

Accounts

Metal Ion-Assisted Weak Interactions Involving Biological Molecules. From Small Complexes to Metalloproteins

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Noncovalent or weak interactions play important roles in molecular recognition and structual organization in chemical and biological systems. Hydrogen bonding and aromatic ring stacking are widely recognized for biomolecules, and these and other types of weak interactions are recently attracting much attention as a clue to understanding the efficiency and specificity of biological reactions. We studied weak interactions around the metal center as factors for mixed ligand (ternary) metal complex formation, molecular recognition, and construction of self-organized structures by potentiometric, spectroscopic, and crystallographic methods. We concluded the electrostatic ligand–ligand interactions within Cu(II) and Pd(II) complexes containing an acidic and a basic amino acid and aromatic ring stacking interactions between the aromatic rings of an aromatic amino acid and a coordinated aromatic ligand such as 2,2′-bipyridine. Selective adduct formation between Pt(II) complexes and uncoordinated mononucleotides were found to occur through stacking and hydrogen bonding. In the systems with aromatic ring stacking, a close metal–aromatic ring contact was revealed in the solid state, and the ^{195}Pt NMR spectra indicated electron density decrease due to stacking. As an extension of such studies, we investigated metalloprotein–charged peptide electrostatic interactions and established the redox partner binding site of plastocyanin and its subtle structural change due to the interactions. The results demonstrated the use of charged oligopeptides as models for the studies on protein–protein binding. Weak interactions between $[Cu(arginine)_2]^{2+}$ and its counterions, such as benzene-1,3-dicarboxylate, led to controlled self-organization of the complex ion, giving crystalline products with handed single-helical or double-helical structures and layer structures, depending on the anions used.

Noncovalent interactions play vital roles in biological systems.[1] Hydrogen bonding and aromatic ring stacking are well recognized for α-amino acids and nucleotides and are essential for the structures and functions of proteins and nucleic acids.[2] Other interactions such as cation–π[3] and CH–π[4] interactions may also be important and are currently attracting much attention. Specificity and efficiency of metalloenzymes owe much to weak interactions involving the side chains of amino acid residues,[1,2] and the central metal ion serves as a site of intermolecular interactions by coordinating molecules in various geometries and thus bringing them close together to interact with each other. In zinc finger proteins, repeats of peptide units with four cysteine (cys) residues or with two cys and two histidine (his) residues bind with zinc ions by a $(cys)_4$ or a $(cys)_2(his)_2$ donor set, respectively, to form finger-like structures with arginine, lysine, and polar amino acid residues at the finger tips,[5] whose charged or polar side chain groups have been revealed to bind with the nucleic bases of DNA.[6]

Formation of mixed ligand (or ternary) metal complexes depends on combinations of donor atoms, steric requirements, and ligand–ligand interactions in the resulting complexes. Preferential formation of certain His-containing ternary Cu(II)-amino acid complexes in blood plasma, as revealed by tracer studies,[7] can be interpreted as due to the effective metal binding by His and ligand–ligand interactions between the side chains of His and the other amino acid.[8] Substrate specificity of metalloenzymes may be regarded as the molecular recognition or the ligand selectivity at the active center of the metal–protein–substrate ternary complex, whose reactivity is based on the reactivity of the central metal ion and protein–substrate noncovalent interactions.[1a,1f] In addition to the ability of serving as a template for intermolecular interactions, transition metal ions may function as Lewis acids, undergo redox reactions, and exert electronic effects. An important feature of metal ions is that they exhibit characteristic electronic spectra in the d-d region and circular dichroism (CD) spectra due to the vicinal effect of coordinated α-amino acids and other optically active ligands, both of which provide valuable information on the mode and energy of weak interactions. With these points in mind we have been studying the structures and prop-

erties of various metal–ligand systems containing amino acids, peptides, and/or diimines, and metal complex–nucleotide and metalloprotein–peptide systems, in order to obtain basic information on interactions in biological systems. This paper deals with weak interactions occurring in and around the metal center of low-molecular-weight complexes and metalloproteins and their biological relevance with emphasis on hydrogen bonds and aromatic ring stacking interactions.

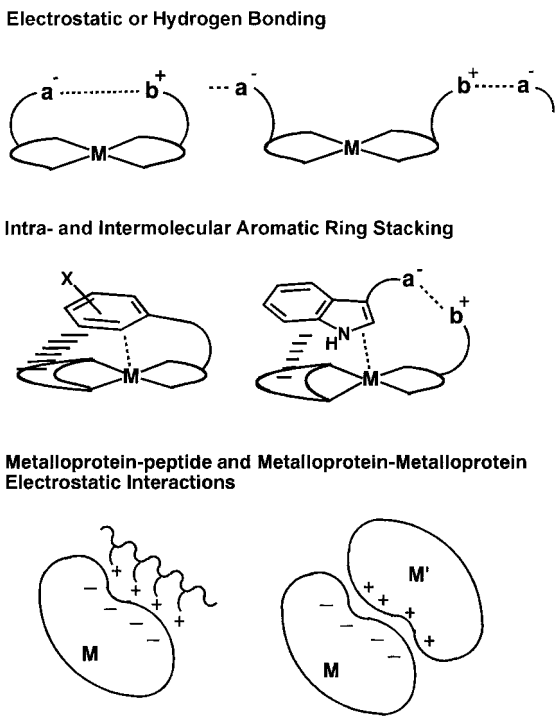
Weak Interactions in and around the Metal Center

The interactions in metal ion-ligand systems may be classified as follows:

(1) Metal–ligand coordinate bonds
(2) Through-metal ligand–ligand interactions
(3) Through-space ligand–ligand interactions
  i) Hydrogen bonds (electrostatic interactions)
  ii) Aromatic ring stacking interactions
  iii) CH–π and other interactions
(4) Metal–aromatic ring close contact
(5) Interactions between a metal complex and an uncoordinated molecule

Interactions of type (2) are regarded as electronic interactions between coordinated ligands mediated by the central metal ion,[9] and this has been shown for ternary Cu(II) complexes experimentally.[9a,10] On the basis of mechanistic considerations, Tanaka presented an equation for estimating the stability constants for ternary complexes of nitrogen- and oxygen-donor ligands such as amino acids by introducing parameters named ligand interaction terms, δ_{ij}, determined by detailed surveys of the reported stability constants.[11,12] The δ_{ij} values accommodate the effects of through-metal ligand–ligand interactions; in the absence of through-space interactions, the equation has been shown to give excellent predictions of stability constants, e.g., for histidine-containing Cu(II) complexes.[13] Here we will focus on weak attractive interactions (3, i), ii)), (4), and (5). Steric effects due to bulky groups may be regarded as through-space interactions (3) but will not be considered in this paper. Scheme 1 illustrates the cases of intra- and intermolecular interactions involving metal complexes.

For amino acids with charged side chains, we may expect electrostatic interactions or hydrogen bonds between the carboxylate group of aspartate (asp) or glutamate (glu) and the ammonio group of monoprotonated lysine (lysH; H denotes a proton attached to the ε-amino group) or the guanidinium group of monoprotonated arginine (argH; H denotes a proton attached to the guanidino group), in such cases as Cu(asp)(argH) and Cu(glu)(lysH) (charges are omitted for clarity; all α-amino acids are L-enantiomers unless otherwise noted). The aromatic side chains of phenylalanine (phe), tyrosine (tyr), and tryptophan (trp) may undergo intramolecular stacking interactions with the aromatic rings of 2,2′-bipyridine (bpy), 1,10-phenanthroline (phen), etc. coordinated to the same metal ion. On the other hand, adducts between a metal complex and an uncoordinated molecule, such as Pt(DA)(argH)···AMP (DA = bpy or phen; AMP = adenosine 5′-monophosphate), may be formed through weak intermolecular interactions. Under favorable conditions, weak interactions between molecules lead to self-organized structures such as helices and sheets, which have been the subjects of great interest in the field of supramolecular chemistry.[14]



Scheme 1. Intra- and intermolecular interactions involving metal complexes.

Evidence for the above interactions may be provided by charge-transfer bands in UV-visible absorption spectra, appearance and magnitude anomaly of CD spectra, and ^{1}H NMR spectra showing changes in coupling constants and upfield shifts due to the ring current effect of aromatic rings. Ligand selectivity in ternary or mixed ligand complex formation is regarded as a result of through-metal (electronic) and through-space ligand–ligand interactions and is reflected on the solution equilibrium:

$$\mathrm{pM + qA + rB + sH} \overset{\beta_{pqrs}}{\rightleftharpoons} \mathrm{M_pA_qB_rH_s}$$
$$\beta_{pqrs} = [\mathrm{M_pA_qB_rH_s}]/([\mathrm{M}]^p[\mathrm{A}]^q[\mathrm{B}]^r[\mathrm{H}]^s) \quad (1)$$

where M, A, B, and H refer to metal ion, ligand A, ligand B, and proton, respectively, and p, q, r, and s are the stoichiometric numbers of the corresponding components in the complex formed.

Isolation of mixed ligand complexes as crystals supports their occurrence in solution, and structural determination by X-ray crystallography provides direct evidence for ligand–ligand interactions and clarifies their details in the solid state. The mode of interactions revealed in the solid state may not be the same as that in solution because of intermolecular interactions necessary for crystal growth.

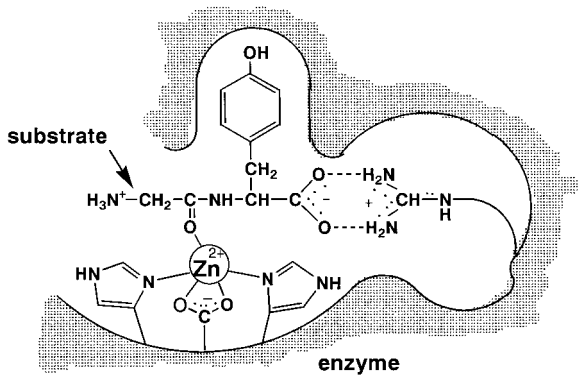


Fig. 1. Schematic representation of the active site structure of CPA–gly·tyr complex.[15]

Ligand–Ligand Electrostatic Interactions or Hydrogen Bonds in Metal(II)-Amino Acid and -Peptide Complexes

Ligand–ligand interactions in metal complexes have been concluded from various lines of evidence.

(1) Synthetic Studies. The X-ray crystal structure analysis of carboxypeptidase A (CPA) and the CPA–gly·tyr complex (gly·tyr = glycyltyrosinate) disclosed for the first time the molecular structures of an enzyme and its complex with a model substrate gly·tyr, which is bound to the enzyme through coordination to the zinc ion and weak interactions of its tyr phenol moiety and carboxylate terminus with the hydrophobic pocket and arg guanidinium moiety of the enzyme molecule, respectively (Fig. 1).[15] This enzyme-substrate complex structure prompted us to work out model mixed amino acid complexes with oppositely charged side chain groups. We could isolate various ternary complexes M(A)(BH), such as Cu(asp)(argH) (M = Cu(II) or Pd(II); A = asp, glu, ethylenediamine-*N*-monoacetate (edma), etc.; BH (ω-protonated basic amino acids (B) = argH, lysH, ornithine (ornH), etc.).[16,17] It was possible along this line to incorporate two interacting groups into one ligand by combining amino acids A and B to form dipeptides BH·A.[18] Thus, arginylaspartate (argH·asp) and arginylglutamate (argH·glu) gave Cu(argH·asp·H_{-1}) and Cu(argH·glu·H_{-1}) (H_{-1} denotes deprotonation from the peptide bond).

