH–$H_2O$ | 10.1 | 624 | 57 | 635 | −0.075 | | | | |
| | | | | | 760 | 0.014 | | | | |
| Cu(L-Pser)$_2$ | 1 M $NaClO_4$ | 10.1 | 630 | 53 | 634 | −0.176 | | | | |
| | | | | | 790 | 0.028 | | | | |
| | 0.1 M $NaClO_4$ | 10.5 | 632 | 55 | 631 | −0.199 | | | | |
| | | | | | 788 | 0.035 | | | | |
| | $H_2O$ | 10.6 | 632 | 55 | 631 | −0.199 | | | | |
| | | | | | 789 | 0.033 | | | | |
| | 50% MeOH–$H_2O$ | 10.8 | 632 | 56 | 632 | −0.227 | | | | |
| | | | | | 782 | 0.033 | | | | |
| Cu(L-Ptyr)$_2$ | 1 M $NaClO_4$ | 9.9 | 622 | 58 | 605 | −0.264 | | | | |
| | | | | | 761 | 0.051 | | | | |
| | 0.1 M $NaClO_4$ | 10.1 | 626 | 58 | 607 | −0.259 | | | | |
| | | | | | 771 | 0.053 | | | | |
| | $H_2O$ | 10.3 | 626 | 58 | 610 | −0.236 | | | | |
| | | | | | 774 | 0.050 | | | | |
| | 50% MeOH–$H_2O$ | 10.8 | 624 | 59 | 612 | −0.197 | | | | |
| | | | | | 754 | 0.045 | | | | |
| Cu(L-Pser)(L-Ala) | 1 M $NaClO_4$ | 7.1 | | | 632 | −0.116 | 0.001 | 0.99 | | |
| | | | | | 785 | 0.018 | 0.002 | 1.1 | | |
| | 0.1 M $NaClO_4$ | 7.5 | | | 630 | −0.128 | 0.005 | 0.96 | | |
| | | | | | 780 | 0.022 | 0.003 | 1.2 | | |
| | 50% MeOH–$H_2O$ | 7.8 | | | 625 | −0.127 | 0.017 | 0.88 | | |
| | | | | | 764 | 0.033 | 0.011 | 1.5 | | |
| Cu(L-Pser)(L-Arg) | 1 M $NaClO_4$ | 7.1 | 626 | 56 | 623 | −0.155 | −0.012 | 1.08 | | |
| | | | | | 774 | 0.028 | 0.001 | 1.0 | | |
| | 0.1 M $NaClO_4$ | 7.7 | 628 | 55 | 618 | −0.190 | −0.042 | 1.28 | | |
| | | | | | 775 | 0.042 | 0.010 | 1.3 | | |
| | $H_2O$ | 7.8 | 632 | 56 | 616 | −0.214 | −0.078 | 1.57 | | |
| | | | | | 774 | 0.050 | 0.020 | 1.7 | | |
| | 50% MeOH–$H_2O$ | 8.1 | 632 | 60 | 610 | −0.273 | −0.134 | 1.96 | | |
| | | | | | 775 | 0.074 | 0.040 | 2.2 | | |
| Cu(L-Pser)(L-Lys) | 1 M $NaClO_4$ | 9.2 | 624 | 55 | 622 | −0.164 | −0.012 | 1.08 | 0.000 | 1.00 |
| | | | | | 773 | 0.029 | 0.000 | 1.0 | 0.000 | 1.0 |
| | 0.1 M $NaClO_4$ | 9.3 | 630 | 57 | 621 | −0.180 | −0.020 | 1.13 | | |
| | | | | | 769 | 0.042 | 0.009 | 1.3 | | |

(*continued*)

TABLE 3. (*continued*)

| Complex | Medium | pH | Absorption | | CD | | CD additivity[a] | | CD additivity[b] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\lambda_{max}$ | $\epsilon$ | $\lambda_{max}$ | $\Delta\epsilon_{obs}$ | $\Delta\Delta\epsilon^c$ | $\Delta\epsilon_{obs}/\Delta\epsilon_{calc}$ | $\Delta\Delta\epsilon$ | $\Delta\epsilon_{obs}/\Delta\epsilon_{calc}$ |
| | $H_2O$ | 9.6 | 630 | 57 | 619 | −0.187 | −0.038 | 1.26 | 0.004 | 0.98 |
| | | | | | 763 | 0.047 | 0.015 | 1.5 | −0.004 | 1.0 |
| | 50% | 9.3 | 632 | 59 | 609 | −0.211 | −0.062 | 1.42 | 0.003 | 0.99 |
| | MeOH–$H_2O$ | | | | 760 | 0.070 | 0.036 | 2.1 | 0.000 | 1.0 |
| Cu(L-Ptyr)(L-Ala) | 1 M $NaClO_4$ | 9.5 | 620 | 56 | 604 | −0.193 | −0.004 | 1.02 | | |
| | | | | | 772 | 0.034 | 0.003 | 1.1 | | |
| | 0.1 M $NaClO_4$ | 9.9 | 624 | 56 | 608 | −0.186 | 0.015 | 1.09 | | |
| | | | | | 770 | 0.039 | 0.008 | 1.3 | | |
| | $H_2O$ | 10.0 | 626 | 55 | 603 | −0.177 | −0.023 | 1.15 | | |
| | | | | | 768 | 0.039 | 0.009 | 1.3 | | |
| | 50% | 10.3 | 622 | 58 | 602 | −0.164 | −0.028 | 1.21 | | |
| | MeOH–$H_2O$ | | | | 759 | 0.043 | 0.010 | 1.3 | | |
| Cu(L-Ptyr)(L-Arg) | 1 M $NaClO_4$ | 9.5 | 620 | 55 | 595 | −0.271 | −0.044 | 1.19 | | |
| | | | | | 765 | 0.064 | 0.015 | 1.3 | | |
| | 0.1 M $NaClO_4$ | 9.9 | 622 | 53 | 586 | −0.361 | −0.175 | 1.94 | | |
| | | | | | 760 | 0.102 | 0.055 | 2.2 | | |
| | $H_2O$ | 10.0 | 624 | 53 | 585 | −0.411 | −0.249 | 2.54 | | |
| | | | | | 759 | 0.115 | 0.073 | 2.7 | | |
| | 50% | 10.4 | 618 | 52 | 581 | −0.500 | −0.357 | 3.50 | | |
| | MeOH–$H_2O$ | | | | 754 | 0.144 | 0.097 | 3.1 | | |
| Cu(L-Ptyr)(L-Lys) | 1 M $NaClO_4$ | 9.4 | 618 | 56 | 598 | −0.265 | −0.038 | 1.17 | | |
| | | | | | 764 | 0.051 | 0.008 | 1.2 | | |
| | 0.1 M $NaClO_4$ | 9.5 | 624 | 55 | 592 | −0.332 | −0.082 | 1.33 | | |
| | | | | | 767 | 0.080 | 0.038 | 1.9 | | |
| | $H_2O$ | 9.7 | 622 | 53 | 591 | −0.382 | −0.114 | 2.27 | | |
| | | | | | 765 | 0.096 | 0.057 | 2.5 | | |
| | 50% | 9.6 | 622 | 55 | 586 | −0.459 | −0.312 | 3.12 | | |
| | MeOH–$H_2O$ | | | | 760 | 0.121 | 0.045 | 2.7 | | |

[a] $\Delta\epsilon_{calc}$ was calculated according to eqn. (2). [b] $\Delta\epsilon_{calc}$ was calculated according to eqn. (3). [c] $\Delta\Delta\epsilon = \Delta\epsilon_{obs} - \Delta\epsilon_{calc}$.

TABLE 4. ESR parameters at 77 K for amino acid–Cu(II) systems

| Complex | pH | $g_{\parallel}$ | $g_{\perp}$ | $\lvert A_{\parallel}\rvert$ (mT) | $\lvert A_{\perp}\rvert$ (mT) | $N^a$ |
|---|---|---|---|---|---|---|
| Cu(L-Pser)$_2$ | 7.9 | 2.247 | 2.056 | 17.5 | 1.2 | 5 |
| Cu(L-Ala)$_2$ | 7.7 | 2.249 | 2.056 | 16.8 | 1.4 | 5 |
| Cu(L-Pser)(L-Ala) | 7.6 | 2.259 | 2.057 | 17.3 | 1.3 | 5 |
| Cu(L-Pser)(L-Arg) | 7.7 | 2.252 | 2.056 | 17.0 | [b] | [b] |
| Cu(L-Pser)(L-Lys) | 7.6 | 2.257 | 2.057 | 17.5 | [b] | [b] |

[a]The number of superhyperfine structures due to nitrogen. [b]Values could not be measured exactly due to broadening.

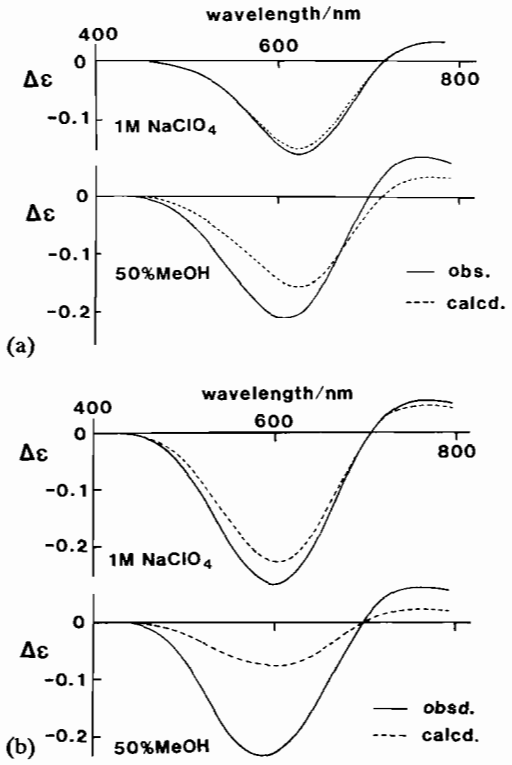


Fig. 1. Observed (——) and calculated (– – –) CD spectra for Cu(L-Pser)(L-Lys) (a) and Cu(L-Ptyr)(L-Lys) (b). Calculated spectra were obtained by eqn. (3).

calculated from the stability constants in Table 5 (Fig. 2), the ternary species (1110 for B = Arg and 1111 for B = Lys) predominates at neutral pH in all the systems studied and at pH 5–6 its protonated species is formed to a small extent. This confirms that the species responsible for the CD magnitudes listed in Table 3 are mainly the ternary species with monoprotonated B.