Solid state structures of Cu(argH·asp·H_{-1}) and Cu(argH·glu·H_{-1}) showed that the interactions between the oppositely charged groups occur intermolecularly and not intramolecularly (Fig. 2),[18] which is probably because interactions between molecules are required for crystallization. However, these structures indicate that intramolecular interactions should occur within the complex molecule in dilute solution, and this is supported by solution equilibrium and CD spectral studies (vide infra).

Exchangeable Cu(II) in human blood plasma is present as complexes of serum albumin and amino acids, and according to Sass-Kortsak et al. the ternary amino acid complexes containing his, Cu(his)(asn), Cu(his)(thr), and Cu(his)(gln) (asn = asparaginate; thr = threoninate; gln = glutaminate), are preferentially formed.[7b] Cu(his)(thr) was isolated from blood plasma as crystals and structurally characterized by X-ray analysis to have a *cis*-configuration of the amino groups in a square-pyramidal geometry having his coordinated as a terdenate ligand with its carboxylate moiety at an axial position.[19] By assuming the same coordination mode of his in the above complexes, we inferred that the polar side chain groups of asn, gln, and thr would interact with the carboxylate oxygens to stabilize the complexes relative to those without such interactions, and isolated as crystals a number of ternary complexes Cu(his)(Aa) by using amino acids with a polar side group (Aa = asn, gln, citrullinate (cit), serinate (ser), homoserinate (hmser)).[8]

The X-ray structure analysis of Cu(his)(asn)[20] revealed that it has the same coordination structure as that of Cu(his)(thr) and suggests that the asn amido NH_2 moiety may form a hydrogen bond with the axially coordinated COO^- group by rotation of the asn side chain around the Cα–Cβ bond (Fig. 3).[8] Cu(his)(ser) was also shown to have the *cis*-configuration.[21]

(2) Circular Dichroism and ^{1}H NMR Spectra. The

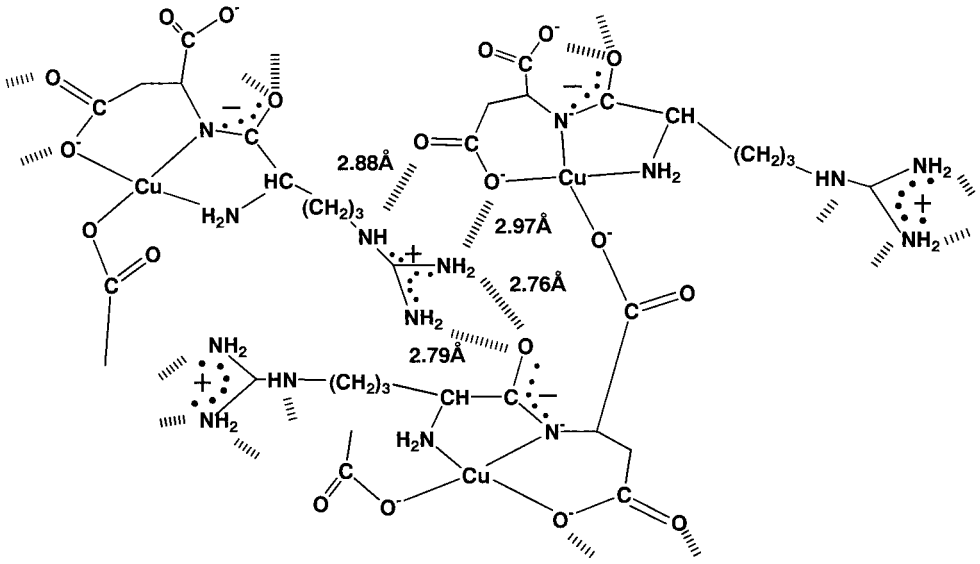
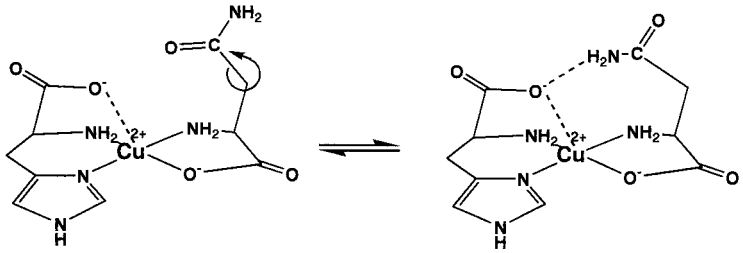


Fig. 2. Molecular structure and hydrogen bonding network in Cu(argH·asp·H_{-1}).[18]



(a) (b)

Fig. 3. Structure of Cu(his)(asn) (a)[20] and possible intramolecular hydrogen bonding by conformational change (b).[8]

Table 1. CD Spectral Data for Ternary Metal(II)–L–Amino Acid Systems in Aqueous Solution[9b,16,17,24,25,31]

System	pH	λ_{max}	$\Delta\varepsilon$	$\Delta\varepsilon_{obsd}/\Delta\varepsilon_{calcd}$	
Cu(ala)(argH)	9.4	600	0.10	1.0	
Cu(ala)(asp)	9.3	630	0.06	1.0	
Cu(asp)(argH)	7.2	630	0.09	1.4	
	9.5	630	0.09	1.3	
Cu(asp)(lysH)	7.2	640	0.08	1.2	
	9.6	640–650	0.08	1.1	
Cu(asp)(ornH)	7.1	640	0.07	1.5	
Cu(glu)(argH)	7.3	600	0.14	1.4	
	8.5	600	0.15	1.3	
Cu(glu)(argH)	8.5	598	0.147	1.26	
	7.8	600	−0.173	1.44	50% aq ethanol
	7.7	599	0.134	1.12	I = 0.1 M $NaClO_4$
	7.8	607	−0.143	1.05	I = 0.5 M $NaClO_4$
Pd(ala)(asp)	6.4	345	0.57	1.00	
		306	−0.84	1.00	
Pd(asp)(lysH)	6.2	351	0.41	0.70	
		308	−0.94	0.80	
Pd($cySO_3$)(lysH)	6.4	349	0.20	0.87	
		306	−0.89	0.90	
Pd(his)(ser)	6.9	321	−0.11	0.58	
Pd(his)(hmser)	7.1	323	−0.10	0.64	
Pd(his)(asn)	6.8	307	−0.12	0.64	
Pd(his)(gln)	7.0	325	−0.15	0.74	
Pd(hisOMe)(gln)	7.0	336	−0.084	1.04	
Cu(ala)(pser)	7.1	623	−0.116	0.99	I = 1 M $NaClO_4$
	7.5	630	−0.128	0.96	I = 0.1 M $NaClO_4$
	7.8	625	−0.127	0.88	50% aq methanol
Cu(pser)(argH)	7.8	616	−0.214	1.57	
	8.1	610	−0.273	1.96	50% aq methanol
		775	0.074	2.2	
Cu(ptyr)(argH)	9.5	595	−0.271	1.19	I = 1M $NaClO_4$
	9.9	586	−0.361	1.94	I = 0.1 M $NaClO_4$
	10.0	585	−0.411	2.54	
	10.4	581	−0.500	3.50	50% aq methanol

1:1:1 Cu(II)–A–BH systems exhibited a similar d-d absorption peak at 600–640 nm and negative CD peaks in the corresponding region due to the vicinal effect of the asymmetric carbons.[16,17,22] According to Martin et al. the CD magnitude of Cu(II)- and Ni(II)–oligopeptide complexes is an additive function of the magnitudes for the binary complexes of the component amino acid residues.[23] We found that in the absence of interacting side chain groups the magnitude $\Delta\varepsilon_{calcd}$ for Cu(ala)(val) (ala = alaninate; val = valinate) can also be estimated on the basis of additivity:[9c,16,17]

$$\Delta\varepsilon_{calcd} = (\Delta\varepsilon_{Cu(ala)_2} + \Delta\varepsilon_{Cu(val)_2})/2 \quad (2)$$

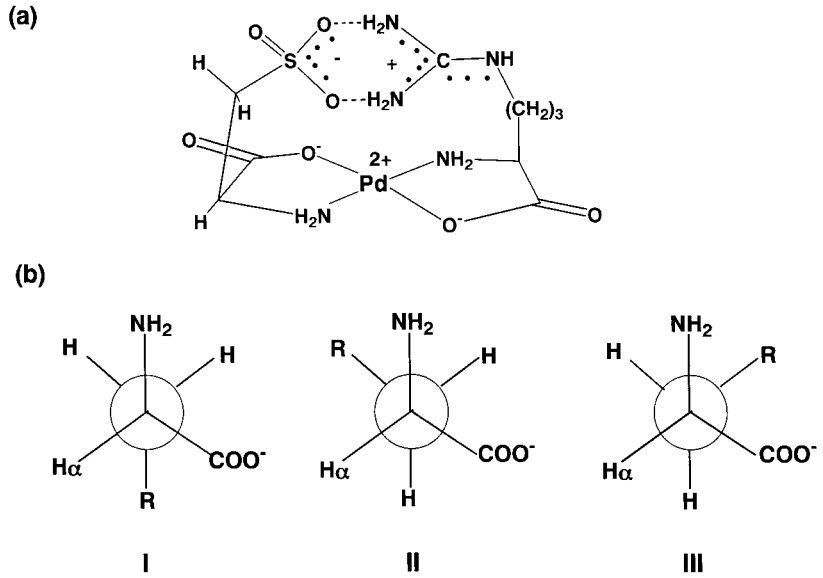


Fig. 4. Intramolecular hydrogen bonds in Pd(L-cySO_3)(L-argH) (a) and three staggered rotamers for α-amino acids (b).

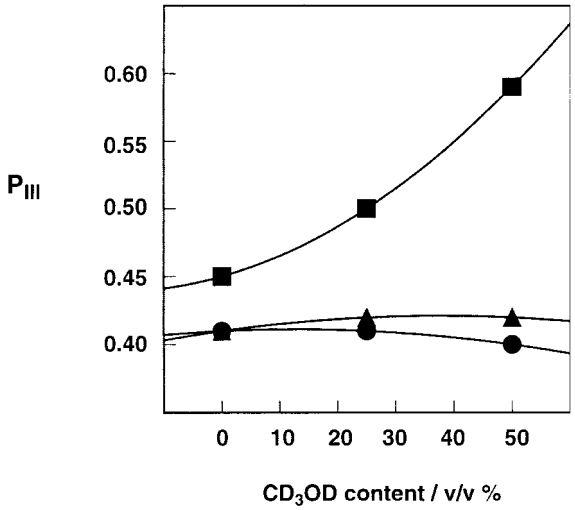


Fig. 5. Solvent dependence of P_{III} for ternary Pd(II)-amino acid systems.[24] ●, Pd(cySO_3)(lysH); ▲, Pd(cySO_3)(ala); ■, Pd(cySO_3)$_2$.

where $\Delta\varepsilon_{Cu(ala)_2}$ and $\Delta\varepsilon_{Cu(val)_2}$ are the CD magnitudes for the binary 1:2 complexes, Cu(ala)$_2$ and Cu(val)$_2$, respectively. The magnitudes for Cu(asp)(ala) and Cu(val)(argH), for example, agreed well with the calculated values. The magnitude additivity has also been found to hold for the Pd(II) complexes such as Pd(ala)(argH).[24] However, when A and BH having oppositely charged groups were used, the magnitudes for 1:1:1 Cu(II)–A–BH such as Cu(asp)(argH) significantly deviated from the $\Delta\varepsilon_{calcd}$ values,[9c,16,17] and the corresponding Pd(II) systems also exhibited anomalous magnitudes.[24] The anomaly became more pronounced in media with lower polarity and decreased at high ionic strengths. The extent of magnitude anomaly can be expressed by a relative magnitude, which is defined as the ratio of the observed magnitude $\Delta\varepsilon_{obsd}$ to the calculated one: $\Delta\varepsilon_{obsd}/\Delta\varepsilon_{calcd}$. Selected CD spectral data and relative magnitudes are summarized in Table 1, which indicates that the asymmetry of the side chains is increased due to the electrostatic side chain–side chain interactions.