The tendency of ternary complex formation can be estimated from the $\Delta\log K$ and $\log K_m$ values defined by eqns. (4) and (5), respectively [28].

$$\mathrm{CuA} + \mathrm{CuB} \overset{10^{\Delta\log K}}{\rightleftharpoons} \mathrm{CuAB} + \mathrm{Cu}$$

$$\Delta\log K = \log \beta_{\mathrm{CuAB}} - \log \beta_{\mathrm{CuA}} - \log \beta_{\mathrm{CuB}} \quad (4)$$

$$\mathrm{CuA_2} + \mathrm{CuB_2} \overset{K_m}{\rightleftharpoons} 2\mathrm{CuAB}$$

$$\log K_m = 2\log \beta_{\mathrm{CuAB}} - (\log \beta_{\mathrm{CuA_2}} + \log \beta_{\mathrm{CuB_2}}) \quad (5)$$

According to Sigel [28b] the statistical value of $\Delta\log K$ is $-0.6$ for square-planar complexes of bidentate ligands and $-0.9$ for distorted octahedral complexes such as known for Cu(II), while the statistical $\log K_m$ value for square-planar complexes is 0.6 [29]. Table 6 summarizes the $\Delta\log K$ and $\log K_m$ values calculated from the stability constants listed in Table 5. The $\Delta\log K$ values are smaller than $-0.9$ for most ternary complexes, but the $\log K_m$ values suggest that the ternary complexes are reasonably stable as compared with the parent complexes.

*Evaluation of the stabilization due to electrostatic interactions involving a phosphoester group*

The stability constants for Cu(L-A)(L-B) reflect various factors which affect the solution equilibrium, so that it is often difficult to ascribe the stabilization of the system to a specific factor. We have previously shown that the stability enhancement mainly due to aromatic ring stacking can be calculated by considering a hypothetical equilibrium involving ternary species with and without expected ligand–ligand interactions [12]. For the systems Cu(L-A)(L-B), we may think of a constant $K$ defined by the following equilibrium

$$\mathrm{Cu(L\text{-}A)(L\text{-}B')} + \mathrm{Cu(L\text{-}A')(L\text{-}B)} \overset{K}{\rightleftharpoons} \mathrm{Cu(L\text{-}A)(L\text{-}B)} + \mathrm{Cu(L\text{-}A')(L\text{-}B')} \quad (6)$$

where A and B are the amino acids with an interacting charged side group, and A′ and B′ are those without such a group. Intramolecular electrostatic interactions are possible only in Cu(L-A)(L-B), and the stability increment can be evaluated by eqn. (7) by using the stability constants determined by separate experiments.

$$\log K = \log \beta_{\mathrm{Cu(L\text{-}A)(L\text{-}B)}} + \log \beta_{\mathrm{Cu(L\text{-}A')(L\text{-}B')}} - \log \beta_{\mathrm{Cu(L\text{-}A)(L\text{-}B')}} - \log \beta_{\mathrm{Cu(L\text{-}A')(L\text{-}B)}} \quad (7)$$

TABLE 5. Stability constants log $\beta_{pqrs}$ for $Cu_p(L\text{-}A)_q(L\text{-}B)_rH_s$ complexes at 25 °C[a]

| Complex | *pqrs* | $I=0.1$ M ($KNO_3$) | $I=1.0$ M ($KNO_3$) |
|---|---|---|---|
| Cu(L-Pser)(L-Ala) | 1110 | 15.631(4) | 15.318(4) |
| Cu(L-Pser)(L-Arg) | 1111 | 21.16(2) | 20.22(2) |
| | 1110 | 15.614(5) | 14.836(5) |
| Cu(L-Pser)(L-Lys) | 1112 | 31.40(3) | |
| | 1111 | 26.066(5) | 25.821(5) |
| Cu(L-Ser)(L-Ala) | 1110 | 15.146(3) | 15.158(3) |
| Cu(L-Ser)(L-Arg) | 1110 | 14.714(4) | 14.645(6) |
| Cu(L-Ser)(L-Lys) | 1111 | 25.217(3) | 25.486(6) |
| Cu(L-Ptyr)(L-Ala) | 1111 | | 20.32(3) |
| | 1110 | 15.289(4) | 15.094(4) |
| Cu(L-Ptyr)(L-Arg) | 1111 | 20.799(5) | 20.152(8) |
| | 1110 | 15.400(1) | 14.801(2) |
| Cu(L-Ptyr)(L-Lys) | 1112 | 31.35(3) | 31.00(04) |
| | 1111 | 26.119(5) | 25.811(6) |
| | 1110 | 16.026(8) | 15.952(9) |
| Cu(L-Tyr)(L-Ala) | 1111 | | 24.956(6) |
| | 1110 | | 15.422(7) |
| Cu(L-Tyr)(L-Arg) | 1111 | | 24.421(3) |
| | 1110 | | 4.943(3) |
| Cu(L-Tyr)(L-Lys) | 1112 | | 35.273(4) |
| | 1111 | | 25.747(6) |
| Cu–L-Ala | 1010 | 8.33[b] | 8.135(0) |
| | 1020 | 15.27[b] | 14.973(0) |
| L-Ala | 0011 | 9.82[b] | 9.826(1) |
| | 0012 | 12.16[b] | 12.276(2) |
| Cu–L-Arg | 1010 | 7.652(1) | 7.670(1) |
| | 1020 | 14.128(1) | 14.178(1) |
| | 1021 | 3.140(2) | 2.964(2) |
| L-Arg | 0011 | 9.104(1) | 9.139(1) |
| | 0012 | 11.099(1) | 11.311(1) |
| Cu–L-Lys | 1011 | 18.239(1) | 18.497(1) |
| | 1022 | 35.318(2) | 35.885(3) |
| | 1021 | 25.348(3) | 25.697(4) |
| | 1020 | 14.872(4) | 15.283(4) |
| L-Lys | 0011 | 10.589(1) | 10.693(1) |
| | 0012 | 19.759(1) | 20.007(1) |
| | 0013 | 21.592(1) | 22.222(1) |
| Cu–L-Pser | 1101 | 14.781(1) | 14.014(1) |
| | 1100 | 9.578(1) | 8.962(1) |
| | 1200 | 15.656(1) | 15.100(1) |
| L-Pser | 0101 | 9.927(0) | 9.592(1) |
| | 0102 | 15.671(0) | 15.097(1) |
| | 0103 | 17.840(1) | 17.230(2) |
| Cu–L-Ser | 1100 | 7.858[c] | 7.821(4) |
| | 1200 | 14.428[c] | 14.428(3) |
| L-Ser | 0101 | 9.073[c] | 9.118(1) |
| | 0102 | 11.024[c] | 11.407(2) |
| L-Tyr | 1011 | 17.99[d] | 17.61(1) |
| | 1010 | 10.64[d] | 10.22(4) |
| | 1022 | 34.90[d] | 34.64(1) |
| | 1021 | 25.47[d] | 25.13(7) |
| | 1020 | 15.36[d] | 15.22(4) |
| | 0011 | 10.142[d] | 9.898(6) |
| | 0012 | 19.170[d] | 19.073(5) |
| | 0013 | 21.051[d] | 21.594(12) |
| Cu–L-Ptyr | 1101 | 14.264(2) | 13.591(1) |
| | 1100 | 9.261(2) | 8.671(1) |
| | 1200 | 14.841(4) | 14.449(1) |
| L-Ptyr | 0011 | 9.535(2) | 9.386(2) |
| | 0012 | 15.283(2) | 14.861(2) |
| | 0013 | 17.527(6) | 17.072(7) |

[a]Values in parentheses denote e.s.d.s. [b]From ref. 25. [c]From ref. 26. [d]From ref. 27.

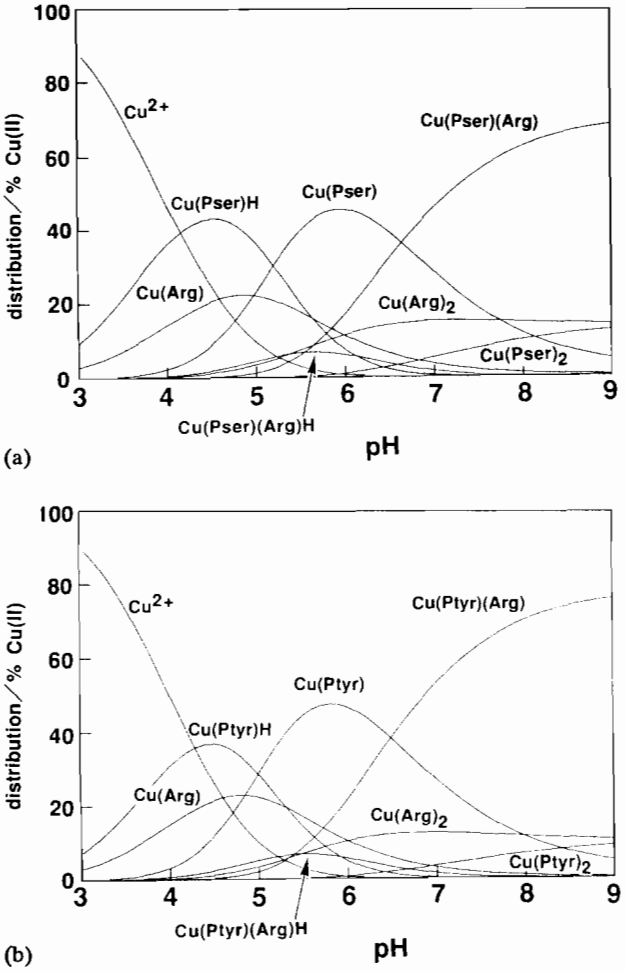


Fig. 2. Calculated species distributions for $Cu_p(L\text{-}A)_q(L\text{-}Arg)_rH_s$. A = Pser (a) and Ptyr (b). Total concentrations: [Cu] = [A] = [Arg] = 1 mM.