While the his-containing Cu(his)(Aa) systems (Aa = asn, ser) showed no appreciable magnitude enhancement in spite of the expected hydrogen bond as shown in Fig. 3b,[8] the $\Delta\varepsilon$ values observed for the corresponding Pd(II) systems were much smaller than the $\Delta\varepsilon$calcd values, which were calculated according to Eq. 3:[25]

$$\Delta\varepsilon_{calcd} = \Delta\varepsilon_{M(DL\text{-}his)(L\text{-}Aa)} + \Delta\varepsilon_{M(L\text{-}his)(DL\text{-}Aa)} \quad (3)$$

No anomaly was observed for the systems containing histidine methyl ester (hisOMe), Pd(hisOMe)(Aa) (Aa = ser, gln), which shows that the charged carboxylate group is necessary for the interactions (Table 1). Other systems such as Cu(his)(lysH) and Cu(his)(AA) (AA = phe, tyr, trp) exhibited anomalous CD magnitudes (vide infra). These results indicate that the CD magnitude may be affected more strongly by interactions between charged groups than by interactions involving a polar group such as amido and hydroxy groups.

Amino acids coordinated to a metal ion may be expressed by three staggered rotamers I, II, and III (Fig. 4b). Solvent dependence of the fractional populations of rotamers calculated from the vicinal coupling constants of the ^{1}H NMR spectra for Pd(II)–asp(or cySO_3)–BH (cySO_3 = cysteate; BH = lysH) clearly indicated that the population calculated according to Pachler and Feeney[26] of rotamer III (P_{III}), which enables the interaction to occur, increases with the CD_3OD content (Fig. 5) and decreases with temperature significantly, supporting the existence of electrostatic interactions within the complex molecule.[24]

(3) Stability Enhancement and Stereoselectivity Due to Ligand–Ligand Interactions. Intramolecular interactions may give rise to *cis–trans* isomerism of planar complexes due to the steric requirements for the interactions.[17] Figure 6

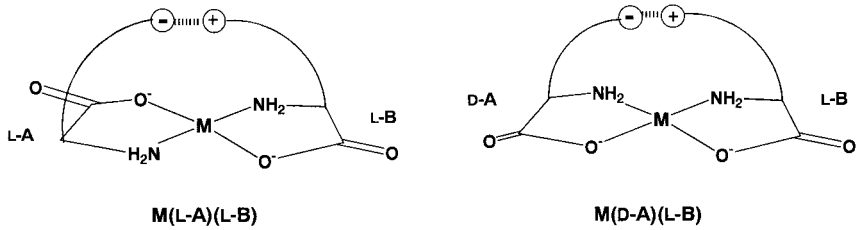


Fig. 6. Geometric isomerism due to steric requirements for ligand–ligand interactions.

shows the configurations favoring the interactions expected for diastereomeric complexes. Brookes and Pettit observed a small but significant difference in the stability constants for Cu(L- or D-his)(L-BH) (B = arg, lys, orn), where complexes Cu(L-his)(L-BH) were more stable than Cu(D-his)(L-BH).[27] They ascribed the difference to the side chain interactions by assuming that the complexes have a *cis*-configuration similar to that shown in Fig. 3. The Cu(L- or D-A)(L-BH) systems (A = asp, glu) did not exhibit the stability enhancement and stereoselectivity due to electrostatic interactions, indicating lack of stability difference between the *cis*- and *trans*-forms (Fig. 6).[28] The pK_a values for the ω-ammonio group of lysH and ornH in Cu(L-A)(L-BH) (A = asp or glu) were higher than those for Cu(L-ala)(L-BH) having ala in place of A, which implies that protonation of this group is favored due to the interactions.[28] No stability differences were detected for the Cu(L- or D-his)(L-Aa) systems (Aa = asn, gln, ser, thr, etc.);[13] as described earlier, the estimates of the stability constants for Cu(L-his)(L-Aa) calculated according to Tanaka's equation[11b] were in excellent agreement with the experimental values, which suggests that there were no influential through-space ligand–ligand interactions under the conditions employed (I = 0.1 M (KNO_3)). Electrostatic interactions between amino acid side chains have also been reported for a Cu(II) complex containing DL-8-*N*-trimethylornithinate and DL-homocysteinate.[29] Probably due to a solubility difference between the geometric isomers as shown in Fig. 6 and as expected from the structural requirement for interactions (Fig. 3), the ternary complexes isolated from Cu(DL-A)(L-BH) and Cu(DL-his)(L-asn) systems in aqueous ethanol or methanol were found to have incorporated D-A and L-his in 39–93[30] and 98%[8] optical purity, respectively. The Pd(DL-A)(L-BH) systems also incorporated L-enantiomers preferentially.[24] These findings serve as indications of the existence and effect of ligand–ligand interactions.

Phosphorylation of the hydroxy group of ser and tyr gives *O*-phosphoserinate (pser) and *O*-phosphotyrosinate (ptyr), respectively. The negatively charged phosphoester group can now interact with the positively charged argH and lysH side chains; the systems such as Cu(pser)(argH) and Cu(ptyr)(argH) exhibited greatly enhanced relative CD magnitudes (Table 1).[31] For the systems Cu(A)(BH), where A denotes pser, ptyr, and edma and BH to argH and lysH, we may consider the following hypothetical equilibrium:[31,32]

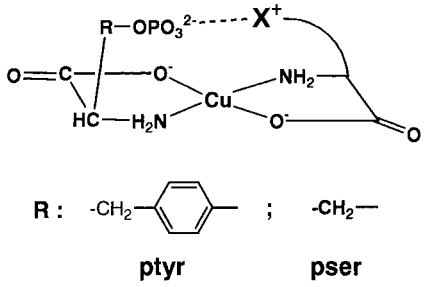
$$\mathrm{Cu(A)(B')} + \mathrm{Cu(A')(BH)} \overset{K}{\rightleftharpoons} \mathrm{Cu(A)(BH)} + \mathrm{Cu(A')(B')}$$
$$\log K = \log \beta_{\mathrm{Cu(A)(BH)}} + \log \beta_{\mathrm{Cu(A')(B')}} - \log \beta_{\mathrm{Cu(A)(B')}} - \log \beta_{\mathrm{Cu(A')(BH)}} \quad (4)$$

where A′ and B′ are amino acids without any interacting side chain group, and ligand–ligand interactions occur only in Cu(A)(BH). Stabilization of this complex relative to others is evaluated by the log K values given by the log β values such as log $\beta_{\mathrm{Cu(A)(BH)}}$, which are overall stability constants for the complex species indicated. In the absence of any electrostatic interactions or hydrogen bonds favoring a particular complex, formation of the four complexes in Eq. 4 occurs statistically, so that log K is equal to zero, but when Cu(A)(BH) is stabilized by the interactions, the equilibrium shifts to the right, giving log $K > 0$. Table 2 shows that complexes such as Cu(pser)(argH) have log K values of 0.36–0.73 at I = 0.1 M (KNO_3) and that the values decrease at I = 1.0 M (KNO_3), indicating that the complexes are stabilized by the electrostatic interactions.[31] Complex stabilization due to electrostatic interactions was also detected for Cu(edma)(argH) etc. relative to Cu(en)(ala), although its crystal structure exhibited intermolecular interactions.[32] Similar electrostatic interactions stabilized the complexes containing the deprotonated form (I_2tyrO, deprotonated from the phenol moiety) of 3,5-diiodotyrosinate (I_2tyr), Cu(I_2tyrO)(BH).[33] Analysis of the solution equilibria of Cu(argH)$_2$ in 0.1 M KNO_3 and 0.1 M guanidinium chloride (Gua·Cl) indicated the formation of an adduct, Cu(argH)$_2$·Gua, with the Gua^+ ion probably bound with the two side chain guanidinium groups of coordinated argH molecules.[34] Pt(bpy)(argH) was inferred to form a dimer through stacking of bpy and H2O-bridged hydrogen bonds between the side chain guanidinium groups of argH.[34]

Table 2. Log K Values[a] for Cu(A)(BH) Systems[31]

System	log K	
	I = 0.1 M	I = 1 M
Cu(ptyr)(argH)	0.65	0.24
Cu(pser)(argH)	0.42	0.03
Cu(ptyr)(lysH)	0.73	0.40
Cu(pser)(lysH)	0.36	0.18

a) Ionic strengths were adjusted with KNO_3.



The Cu(II) complexes of dipeptides (L = BH·A) incorporating charged amino acid residues within one molecule may be

stabilized relative to Cu(II)–gly·gly (gly·gly = glycylglycinate) because of the Cu(II)–side chain and side chain-side chain interactions in complexes CuL, where L coordinates to Cu(II) through the amino nitrogen and the peptide oxygen. As compared with Cu(gly·gly), the Cu(BH·A) complexes (BH = argH or lysH; A = asp or glu) exhibited higher stabilities, as evaluated by the log β_{110} values for $Cu_pL_qH_r$, the order being BH·asp ≥ BH·glu ≥ BH·gly.[18] This means that the electrostatic interactions between the oppositely charged side chains of argH or lysH and asp or glu stabilize the resulting complexes Cu(BH·A). Contribution of the interactions may also be evaluated by the following hypothetical ligand exchange reaction:[18]

$$\mathrm{Cu(gly{\cdot}gly{\cdot}H_{-1})} + \mathrm{BH{\cdot}A} \overset{K_{ex}}{\rightleftarrows} \mathrm{Cu(BH{\cdot}A{\cdot}H_{-1})} + \mathrm{gly{\cdot}gly}$$

$$\log K_{ex} = \log \beta_{11\text{-}1(\mathrm{Cu(BH{\cdot}A{\cdot}H\text{-}1))}} + \log \beta_{011(\mathrm{gly{\cdot}gly})} - \log \beta_{11\text{-}1(\mathrm{Cu(gly{\cdot}gly{\cdot}H\text{-}1))}} - \log \beta_{011(\mathrm{BH{\cdot}A})} \quad (5)$$