By definition log $K$ is equal to 0 when there is no ligand–ligand interaction. The log $K$ values then show stabilization of Cu(L-A)(L-B) with Cu(L-A′)(L-B′) as standard. In the present systems A′ ligands are Ser and Tyr for Cu(L-Pser)(L-B) and Cu(L-Ptyr)(L-B), respectively, B′ being Ala in both systems. An advantage of eqn. (7) is that the ligand field of the complexes can be maintained nearly constant, thus making it possible to calculate the stability difference. The values calculated from the stability constant log $\beta_{pqrs}$ are listed in Table 7. The log $K$ values are all positive, indicating that the electrostatic interactions between the side chains of L-A and L-B stabilize the ternary complexes involving both ligands. As expected, the values are dependent on the ionic strength of the medium, and the stabilization is greater at lower ionic strength, which parallels the CD spectral magnitude enhancement and supports that the complexes are stabilized by electrostatic interactions. On the basis of the stability constants and the spectral observations we propose the ternary

TABLE 6. Tendency of ternary complex formations in $Cu_p(L\text{-}A)_q(L\text{-}B)_rH_s$ systems at 25 °C and $I=0.1$ M and 1.0 M ($KNO_3$)

| Complex | Species *pqrs* | $\Delta\log K$[a] | | $\log K_m$[b] | |
|---|---|---|---|---|---|
| | | $I=0.1$ | $I=1.0$ | $I=0.1$ | $I=1.0$ |
| Cu(L-Pser)(L-Ala) | 1110 | −2.28 | −1.78 | 0.34 | 0.56 |
| Cu(L-Pser)(L-Arg) | 1111 | −1.27 | −1.46 | | |
| | 1110 | −1.62 | −1.80 | 1.44 | 0.39 |
| Cu(L-Pser)L-Lys) | 1112 | −1.62 | | | |
| | 1111 | −1.75 | −1.64 | 1.16 | 0.66 |
| | 1110 | | | 1.42 | 0.95 |
| Cu(L-Ala)(L-Arg) | 1110 | −1.01 | −0.89 | 0.55 | 0.69 |
| Cu(L-Ala)(L-Lys) | 1111 | −1.03 | −0.89 | 0.55 | 0.69 |
| Cu(L-Ser)(L-Ala) | 1110 | −1.04 | −0.80 | 0.59 | 0.92 |
| Cu(L-Ser)(L-Arg) | 1110 | −0.80 | −0.85 | 0.87 | 0.68 |
| Cu(L-Ser)(L-Lys) | 1111 | −0.88 | −0.83 | 0.69 | 0.66 |
| Cu(L-Ptyr)(L-Ala) | 1111 | | −1.41 | | |
| | 1110 | −2.30 | −1.71 | 0.47 | 0.77 |
| Cu(L-Ptyr)(L-Arg) | 1111 | −1.12 | −1.11 | | |
| | 1110 | −1.51 | −1.54 | 1.83 | 0.98 |
| Cu(L-Ptyr)(L-Lys) | 1112 | | −1.09 | | |
| | 1111 | −1.38 | −1.36 | 2.08 | 1.29 |
| | 1110 | | | 2.34 | 2.17 |
| Cu(L-Tyr)(L-Ala) | 1111 | −0.74 | −0.79 | 1.00 | 0.30 |
| | 1110 | −2.92 | −2.93 | 1.47 | 0.65 |
| Cu(L-Tyr)(L-Arg) | 1111 | −0.60 | −0.86 | 0.99 | 0.02 |
| | 1110 | −2.66 | −2.95 | 1.71 | 0.49 |
| Cu(L-Tyr)(L-Lys) | 1112 | −0.54 | −0.83 | 1.16 | 0.02 |

[a]Calculated according to eqn. (4). [b]Calculated according to eqn. (5).

complex structure with ligand–ligand interactions as shown in Fig. 3.

The log $K$ values in Table 7 further reveal that the Ptyr-containing complexes are more stable than the Pser-containing ones. This may be interpreted as due to a local hydrophobic environment resulting from the aromatic ring. It is possible to estimate from the log $K$ values the fractional population $P$ of bound (b) and unbound (u) forms of Cu(L-A)(L-B) in the manner employed for the systems with aromatic ring stacking. The population for the bound form, $P_b$, is expressed as follows [25, 30]:

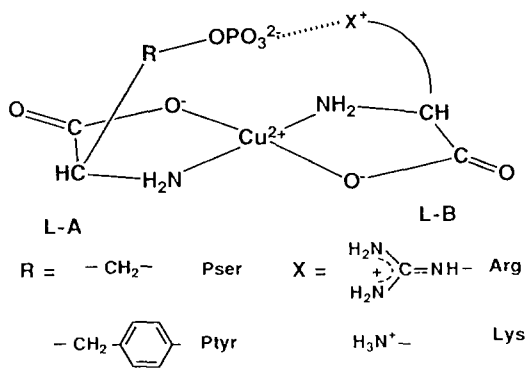


Fig. 3. Proposed electrostatic interactions between the phosphate group of A and the positively charged side chain of B.

TABLE 7. Stabilization of Cu(L-A)(L-B) due to electrostatic interactions expressed by log $K$ values and $P_b$ values

| L-A | L-B | Species *pqrs* | log $K$[a] | | $P_b$ (%)[b] | |
|---|---|---|---|---|---|---|
| | | | $I=0.1$ | $I=1.0$ | $I=0.1$ | $I=1.0$ |
| Ptyr | Arg | 1110 | 0.65 | 0.24 | 78 | 42 |
| | Lys | 1111 | 0.73 | 0.40 | 81 | 60 |
| Pser | Arg | 1110 | 0.42 | 0.03 | 62 | 7 |
| | Lys | 1111 | 0.36 | 0.18 | 56 | 34 |

[a]Calculated according to eqn. (1). [b]Calculated according to eqn. (8).

$$P_b = \frac{[Cu(L\text{-}A)(L\text{-}B)_b]}{[Cu(L\text{-}A)(L\text{-}B)_b]+[Cu(L\text{-}A)(L\text{-}B)_u]} = 1-\frac{1}{K} \quad (8)$$

We see from Table 7 that as high as 80% of Cu(L-A)(L-B) is in the bound form at $I=0.1$ M. This suggests that such electrostatic interactions can exist to a considerable extent under physiological conditions of $I=0.15$ M.