The log K_{ex} values which were calculated from the log β values were found to be positive and in the order argH·asp > argH·glu ≃ lysH·asp > lysH·glu > gly·asp > argH·gly ≃ lysH·gly > gly·glu, which indicates that complexes with at least one charged side chain are preferred to Cu(gly·gly·H_{-1}) without any side chain. The thermodynamic parameters revealed that the ΔH^0 and ΔS^0 values were both positive, which shows that the entropy factor contributes to the hypothetical ligand exchange reaction and thus the stabilization of Cu(BH·A·H_{-1}). The entropy effect may be ascribed to liberation of water molecules from the interacting side chain groups, supporting the conclusion that intramolecular electrostatic interactions are important. In this connection, Kozlowski et al. detected the strong effect of the peptide side chain groups in the Cu(II) complex of asn·ser·phe·arg·tyr-NH_2 (= asparaginylserylphenylalanylarginyltyrosinamide), where the side chains were concluded to form hydrogen bonds and/or fences around Cu(II), thus shielding the coordination plane from the bulk of solution.[35,36]

Intramolecular Aromatic Ring Stacking Interactions

Aromatic ring stacking has been recognized as a principal mode of interactions in biological systems such as DNA structuring, intercalative binding of planar organic molecules, and molecular recognition. Stacking of the side chain aromatic rings of aromatic amino acid residues is important for stabilizing the tertiary structure of proteins,[1d] and stacking has been observed, e.g., for the active sites of galactose oxidase[37] and cytochrome *c* oxidase[38] as well as the proposed electron transfer pathway in the cytochrome *c* (cyt *c*)–cytochrome *c* peroxidase (C*c*p) couple.[39] While the aromatic–aromatic interactions were interpreted as due the entropy effect of the classical hydrophobic interactions, they are still debatable, and for nucleic bases and aromatic amino acids electrostatic factors may be more important. Hunter et al. qualitatively explained the π–π interactions between porphyrins and between phenyalanine residues in proteins in terms of electrostatic interactions.[40] Interest in stacking in the coordination sphere prompted us to obtain detailed information on the structures and stabilities of ternary complexes containing aromatic diimines (DA) and aromatic amino acids.

In order to obtain detailed information on aromatic rings in complexes in solution we studied various ternary M(II)-ligand systems with aromatic rings by various methods, and established the existence of aromatic ring stacking in metal complexes containing aromatic amino acids and its contribution to stabilization of complexes. We also found that stacking between DA and a side chain aromatic ring decreases the electron density of the central metal ion.

(1) Evaluation of Stability Enhancement Due to Stacking. Stability of complexes with aromatic rings may be affected by stacking between coordinated ligands, metal–aromatic ring interactions, etc.[9a,9b,41] Aromatic rings in metal complexes may exert electronic or solvophobic effects on the central metal ion. Sigel et al. established the intramolecular stacking of the nucleic bases of coordinated nucleotides and arylcarboxylates with DA coordinated to the same metal ion.[9b,42,43] They also evaluated the stabilization due to stacking of his-containing ternary Cu(II) complexes with tyr, trp, etc. by calculating the equilibrium constants such as $10^{\Delta\Delta\log K}$ for an equilibrium typically shown for Cu(his)(trp) and concluded the stacking interaction in these complexes:[44,45]

$$\mathrm{Cu(trp)} + \mathrm{Cu(his)(ala)} \overset{10^{\Delta\Delta\log K}}{\rightleftarrows} \mathrm{Cu(his)(trp)} + \mathrm{Cu(ala)} \quad (6)$$

Aromatic ligands bpy and phen (denoted as DA) are excellent ligands for Cu(II), Pd(II), Fe(II), etc. When coordinated to Cu(II) in a planar geometry, DA serves as acceptors of the π-back donation by Cu(II) and favors coordination of σ- and π-donor ligands such as carboxylates.[9a] Amino acids favorably bind with Cu(DA) to form ternary complexes. The situation is most favorable for coordination of aromatic amino acids AA (= phe, tyr, and trp), because there can be additional stabilization in the resulting complex M(DA)(AA) (M = Cu(II), Pd(II)) by the stacking interaction between DA and the side chain aromatic ring of AA. We determined the stability constants for a number of Cu(DA)(AA) systems (DA = bpy, phen, histamine (hista), 2-aminomethylpyridine (ampy), ethylenediamine (en); AA = phe, tyr, trp, tyr with deprotonated phenol OH group (tyrO), ptyr) by pH titration at 25 °C and I = 0.1 M (KNO_3) and evaluated the stability enhancement due to the stacking interactions by Eq. 7, which is a modification of Eq. 4 :[46,47]

$$\mathrm{Cu(DA)(ala)} + \mathrm{Cu(en)(AA)} \overset{K}{\rightleftarrows} \mathrm{Cu(DA)(AA)} + \mathrm{Cu(en)(ala)}$$

$$\log K = \log \beta_{\mathrm{Cu(DA)(AA)}} + \log \beta_{\mathrm{Cu(en)(ala)}} - \log \beta_{\mathrm{Cu(DA)(ala)}} - \log \beta_{\mathrm{Cu(en)(AA)}} \quad (7)$$

where DA and AA are bidentate ligands with aromatic rings which can undergo stacking interactions while en and ala have no such rings. Therefore, stacking occurs only in Cu(DA)(AA), whose stabilization is evaluated by the log K value by assuming that all the complexes have comparable coordination structures and donor sets. Equation 7 is similar in context to Eq. 6 except that the donor set in each complex is maintained nearly the same in Eq. 7.

(2) Structure–Stability Relationship. Table 3 summarizes the log K values calculated for various Cu(DA)(AA) complexes from the relevant log β values with Cu(en)(ala) as standard. It is clearly seen from Table 3 that the complexes with

Table 3. Log K Values[a] for Cu(DA)(AA)[46,49]

L-AA	Side chain	DA			
		hista	ampy	bpy	phen
val	$-CH(CH_3)_2$	0.06	0.12	0.02	0.08
phe	$-CH_2-C_6H_5$	0.26	0.22	0.60	0.64
tyr	$-CH_2-C_6H_4-OH$	0.51	0.55	0.90	1.05
tyrO$^-$	$-CH_2-C_6H_4-O^-$	0.11	0.38	0.25	
I_2tyr	$-CH_2-C_6H_2I_2-OH$	0.84	0.69	1.88	2.18
I_2tyrO$^-$	$-CH_2-C_6H_2I_2-O^-$	0.28	0.50	1.20	1.38
trp	$-CH_2-C_8H_6N$	0.60	0.48	1.19	1.39
htrp	$-CH_2-C_8H_5N(OH)$	0.87	0.82	1.80	2.22
ptyr	$-CH_2-C_6H_4-O-PO_3^{2-}$	−0.15		−0.14	−0.02

a) Calculated according to Eq. 7.

large interacting aromatic rings have higher log K values as compared with those with small aromatic rings. Thus, Cu(phen)(trp) (log K = 1.39) is more stabilized than Cu(hista)(trp) (0.60), Cu(ampy)(trp) (0.48), and Cu(phen)(tyr) (1.05), all of which have a smaller DA or AA.[46] When the aromatic rings are of comparable size, the electron density difference due to substituents affects the log K values; the value for Cu(phen)(htrp) (htrp = 5-hydroxytryptophanate) is 0.83 log unit higher than that for Cu(phen)(trp). Since complexes with valinate (val), Cu(DA)(val), have log K values $\leq$ 0.12, the results indicate that there is a stacking interaction in the ternary complexes when both DA and AA have aromatic rings, and this interaction stabilizes the complexes. The stability sequence as expressed in terms of the side chain aromatic rings of AA is as shown in Chart 1.

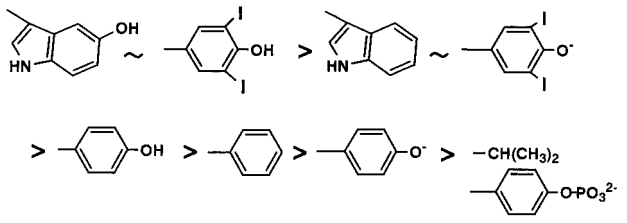


Chart 1. Stability sequence expressed in structures of AA.

Stabilization of ternary complexes with *p*-substituted phenylalaninate (X-phe) Cu(DA)(X-phe) (DA = bpy, phen; X-phe = DL-F-, DL-Cl-, DL-Br-, I-, NO_2-, and NH_2-phe) was also evaluated by the log K values. The stability sequence was found to be Br > OH > Cl $\simeq$ NH_2 > H > F, although there were some ambiguities regarding the order of X = I and

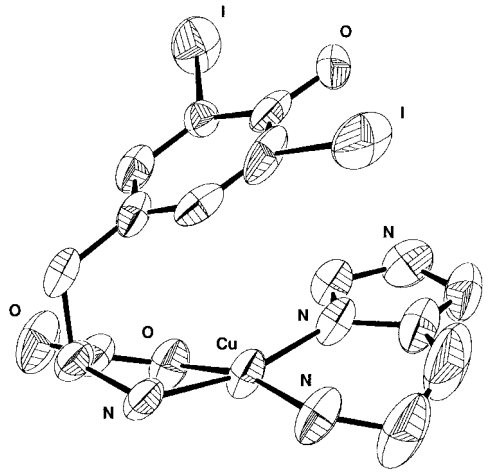


Fig. 7. Molecular structure of Cu(hista)(I_2tyrO).[50]

NO_2.[48] However, the ternary complexes containing I_2tyr and its deprotonated form I_2tyrO, Cu(DA)(I_2tyr) and Cu(DA)-(I_2tyrO), respectively, were disclosed to have large log K values which are much larger than those for tyr (Table 3),[49] suggesting that the iodine atoms strongly assist the stacking interactions. The molecular structures of Cu(bpy)(tyr),[31] Cu(bpy)-(I_2tyr),[49] etc. in the solid state have been disclosed to have a stacked structure as shown in Fig. 7 for Cu(hista)(I_2tyrO),[50] and the stacking in Cu(bpy)(I_2tyr) was not affected by deprotonation of the OH group.[49] Other complexes having medium to large log K values, such as Cu(bpy)(trp)[51] and Cu(phen)(trp),[52] have been shown to have a stacked structure by X-ray analysis. Cu(phen)(phe) (log K = 0.64) was found to have both the stacked and unstacked structures, while Cu(bpy)(phe) (0.60)

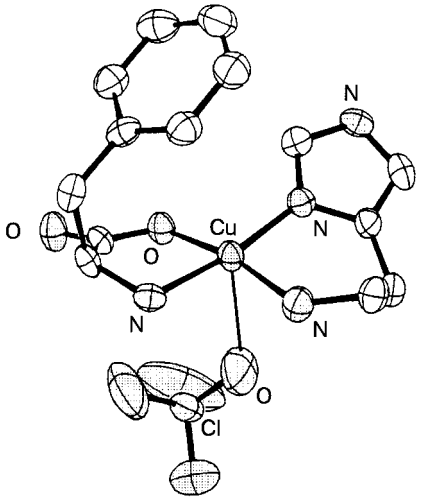


Fig. 8. Molecular structure of [Cu(hista)(phe)(ClO_4)].[53]

showed only the unstacked structure,[48] which suggests that the log K value of 0.6 for Cu(bpy or phen)(AA) could be a borderline value for intramolecular stacking to occur in the solid state. In contrast stacking with a coordinated imidazole ring in Cu(hista)(phe) (Fig. 8) and Cu(hista)(tyr) having rather small log K values of 0.3–0.5 (Table 3) was established by X-ray crystal structure analysis,[53] supporting the previous conclusions from the solution equilibrium and spectral studies.[41,42,44,47]