### *Aromatic ring stacking in the complex of non-phosphorylated tyrosine*

It was concluded in a previous study that Cu(bpy)(L-Tyr) with log $K=0.90$ as well as other complexes is

stabilized by stacking [12a]. Aromatic ring stacking in ternary Cu(II) complexes containing a coordinated aromatic heterocycle and an aromatic amino acid has since been established by X-ray analysis of Cu(phen)-(L-Trp) (Trp = tryptophan) [31], Cu(histamine)(Phe) (Phe = phenylalanine), Cu(histamine)(Tyr) [13] and Cu(bpy)(L-Trp) [32], all of which had been shown to have positive log $K$ values and hence to be stabilized by stacking interactions. We now isolated crystals of $[Cu(bpy)(L\text{-}Tyr)ClO_4]\cdot 2H_2O$ suitable for X-ray analysis, determined the molecular structure, and again confirmed the presence of stacking between bpy and the Tyr phenol ring.

*Structure of $[Cu(bpy)(L\text{-}Tyr)ClO_4]\cdot 2H_2O$*

The crystal structure is depicted in Fig. 4. The unit cell contains one $[Cu(bpy)(L\text{-}Tyr)ClO_4]$ complex. The central Cu(II) ion, five-coordinated, has a tetrahedrally distorted square-pyramidal geometry, where the two nitrogen atoms of bpy and the nitrogen and oxygen atoms of L-Tyr coordinated at the equatorial positions and one oxygen atom of the perchlorate ion at the axial position, as shown in Fig. 5(a). The copper coordination plane formed by three nitrogens and one oxygen is ruffled within 0.28 Å. The dihedral angle between the planes, defined by the copper and the two nitrogen atoms of the bpy molecule and by the copper and the nitrogen and oxygen atoms of the L-Tyr molecule, is 24.5°.

The equatorial Cu–N and Cu–O bond lengths (Cu–N(1) = 1.983(8), Cu–N(2) = 1.969(11), Cu–N(3) = 1.987(7) and Cu–O(1) = 1.943(9)) (Table 8) agree well with those generally found in square-planar Cu(II) complexes [33]. The axial Cu–O(7) bond length (2.891(12) Å) is considerably longer than the sum of the ionic radii for Cu(II) (0.72 Å) and $O^-$ (1.40 Å) [34], and the O(7) atom is considered to be weakly coordinated. Most important is that the opposite site of the axial O(7) position is occupied by the phenol ring of the intramolecular L-Tyr group, which is approximately parallel to the Cu(II) coordination plane with the average spacing of 3.35 Å (Fig. 5(b)). There are two close approaches between the Cu(II) ion and carbon atoms of the phenol group (Cu(II)···C(14): 3.24 Å and Cu(II)···C(19): 3.11 Å). Part of the phenol ring is located over one of the pyridine ring of bpy, showing an intramolecular aromatic ring stacking interaction. Large deviation of the coordinated atoms from the Cu(II) plane makes this parallel stacking

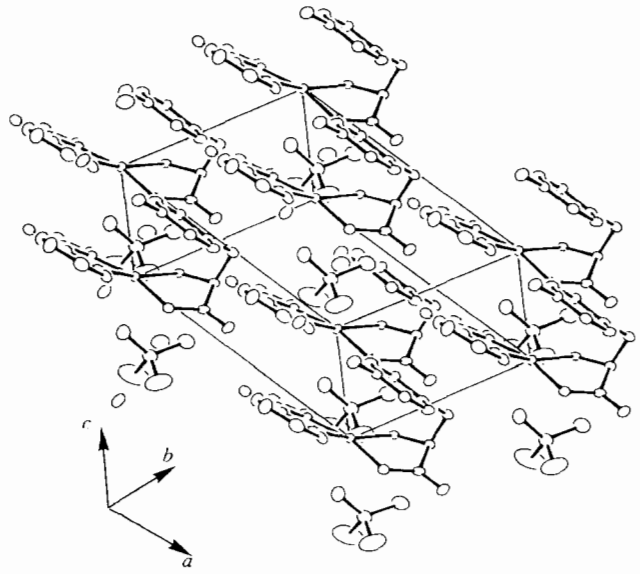


Fig. 4. Crystal structure of $[Cu(bpy)(L\text{-}Tyr)ClO_4]\cdot 2H_2O$.