Stacking of aromatic rings has also been concluded for Cu(DA)(L) complexes, where DA refers to bpy or phen and L to tyr-containing dipeptides, tyr·X (X = gly, L/D-ala, -tyr, -trp, and -phe).[54] The log K values were calculated from Eq. 7′ by replacing AA and ala with dipeptides tyr·X and gly·gly, respectively:

$$\text{Cu(DA)(gly·gly)} + \text{Cu(en)(L)} \overset{K}{\rightleftharpoons} \text{Cu(DA)(L)} + \text{Cu(en)(gly·gly)} \quad (7')$$

where stacking is possible only in Cu(DA)(L). The coordination modes of dipeptides depend on the pH of the solution; at pH 5–8, L is bound to Cu(II) through the amino nitrogen and peptide carbonyl oxygen atoms to form Cu(DA)(L). The log K values calculated with Cu(en)(gly·gly) as standard were larger than 0.91, showing that Cu(DA)(L) complexes are strongly stabilized by stacking. At pH 8–10, L coordinates to Cu(II) through the amino nitrogen, peptide nitrogen, and carboxylate oxygen atoms in the Cu(II) plane as a terdentate ligand to form Cu(DA)(L·H_{-1}) such as Cu(phen)(tyr·gly·H_{-1}), which has been revealed to have a square-pyramidal structure with stacking between the phenol moiety of the tyr residue and phen coordinated at an equatorial and an axial position (Fig. 9)[54] as previously shown for Cu(phen)(gly·gly·H_{-1}).[55] A corresponding Pd(II) complex was shown to have a planar structure with N_4 coordination (Fig. 10).[54] The Cu(DA)(L·H_{-1}) complexes are similarly stabilized, but the log K values calculated with Cu(en)(gly·gly·H_{-1}) as standard (0.35–1.33) were smaller than those for Cu(DA)(L).

Table 3 shows that complexes Cu(DA)(ptyr) (ptyr = O-phosphorylated tyrosinate) have small negative log K values,

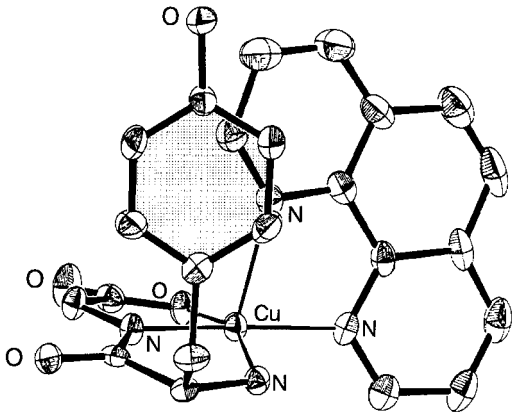


Fig. 9. Molecular structure of Cu(phen)(tyr·gly·H_{-1}) showing stacking between the tyr phenol ring and axially coordinated phen.[54]

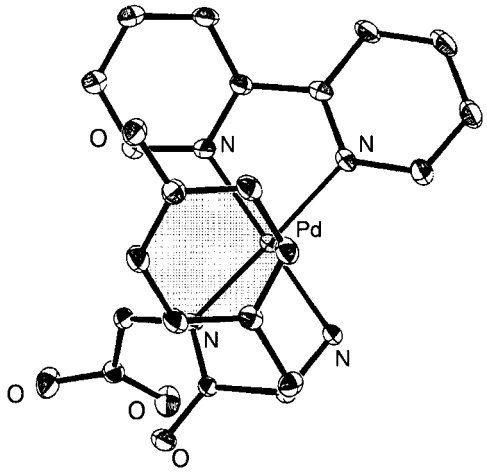
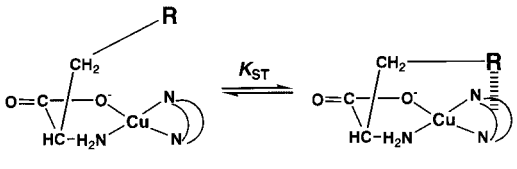


Fig. 10. Molecular structure of Pd(bpy)(tyr·gly·H_{-1}).[54]

indicating that the stacking interaction is virtually cleaved by phosphorylation of the tyrosine phenol OH group. Deprotonation of tyr to give tyrO also decreased the values. The stacked or closed species of Cu(DA)(AA) in solution is considered to be in equilibrium with the unstacked or open species, and its fractional population P_{ST} may be calculated from log K by the following relationship:[9b,44,47b,56]



(8)

unstacked form stacked form

$K_{ST} = K - 1$; $P_{ST} = 1 - 10^{-\log K}$

For Cu(bpy)(tyr) we have $P_{ST} = 0.87$, but for Cu(bpy)(tyrO) and Cu(bpy)(ptyr) the P_{ST} values are 0.44 and 0.0, respectively (Table 4). The destabilizing effect of introducing negative charges into the aromatic ring can probably be ascribed to the hydrophilicity and/or bulkiness of the modified (and hydrated) group, which destroys the stacked structure.

Table 4. Populations of Stacked Species, P_{ST}, for Cu(DA)(AA) Systems (I = 0.1 M (KNO_3); 25 °C)[47b]

AA	Species	DA			
	pqrs	phen	bpy	ampy	hista
phe	1110	0.77	0.75	0.40	0.45
tyr	1111	0.91	0.87	0.72	0.69
tyrO	1110		0.44	0.58	0.22
ptyr	1110	0.00	0.00		0.00
trp	1110	0.96	0.94	0.67	0.75
htrp	1111	0.99	0.98	0.85	0.87

(3) Absorption, CD, and NMR Spectral Properties. Stacking of DA with nucleotides in Cu(II) complexes has been found to give rise to a charge-transfer (CT) band in the region 300–400 nm,[57] and similar bands were also observed for Cu(DA)(AA), etc. For example, the complexes Cu(DA)(X-phe) (X-phe = *p*-H-, HO-, Br-, I-, and NH_2-substituted phe) exhibited difference spectra with weak broad bands at 310–400 nm.[48,58] The bands have been assigned to CT between the stacked rings and hence support the existence of stacking interactions in Cu(DA)(AA) in solution. In this connection the (protonated phen)–(protonated trp or htrp) systems, which were without Cu(II), exhibited a well-resolved CT peak at 360–370 nm.[46] The CD spectra of Cu(DA)(AA) with stacking exhibited a negative peak at 570–600 nm, whose magnitude was much stronger than that for Cu(en)(AA) and Cu(DA)(val) and was in the order of AA, phe < tyrO < tyr < trp, reflecting the distortion and increased rigidity of the side chain conformation due to stacking.[46] CD magnitude anomaly[47a] and stability difference[27,47a] between Cu(L-his)(AA) and Cu(D-his)(AA) have been detected for Cu(his)(AA) (AA = phe, tyr, and trp) due to stacking interactions, which is in agreement with the conclusion from the $\Delta\Delta\log K$ values (Eq. 6).[44]

^{1}H NMR upfield shifts due to the ring current effect serve as a strong support for stacking interactions in solution. Studies on the ternary Pd(II) complexes of aromatic amino acid-containing dipeptides L clearly showed that stacking exists in Pd(DA)(L) (DA = bpy, 4,7-diphenyl-1,10-phenanthroline-4′,4″-disulfonate (bphen); L = tyr·gly, tryptophylglycinate (trp·gly), phenylalanylglutamate (phe·glu), etc.)[59] as later revealed for Pd(bpy)(tyr·gly) in the solid state (Fig. 10).[54] From the observed upfield shifts for the aromatic side chain protons and the shifts for complete stacking calculated from the Johnson–Bovey diagram,[60] the P_{ST} values were calculated to be as high as 0.98 for Pd(bphen)(trp·gly) at 25 °C, the structural dependence of P_{ST} being similar to that determined by pH titration (Table 4).

Metal Ion–Aromatic Ring Interactions

The side chain aromatic ring of $Cu(tyr)_2$[61] and Cu(II)–gly·trp (gly·trp = glycyltryptophanate)[62] has long been known to be tilted over the Cu(II) coordination plane in the solid state. Martin pointed out that the ratio of the stepwise stability constant for $Cu(phe)_2$ to that of Cu(phe), K_2/K_1, is higher than that for the Cu(II)–gly and -ala complexes.[63] As a result of intramolecular stacking as seen in Cu(bpy)(tyr) the side chain aromatic ring is located close to and above the Cu(II) plane, to be within a weak bonding distance (3–3.5 Å). In fact the enthalpy change for log K_2 of $Cu(trp)_2$ has been found to be more negative than that for $Cu(ala)_2$,[64] showing a stronger Cu(II)-ligand bond. These observations may indicate a Cu(II)-aromatic ring interaction, which should affect the electron density of the metal ion and therefore the stability constants of the complex formed. Our preliminary ab initio molecular orbital calculation by the density functional method of $[Cu(bpy)(trp)(H_2O)]^+$ which involves a bpy-indole stacking interaction showed that there are bonding orbitals formed between Cu(II) and the pyrrole moiety of the indole ring.[65]

The Cu(II) complexes of tyr-containing dipeptides, $Cu(tyr{\cdot}X{\cdot}H_{0(or\ -1)})$ (tyr·X = tyr·gly, tyrosylphenylalaninate (tyr·phe), tyrosyltyrosinate (tyr·tyr), tyrosyltryptophanate (tyr·trp), etc.), formed at neutral pH easily dimerize to $Cu_2(tyrO{\cdot}X{\cdot}H_{0(or\ -1)})_2$ upon dissociation of the phenol OH group at pH 8–11 through the phenoxide bridge,[66] as previously reported by Hefford and Pettit for tyr·gly.[67] With active dipeptides L-tyr·L-X, this reaction was accompanied by a remarkable magnitude change and sign inversion of the CD spectra at 650–690 nm, whereas no such change was observed for the *meso* dipeptides L-tyr·D-X. Assuming that the monomeric complex $Cu(L\text{-}tyr{\cdot}L\text{-}X{\cdot}H_{(0\ or\ -1)})$ has a structure with the C-terminal aromatic ring R tilted over Cu(II), we concluded that this conformation is distorted upon dimerization to $Cu_2(L\text{-}tyrO{\cdot}L\text{-}X{\cdot}H_{(0\ or\ -1)})_2$ due to steric hindrance (Fig. 11), resulting in the drastic CD spectral change. No conformational distortion and therefore no CD spectral change would occur with

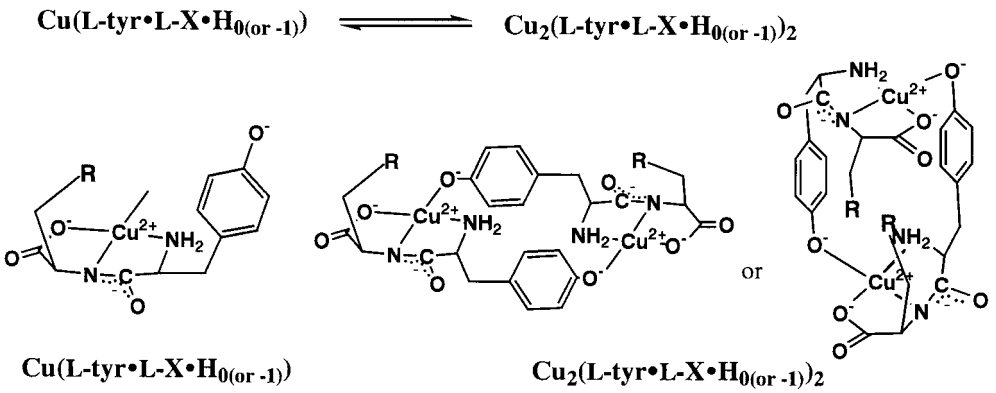


Fig. 11. Dimerization of $Cu(tyrO{\cdot}X{\cdot}H_{-1})$ and concomitant conformational change.[66]

Cu(L-tyr·D-X·H_{-1}) where R is on the other side of the coordination plane and not affected by the phenoxide bridging. Vestues and Martin concluded from the ^{1}H NMR spectra that the aromatic side chain of Pd(II)-phenylalanylphenylalaninate in solution prefers to be above the metal coordination plane.[68] The stability constants for the ternary complexes Cu(DA)(AA) (DA = en, *N*,*N*′-dimethyl- or -dibenzyl-substituted en; AA = phe, tyr, trp, etc.), where there is no aromatic ring stacking and Cu(II)–aromatic ring contact is rather limited, indicated that the complexes are slightly stabilized, and this has been ascribed to the tilted conformation of the aromatic ring and the resulting effect on hydration on Cu(II).[69] The effect of stacking on the electron density of the central metal ion has been shown for Pt(II) by ^{195}Pt NMR (vide infra).