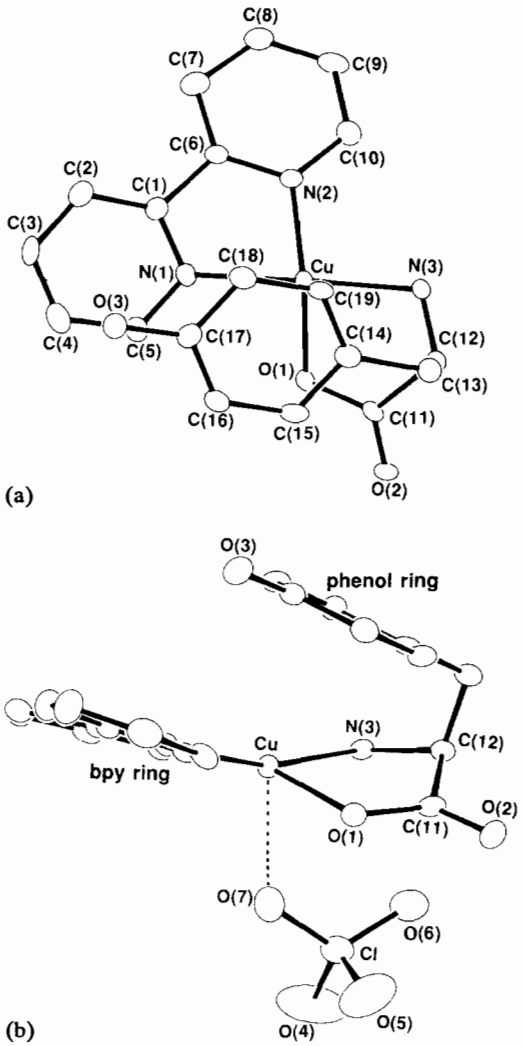


Fig. 5. Diagram showing the stacking interaction between the bpy and phenol rings in $[Cu(bpy)(L\text{-}Tyr)ClO_4]$ as viewed upright to the mean plane of the bpy ligand (a) and parallel to the same plane (b). Thermal ellipsoids are drawn at the 30% probability level. The labeling scheme used for the atoms in the molecule is also shown.

TABLE 8. Bond lengths (Å) and angles (°) for [Cu(bpy)-(L-Tyr)ClO$_4$]

| | | | |
|---|---|---|---|
| Cu–N(1) | 1.983(8) | Cu–N(2) | 1.969(11) |
| Cu–N(3) | 1.987(7) | Cu–O(1) | 1.943(9) |
| Cl–O(4) | 1.512(18) | Cl–O(5) | 1.471(14) |
| Cl–O(6) | 1.435(12) | Cl–O(7) | 1.419(11) |
| C(1)–C(2) | 1.358(16) | C(1)–C(6) | 1.485(17) |
| C(1)–N(1) | 1.348(17) | C(2)–C(3) | 1.408(22) |
| C(3)–C(4) | 1.364(23) | C(4)–C(5) | 1.398(17) |
| C(5)–N(1) | 1.368(16) | C(6)–C(7) | 1.402(20) |
| C(6)–N(2) | 1.333(14) | C(7)–C(8) | 1.442(18) |
| C(8)–C(9) | 1.339(18) | C(9)–C(10) | 1.373(21) |
| C(10)–N(2) | 1.357(16) | C(11)–C(12) | 1.580(15) |
| C(11)–O(1) | 1.297(12) | C(11)–O(2) | 1.195(16) |
| C(12)–C(13) | 1.540(16) | C(12)–N(3) | 1.478(16) |
| C(13)–C(14) | 1.481(15) | C(14)–C(15) | 1.419(18) |
| C(14)–C(19) | 1.377(19) | C(15)–C(16) | 1.392(17) |
| C(16)–C(17) | 1.365(20) | C(17)–C(18) | 1.436(18) |
| C(17)–O(3) | 1.329(13) | C(18)–C(19) | 1.392(16) |
| N(1)–Cu–N(2) | 81.6(4) | N(1)–Cu–N(3) | 162.5(4) |
| N(1)–Cu–O(1) | 95.7(4) | N(2)–Cu–N(3) | 102.4(4) |
| N(2)–Cu–O(1) | 164.3(3) | N(3)–Cu–O(1) | 84.8(4) |
| O(4)–Cl–O(5) | 105.0(11) | O(4)–Cl–O(6) | 105.0(9) |
| O(4)–Cl–O(7) | 108.5(8) | O(5)–Cl–O(6) | 113.5(8) |
| O(5)–Cl–O(7) | 113.1(9) | O(6)–Cl–O(7) | 111.1(7) |
| C(2)–C(1)–C(6) | 124.4(13) | C(2)–C(1)–N(1) | 123.1(12) |
| C(6)–C(1)–N(1) | 112.5(9) | C(1)–C(2)–C(3) | 118.1(14) |
| C(2)–C(3)–C(4) | 120.0(11) | C(3)–C(4)–C(5) | 119.3(13) |
| C(4)–C(5)–N(1) | 120.4(13) | C(1)–C(6)–C(7) | 120.8(10) |
| C(1)–C(6)–N(2) | 115.7(11) | C(7)–C(6)–N(2) | 123.6(11) |
| C(6)–C(7)–C(8) | 114.6(11) | C(7)–C(8)–C(9) | 120.9(14) |
| C(8)–C(9)–C(10) | 120.6(12) | C(9)–C(10)–N(2) | 120.6(11) |
| C(12)–C(11)–O(1) | 114.0(10) | C(12)–C(11)–O(2) | 120.2(9) |
| O(1)–C(11)–O(2) | 125.8(10) | C(11)–C(12)–C(13) | 108.1(9) |
| C(11)–C(12)–N(3) | 108.5(8) | C(13)–C(12)–N(3) | 113.1(10) |
| C(12)–C(13)–C(14) | 113.3(8) | C(13)–C(14)–C(15) | 119.1(12) |
| C(13)–C(14)–C(19) | 124.0(11) | C(15)–C(14)–C(19) | 116.9(10) |
| C(14)–C(15)–C(16) | 120.5(13) | C(15)–C(16)–C(17) | 122.2(12) |
| C(16)–C(17)–C(18) | 118.3(10) | C(16)–C(17)–O(3) | 121.0(12) |
| C(18)–C(17)–O(3) | 120.7(12) | C(17)–C(18)–C(19) | 118.6(13) |
| C(14)–C(19)–C(18) | 123.4(12) | Cu–N(1)–C(1) | 115.0(7) |
| Cu–N(1)–C(5) | 125.4(8) | C(1)–N(1)–C(5) | 119.0(9) |
| Cu–N(2)–C(6) | 114.5(8) | Cu–N(2)–C(10) | 125.8(8) |
| C(6)–N(2)–C(10) | 119.7(11) | Cu–N(3)–C(12) | 107.5(6) |
| Cu–O(1)–C(11) | 115.9(7) | | |