The indole ring is an electron-rich aromatic ring, and as seen from Table 3 it stabilizes the ternary complexes by stacking most effectively. Although it does not bind with a metal ion under usual conditions, it was found to coordinate to Pd(II) through the nitrogen[70] to form Pd–3*H*-indole complex having the NH proton at the C3 position, which was also observed to bind with Pd(II)[71a,71b] and Pt(II).[71b] By designing tripodal N_3-donor ligands with a pendent indole ring and changing the electron density of the central metal ion from Cu(II) to Cu(I), we synthesized the Cu(I) complex of a tripodal ligand, *N*-[2-(3-indolyl)ethyl]bis(6-methyl-2-pyridylmethyl)amine (Me_2-IEP), and revealed that it has a tetrahedral structure with a unique Cu(I)-indole η^2-type bond involving the C2–C3 moiety (Fig. 12).[72] The bond is stable in methanol but is broken in CH_3CN, indicating that it is rather weak. The redox potential of this complex was determined to be slightly higher than a similar complex without the Cu(I)–indole bonding, which may indicate stabilization of the Cu(I) state due to the π-back donation from Cu(I) to the indole ring. Recently a Cu(I)–macrocyclic NS_2 ligand complex with a pendent naphthyl ring was found to form a weak Cu(I)-naphthyl η^2-type bond.[73]

These results suggest that aromatic rings in the vicinity of the metal coordination sphere may have an electronic effect on the central metal ion and affect its reactivity either through direct bonding or through indirect close contact. The metal ion in turn may affect the reactivity of the aromatic rings. Apart from direct metal ion–aromatic ring interactions, cation-π interactions between a cationic portion of a coordinated ligand and an aromatic ring in the side chain have been reported.[74]

Interactions between Metal Complexes and Uncoordinated Molecules

Weak interactions around the central metal ion do not always involve coordination to the metal ion. Ligand–ligand interactions may be generalized as intermolecular interactions around the metal center, where the roles of the metal ion may be to exert structural and electronic effects and transmit relevant information to the outside. Metal complexes can interact with noncoordinating molecules or ions in solution. When they are exchange-inert or stable enough to maintain their structural identity, it is possible to acquire information on the modes of interactions by spectroscopy and on the structural dependence and energy of adduct formation by measuring the equilibrium constants. From this point of view, we studied the adduct formations in metal-containing systems, such as Pt(II) complex–nucleotide systems. Studies on intermolecular interactions between small molecules were then extended to the metalloprotein–charged peptide systems, with a view to clarifying the biological recognition site. As a step toward rational construction of supramolecular assemblies, we studied self-organization of molecules in the systems composed of the 1:2 Cu(II)–arg complex ion, $Cu(arg)_2^{2+}$, and its aromatic and other dinegative counterions such as isophthalate (= benzene-1,3-dicarboxylate).

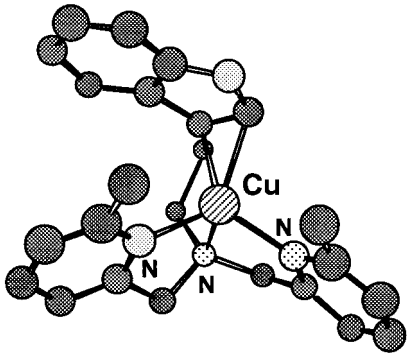


Fig. 12. Unique η^2-type bonding between Cu(I) and the indole ring in Cu(Me_2IEP).[72]

(1) Adduct Formation between Platinum DNA Intercalators and Mononucleotides. Pt(II) complexes with a planar aromatic ligand such as bpy, phen, and 2,2′:6′,2″-terpyridine (terpy) are known to intercalate into DNA base-pairs by stacking and electrostatic interactions, and *Δ*- or *Λ*-Ru(phen)$_3^{2+}$ and other Ru(II) complexes have been revealed to recognize right-handed and left-handed DNA structures by groove binding by weak forces.[75] Stacking is regarded as an important driving force for the intercalative binding of Pt(II)–aromatic ligand complexes into the nucleic base-pairs. Considering that the energy and structural dependence of the interactions determine the site specificity of intercalator– and protein–DNA binding, we studied the adduct formation between a Pt(II) complex Pd(DA)(en) (DA = bpy, phen, etc.) and a mononucleotide NMP (= AMP, guanosine 5′-monophosphate (GMP), cytidine 5′-monophosphate (CMP), etc.) by spectroscopic and calorimetric measurements.[76] The first indication that they form adducts was given by the CD spectra of the Pt(bpy)(en)–NMP systems (NMP = AMP, GMP, CMP) at pH 7–8, which exhibited a positive peak at ~320 nm due to the vicinal effect of the asymmetric carbons of NMP.[77] The CD peak was much weaker with adenosine used in place of NMP, and the peak observed for AMP decreased with the increase of the ionic strength adjusted by $NaClO_4$, which indicates that the adduct formation is partly due to the electrostatic interactions between the Pt(II) complex and the ribose phosphate moiety. Addition of NMP to Pt(DA)(en) (DA = bpy, phen, 3,4,7,8-tetramethyl-1,10-phenanthroline (Me_4phen), 5-nitro-1,10-phenanthroline (nphen)) caused the absorption spectral changes, as revealed by the difference spectra in the aromatic π-π* transition and CT regions (290–340 nm), indicating that the electronic states of the aromatic rings were affected by the adduct formation.[78] The ^{1}H NMR spectra of NMP in the Pt(DA)(en)–NMP systems exhibited upfield shifts of the purine (H2 and/or H8) or pyrimidine (H5 and H6) signals and the ribose (H1′) signals due to the

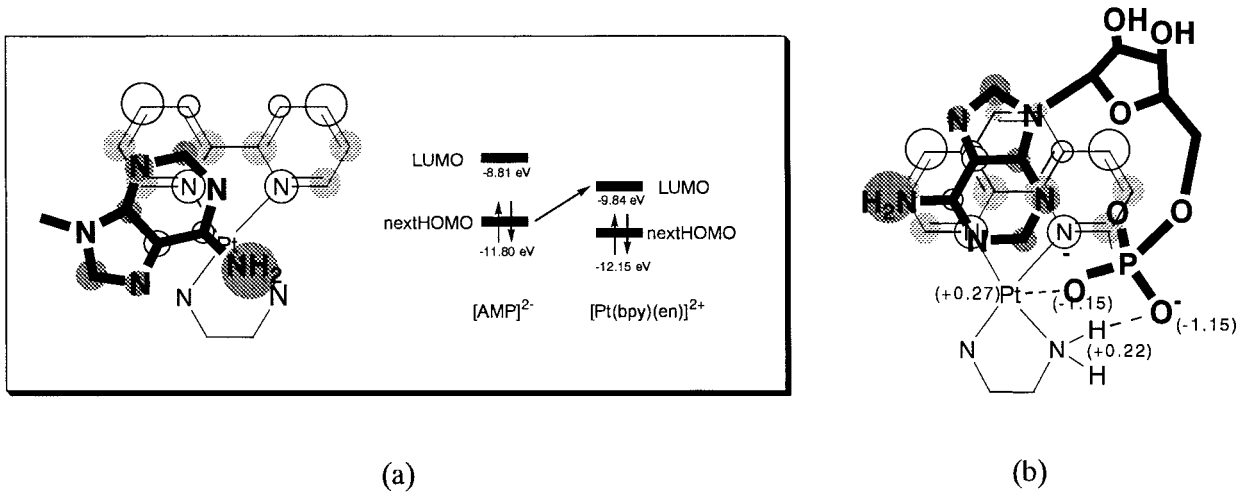


Fig. 13. Extended Hückel MO calculations (a) and the proposed binding mode (b) for [Pt(bpy)(en)]–AMP and [Pt(phen)(en)]–AMP, respetively.[78]

ring current effect, but no upfield shifts were detected for $Pt(en)_2$–NMP.[78,79] X-ray structure analysis of [Pt(bpy)(en)]·AMP·$10H_2O$ revealed the stacking interaction between the adenine ring of AMP and coordinated bpy.[80] These observations established that Pt(DA)(en) and NMP form adducts through stacking between the aromatic rings of the complex and NMP and the electrostatic interactions involving the phosphate group. Extended Hückel molecular orbital calculations using the reported parameters showed the CT interaction between the π orbital of AMP (next HOMO) and the π^* orbital of [Pt(bpy)(en)] (LUMO) (Fig. 13a). The proposed binding mode, which shows that the two oxygen atoms of the phosphate group interact with the Pt(II) ion and the coordinated amino group, is compatible with the calculated net positive charges of these groups (Fig. 13b).[78]

The equilibrium for the adduct formation is given as follows:

$$\text{Pt(DA)(en)} + n\text{NMP} \overset{\beta_n}{\rightleftharpoons} \text{Pt(DA)(en)}\cdots(\text{NMP})_n$$
$$\beta_n = [\text{Pt(DA)(en)}\cdots(\text{NMP})_n]/[\text{Pt(DA)(en)}][\text{NMP}]^n \quad (9)$$

Insights into the structural and thermodynamic aspects of the adduct stability were provided by the stability constants log β_n (n = 1, 2) and thermodynamic parameters for various Pt(DA)(en)–NMP systems determined by the spectroscopic and calorimetric measurements.[78,79,81,82] Table 5 summarizes the log β_1 values and the relevant parameters ΔH^0 and ΔS^0 determined at 25 °C. The stability constants log β_1 are in the range 2–3 at I = 0.1 M ($NaClO_4$ or NaCl) and are dependent on both the DA and NMP structures. Pt(II) complexes with a large DA such as Me_4phen exhibited a log β_1 value as large as 3.07. The order of the stability with respect to NMP was found to be AMP $\simeq$ GMP > IMP > CMP (IMP = inosine 5′-monophosphate), which shows that purine nucleotides form more stable adducts than pyrimidine nucleotides. Adenosine (Ado) and cytidine (Cyt), which do not have the phosphate moiety, form adducts that are 0.3–0.5 log unit less stable, indicating that the electrostatic interactions as shown in Fig. 13b partly contribute to the adduct formation.

Table 5. Stability Constants, log β_1, and Thermodynamic Parameters, ΔH^0 and ΔS^0, for Formation of Pt(DA)(en)–NMP Adducts in Water at 25 °C[79,81,82]

DA	NMP	I	log β_1	ΔH^0/kJ mol^{-1}	ΔS^0/J mol^{-1} K^{-1}
phen	AMP	0.1	2.51	−25.6	−38
phen	AMP	0.2	2.48	−21.1	−23
phen	Ado	0.1	2.21	−15.8	−11
phen	Ado	0.2	2.24	−14.4	−6
phen	GMP	0.1	2.49	−26.2	−40
phen	GMP	0.2	2.47	−20.0	−20
phen	IMP	0.1	2.34	−11.9	5
phen	CMP	0.1	2.17	−6.5	20
phen	Cyt	0.1	1.63	−5.0	15
Me_4phen	AMP	0.1	3.07	−22.9	−18
Me_4phen	Ado	0.1	2.74	−16.1	−2
Me_2phen	AMP	0.1	3.00	−23.3	−21
nphen	AMP	0.1	2.44	−22.7	−30
bpy	AMP	0.1	2.30	−18.0	−16
bpy	Ado	0.1	1.91	−14.2	−11

The thermodynamic parameters shown in Table 5 reveal that the reaction is always accompanied by negative enthalpy changes and negative entropy changes ($\Delta H^0 < 0$ and $\Delta S^0 < 0$) for the purine nucleotides. No liberation of heat was observed for uridine 5′-monophosphate. The results clearly show that the adduct formation is enthalpically driven, implying that the stacking may be regarded as a weak bonding interaction, i.e. a nonclassical hydrophobic interaction. This is not surprising because the enthalpy contribution has already been pointed out e.g. for Cu(his)(trp), Cu(ATP)(his), and related systems by Arena, Rizzarelli, et al.[64,83] The stacking in Pd(DA)(tyr·gly) was also found to be enthalpically driven from the temperature dependence of the equilibrium constants for stacking.[59]

Although the log β_1 values for AMP, GMP, and IMP are in the range 2.3–2.5 for Pt(phen)(en), a closer look at the thermodynamic parameters reveals that the ΔH^0 values are much more negative for AMP and GMP (~ -26 kJ mol^{-1}) than the value for IMP (-11.9 kJ mol^{-1}) and that the ΔS^0 values were negative for AMP and GMP but positive for IMP (Table 5).[82] This

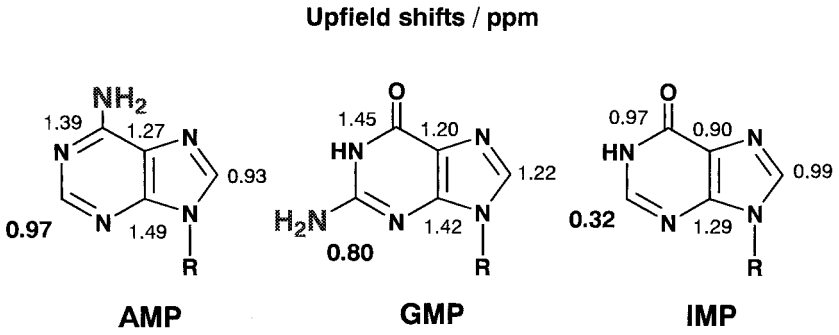


Fig. 14. Comparison of the structures and ^{13}C NMR upfield shifts due to stacking of the nucleic bases in [Pt(Me_2phen)(en)] systems.[82]

indicates that stacking interaction is stronger with AMP and GMP than with IMP. The difference in the thermodynamic properties between the purine nucleotides may be explained by the amino group, which is present in adenine and guanine but absent in IMP (Fig. 14) and which is considered to increase the electron density of the purine ring, because the amino group in adenine and guanine has almost no basic character. This electron density increase is favorable for stacking because the aromatic rings of DA are electron-deficient due to coordination to Pt(II). The ^{13}C NMR spectra of Pt(Me_2phen)(en)–NMP showed that the upfield shifts ($\Delta\delta$) due to stacking of the C2 moiety were much larger for AMP and GMP than for IMP, which is in line with the conclusion from the thermodynamic considerations (Fig. 14).[82] The above results may suggest that intercalative binding occurs preferentially at the guanine-cytosine base-pair where both bases have an amino group.

All the adducts have the stability constants which are much larger than the constants for self-association of NMP (usually less than 1 log unit)[76] and the Pt(II) complexes (0.25 for Pt(phen)(en)),[79] demonstrating the important contribution of the Pt(II) ion to the adduct stability by changing the electron density, increasing the structural rigidity, and thus favoring the adduct formation. When Pt(phen)(en) formed an adduct with AMP, the ^{195}Pt signal suffered downfield shifts relative to that of Pt($en)_2$, and the shifts were linearly correlated with the enthalpy changes of the adduct formation, i.e., 21.5, 23.2, and 9.8 ppm for AMP, GMP, and IMP, respectively.[79] This suggests that the electron density of the Pt(II) center is decreased due to delocalization of the electrons over the aromatic rings interacting with each other through stacking, resulting in stabilization of the Pt(II) complex–AMP adduct.

(2) Interactions between Charged Side Chain Groups of Metal Complexes and Uncoordinated Molecules. Palladium(II) forms stable planar complexes with N-donor ligands such as bpy, en, and amino acids. Although Pd(II) complexes are not exchange-inert, their existence in solution is long enough to be detected separately in the NMR time scale. Possible electrostatic interactions or hydrogen bonds were studied for the systems involving Pd(II), diaminoalkanecarboxylates (A = L- and DL-forms of 2,3-diaminopropionate (dap) or 2,4-diaminobutyrate (dab)), and a biogenic polyamine spermidine (spd = *N*-(3-aminopropyl)-1,4-diaminobutane) as a basis for the intermolecular side chain-side chain interactions.[84] ^{13}C NMR spectra of the 1:1 Pd(II)-spd system revealed that Pd(spd) with spd as a terdentate ligand is the major species at neutral pH, whereas for the 1:2 Pd(II)-spd system, Pd($spdH)_2$ with N_4 coordination (spdH = spd with one of the terminal amino groups protonated) is also formed. For the ternary systems 1:1:1 Pd(II)–A–spd no mixed ligand complexes were formed, but instead Pd($A)_2$ and Pd($spd)_2$, both with N_4 coordination, were the main species. The only detectable species in the systems 1:2:*x* Pd(II)–A–spd ($x \leq 10$) was Pd($A)_2$ with spd uncoordinated, and the positive CD peak of Pd(L-$A)_2$ at 280–290 nm suffered a magnitude decrease with addition of spd while its absorption spectrum stayed unchanged, which indicates that the conformation of L-A is affected by hydrogen bonds or electrostatic interactions with fully protonated spd, $spdH_3$, as shown in Fig. 15. The approximate log β_1 values for Pd($A)_2$–$spdH_3$ adducts were 2.4 and 2.6 for A = L-dpa and L-dba, respectively, at *I* not fixed. The population of the *trans* form of Pd(L-$dba)_2$ was estimated to increase as compared with the *cis* form due to the interactions. The effect on the CD magnitude was more pronounced when diamines, 1,3-diaminopropane and 1,4-diaminobutane (putrescine), were used in

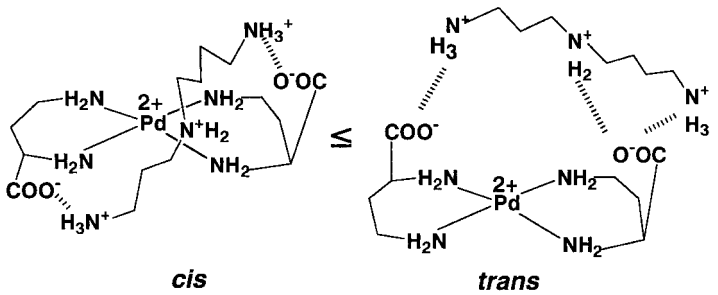


Fig. 15. Intermolecular interactions between Pd($dab)_2$ and protonated spd.[84]

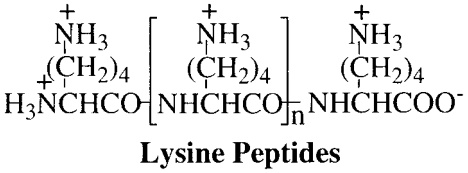
place of spd, probably because they interact with the carboxylate oxygens more effectively.

The mentioned mode of interactions shows an intrinsic affinity of biogenic amines for negatively charged groups, such as the phosphoester groups of DNA, and further suggests that interactions between charged groups exposed to the outside are important for stabilization of molecules and molecular recognition.

(3) Metalloprotein–Charged Peptide Interactions, Their Structural Effects, and Molecular Recognition. Intramolecular ligand–ligand electrostatic interactions or hydrogen bonds have been concluded for complexes involving an acidic amino acid with a negatively charged side chain and a basic amino acid with a positively charged side chain, such as Cu(L-glu)(L-lysH) and Cu(L-asp)(L-lysH) (vide supra). The Pd(II) complex–polyamine adduct formation mentioned above presents a different mode of interactions.[84]

Electrostatic interactions are one of the essential forces for complex formation or molecular recognition between electron transfer proteins. Plastocyanin (PC), a Cu-containing mobile electron transfer protein existing in the thylakoid lumen of photosynthetic organisms, is negatively charged at neutral pH. It contains consecutive acidic residues (negative patch) located at a solvent-accessible site near a tyrosine residue remote from the Cu center.[85] PC receives an electron from cytochrome *f* (cyt *f*) of the cytochrome b_6f complex, which is an integral oligomeric membrane protein complex existing in the photosynthetic organism. A lysine residue-rich positively charged site (positive patch) has been revealed to exist at the solvent-exposed site of cyt *f* by X-ray structural studies,[86] and the electrostatic interaction between the negative patch of PC and the positive patch of cyt *f* has been inferred to be the driving force for the formation of the cyt *f*-PC complex.[87] According to the X-ray crystal structure analysis, the cyt *c*–C*c*P complex is also formed by the electrostatic interaction of the positively charged sites of cyt *c* with the negatively charged sites of C*c*P.[39]

As an extension of intermolecular electrostatic interactions between small molecules, we studied electron transfer protein–charged peptide systems in order to clarify the partner recognition sites and the effects of intermolecular interactions on the metal site structures in the reactions between electron transfer proteins. To investigate the molecular recognition character of proteins, we used the charged peptides schematically shown in Fig. 16 as recognition site models for one of the proteins and determined the electron transfer rates between the redox couple in the presence and absence of the charged peptides, where positively charged peptides interact with the negatively charged sites of proteins while negatively charged peptides interact with the positively charged sites.[88] Thus, lysine peptides interacted with the consecutive asp and glu residues of the negative patch of PC and competitively inhibited electron transfer from reduced cyt *f* or cyt *c* to oxidized PC.[89,90] The inhibitory effect of lysine peptides were explained as competitive inhibition due to neutralization of the PC negative patch by formation of PC·lysine peptide complexes (Fig. 17), which demonstrates that lysine peptides function as models for the PC interacting site of proteins. Sykes et al. showed that small redox-inactive inorganic compounds inhibit electron transfer between



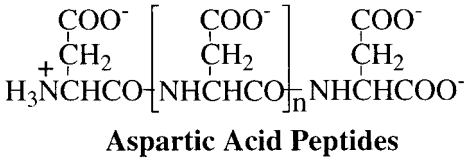


Fig. 16. Structures of charged peptides used.