arrangement feasible. Similar intramolecular stacking has also been found in the previously reported copper complexes [13, 31, 32]. The phenol ring is further stacked with the bpy ring of the neighboring complex with the average separation of 3.02 Å (Fig. 4).

*Control of side chain conformation by tyrosine phosphorylation*

The tyrosine phenol ring can be involved in the stacking interactions with the aromatic heterocycles in ternary Cu(II) and Pd(II) complexes. The structure of [Cu(bpy)(L-Tyr)ClO$_4$] (Fig. 5) clearly shows that the stacking exists within the complex as concluded earlier from the log $K$ value [12]. Convincing evidence for stacking interactions in solution has been obtained from the $^1$H NMR study of ternary Pd(II) complexes involving aromatic heterocycles and dipeptides with an aromatic side group [14]. Hence, Pd(L-Tyr·Gly)(bphen) (Tyr·Gly = tyrosylglycine; bphen = 4,7-diphenyl-1,10-phenanthroline-4′,4″-disulfonate), for example, exhibited a large upfield shift (1.27 ppm) of the phenol ring H(16 or 18) atom due to the ring current effect of bphen. On the other hand, Pd(L-TyrO$^-$·Gly)(bphen) (TyrO$^-$·Gly = tyrosylglycine with the deprotonated phenol group) and Pd(L-Ptyr)(bphen) exhibited much smaller upfield shifts, 0.57 and 0.24 ppm, respectively. The difference between the log $K$ values for Cu(DA)(L-Tyr) and Cu(DA)(L-Ptyr) have been found to be 1.0 ~ 1.1 for the systems with DA = bpy and phen, indicating that stacking is essentially inhibited by introducing a negatively charged group by phosphorylation or deprotonation [12].

The above findings demonstrate that phosphorylation of the Ser- and Tyr-OH group in Cu(L-A)(L-B) gives rise to electrostatic interactions with the side chain of protonated B, which results in stability enhancement as evaluated from the log $K$ values. All the observations are interpreted as demonstrating conversion of aromatic ring stacking to electrostatic bonding or, in other words, regulation of stacking by Tyr phosphorylation. It is of interest to note that protein phosphorylation is of prime importance in regulation of metabolisms, signal transduction and other biological processes [1, 2]. Tyr phosphorylation has been reported to be a key step in cancer cell growth [3]. The structural changes in glycogen phosphorylase [4] and isocitrate dehydrogenase [5] induced by phosphorylation of Ser at an allosteric site and a ligand binding site, respectively, have dramatically shown the effects of introducing a negative group on enzyme activity. We suggested earlier that phosphorylation of a Tyr residue might be a biological way of on–off switching of stacking in proteins [12a, 14]. On the basis of the present observations, we propose a possible scheme of conformational modification of proteins by conversion of stacking to electrostatic bonding or vice versa through Tyr phosphorylation by protein kinase (Fig. 6). Information on simplified systems such as reported here may indicate the basic principle underlying relevant biological reactions.

## Supplementary material

Tables of anisotropic thermal parameters, hydrogen coordinates and structure factor amplitudes are available from the authors on request.

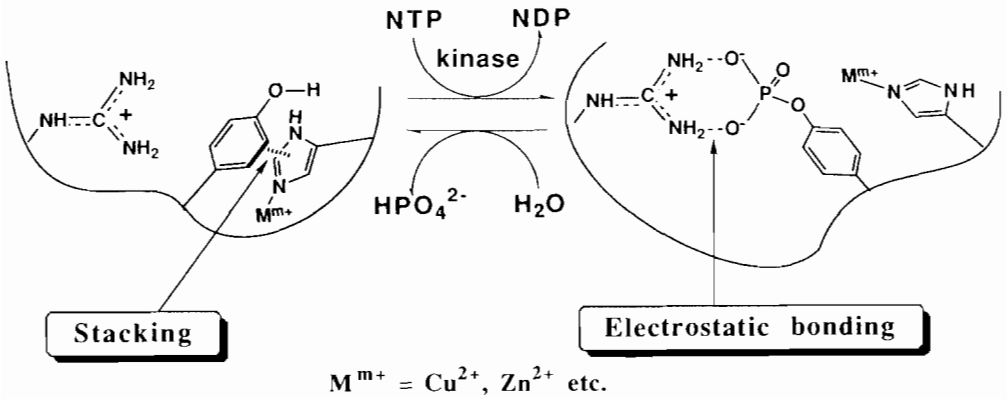


Fig. 6. Proposed conformational change due to conversion of stacking to electrostatic bonding by tyrosine phosphorylation. NTP = nucleotide triphosphate; NDP = nucleotide diphosphate.

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