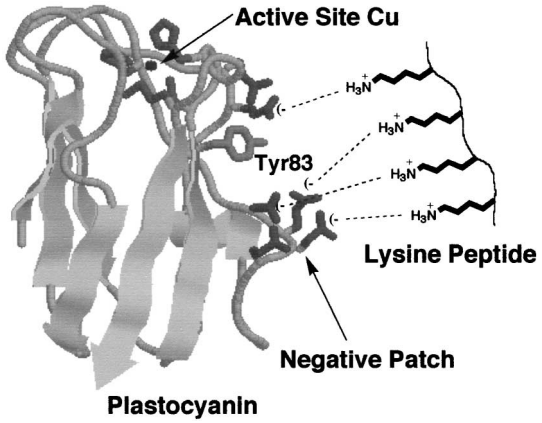


Fig. 17. Schematic representation of PC (PDB 1BYO)[85d] interacting with *tetra*-lys.

PC and cyt *c*,[91] cyt *f*,[92] or inorganic redox partners,[93,94] which has been explained by competitive inhibition due to the interaction between the positive charges of the redox-inactive inorganic compounds with the negative charge of PC. In line with these conclusions, decreasing the net charge of the PC negative patch by mutation resulted in the decrease in the electron transfer rate from reduced cyt *c* to oxidized PC and the inhibitory effect of lysine peptides.[89] These observations strongly support the conclusion that the negative patch of PC is the dominant cyt *c*/*f* molecular recognition site. Likewise, negatively charged aspartic acid peptides (asp peptides) up to pentaaspartate (*penta*-asp) served as competitive inhibitors of the electron transfer from cyt *c* or cyt *f* to PC,[90] which is ascribable to the cyt *c* or cyt *f*-asp peptide complex formation similar to the PC–lysine peptide complex. Lysine peptides, however, promoted electron transfer from $[Fe(CN)_6]^{4-}$ to oxidized PC,[95] which may be explained as due to formation of PC–lysine peptide or lysine peptide–$[Fe(CN)_6]^{4-}$ complexes subsequently forming an electron transferring complex, PC–lysine peptide–$[Fe(CN)_6]^{4-}$, without repulsion of the negative charges.

Lysine peptides have been found to be very useful for investigating the molecular interaction induced structural changes of metalloproteins having absorption bands in the visible region, where the peptides do not have any absorption. The absorption spectra of oxidized PC with and without pentalysine

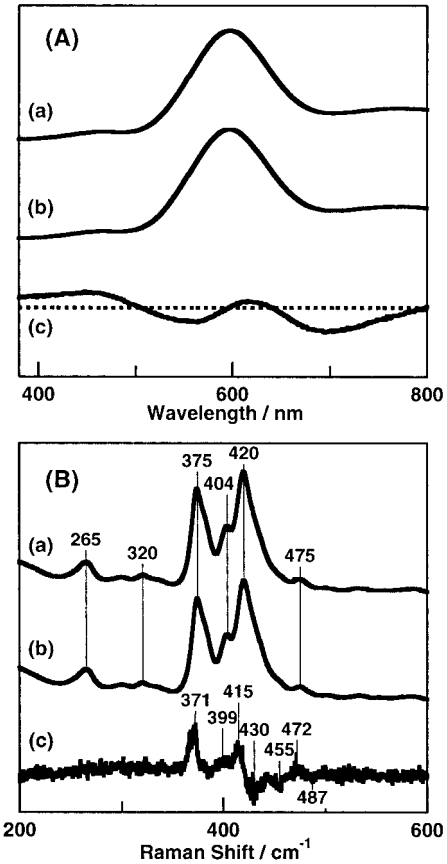


Fig. 18. Absorption and resonance Raman spectra of oxidized PC.[89] (A): Absorption spectra of PC with (a) and without (b) *penta*-lys and their difference spectrum (a–b) multiplied by 20 (c). The baseline of the difference spectrum is shown as a dotted line. (B): Resonance Raman spectrum in the 200–600 cm^{-1} region for oxidized PC with (a) and without (b) *penta*-lys and the difference spectrum obtained by subtracting this spectrum from that with *penta*-lys and multipying it by 10 (c).

(*penta*-lys) and their difference spectrum are shown in Fig. 18A. Peaks and troughs detected in the difference spectrum at about 460 and 630 nm and at about 560 and 700 nm, respectively,[89] revealed that the absorption band centered at about 600 nm shifts to a longer wavelength and that the 460- and 700-nm absorption band intensities increase and decrease, respectively, on interaction with the lysine peptide. Since the 600-nm band is assigned to the S(Cys)-to-Cu(II) LMCT band,[96] the result indicates that the structure of the Cu site (at least the Cu–S(Cys) bond) should be altered. Some of the Raman bands at 375–475 cm^{-1} slightly shifted to lower frequencies, and the relative intensities of some of the lower frequency bands in this region increased slightly when *tetra*-lys was added (Fig. 18B). The $\nu_{Cu\text{-}S}$ related 375- and 420-cm^{-1} bands were especially affected, indicating that the Cu–S(Cys) bond was weakened on addition of lysine peptides. Binding of lysine peptides also caused the redox potential shift to a higher value. These findings suggest that the peptides induce a structural change in PC to make the copper site adapted for facile electron transfer.[89,97,98] The structural change might be related to the report by Kostic et al., who proposed that PC and cyt *c* or cyt *f* bind and react with each other in different configurations resulting from the protein-protein interaction termed as the gating process for electron transfer,[99–101] showing possible configurations for the diprotein complex by computer simulation.[102,103] Structural changes were also observed for cyt *f*, cyt *c*, and C*c*P on interaction with charged peptides.[90,104]

We extended our study to a system composed of two proteins, PC–cyt *c*.[89,90] Interaction of PC with cyt *c* caused changes in the resonance Raman spectrum of PC, which were similar to those observed for the PC–lysine peptide interactions. This indicates that PC interacts with cyt *c* and lysine peptides in a similar way and that lysine peptides can be excellent models for the PC recognition site of cyt *c*.[89] In connection with the absorption spectral change by lysine binding, the association constants between PC and cyt *f* or cyt *c* have been obtained by measuring the increase of the Soret band intensity of cyt *f* or cyt *c*.[105–107] When asp peptides up to *penta*-asp were added to the cyt *c* solution, changes in the absorption spectrum in the Soret region were detected, and the difference spectral pattern was similar to that observed for cyt *c*–PC interactions,[90] which demonstrates that asp peptides mimic PC in the interaction with cyt *c*.

(4) Self-Organization of Complex Molecules through Guanidinium–Carboxylate Hydrogen Bonding. Intramolecular ligand–ligand interactions in small complex molecules may be converted to intermolecular interactions in concentrated solution and in the solid state (Scheme 1). In fact, the electron spin resonance spectrum of Cu(II)–argH·asp exhibited the broadening of the Cu(II) hyperfine structures already in 2 mM solution, indicating that the complex species associate with each other even in dilute solution through hydrogen bonding as shown in Fig. 2.[18] The crystal structures of this and other complexes such as the [Pt(bpy)(en)]···AMP adduct[80] exhibit infinite structures formed by hydrogen bonding and/or aromatic ring stacking. They form three-dimensional (3D) structures, whose architecture is determined by combinations of a variety of factors such as the molecular structure, chemical bonding, and topology. Supramolecular chemistry has attracted a great deal of attention in recent years, and interesting structures such as helices composed of metal ions with a tetrahedral geometry and oligobipyridines etc. have been reported.[14] In view of the functions of organized molecules in chemical and biological systems, we studied self-organization of Cu(II)–amino acid complex molecules.[108]

The guanidinium group of arg is well known as the hydrogen bonding partner for the carboxylate and phosphate moieties in proteins. It can form two types of two parallel hydrogen bonds with a carboxylate group.[109] In the course of the studies on ligand–ligand interactions in systems involving argH, the molecular structure of $[Cu(L\text{-}argH)_2](NO_3)_2$ was revealed to have two arg molecules in the *cis*-configuration in the coordination plane, probably owing to the hydrogen bonds between the coordinated amino groups and a nitrate ion.[32] The crystal structure is contructed by a 3D infinite network of hydrogen bonds involving the guanidinium group, nitrate ion, and water molecules. When the nitrate ion was replaced by benzene-1,3-dicarboxylate (mba), the resulting complex $[Cu(L\text{-}argH)_2](mba)$ also showed a *cis*-configuration fixed by

the hydrogen bonds between the coordinated amino groups and the oxygen atoms from the two carboxylate groups of mba, and the crystal structure was revealed to have a 1D infinite $\{-[Cu(L\text{-}argH)_2]^{2+}-mba^{2-}-\}_n$ chain of complex molecules connected by the hydrogen bonds between the arg guanidinium groups of the complex and the carboxylate groups of mba (Fig. 19).[110] The hydrogen bonds between the coordinated amino groups and the dicarboxylates bind two nearby parallel helices together to form a right-handed double-helical structure. With the use of D-argH in place of L-argH a left-handed double-helical structure was formed in $[Cu(D\text{-}argH)_2](mba)$, which suggests that the handedness of the helix is determined by the chirality of the α-carbon atom of arg. The same double-helical structure was formed with aromatic dianions pyridine-2,6-dicarboxylate (2,6-dpc) and pyridine-3,5-dicarboxylate (3,5-dpc), where no nitrogen coordination was observed.[110] The $[Cu(L\text{- or }D\text{-}argH)_2]^{2+}$ ion in these structures serves as a building block, having two extended arg side chains which are connected by the counterions (and water molecules), leading to the self-organization.

One of the key factors for the unique double-helical structure is that all the dicarboxylates used have the interacting groups in the *meta*-positions, so that the oxygen atoms from the two carboxylate groups are in positions suitable for hydrogen bonding with the coordinated amino groups in the *cis*-positions. As a result of this and because of the α-carbon chirality, the side chains of the two arg molecules are extended in the opposite directions to be hydrogen bonded with the neighboring aromatic dicarboxylates, and the Cu(II) coordination structure is distorted from the plane by the bridge formation. On the other hand, benzene-1,4-dicarboxylate (pba), benzene-1,3-disulfonate (mbs), and benzene-1,2,4,5-tetracarboxylate (pyromellitate) (pma) used as counteranions gave a 1D infinite $\{-[Cu(L\text{-}argH)_2]^{2+}-\}_n$ tape structure formed by the hydrogen bonds between the guanidinium and coordinated carboxylate groups.[111] For the systems with pba, the Cu(II) center exhibited a *trans*-geometry, and the two arg side chains were extended sideways on the same side of the coordination plane, resulting in a tape rather than a helical structure. The tapes are hydrogen-bonded by pba tilted and existing between them to form a 2D sheet, which then form a 3D layer structure through the hydrogen bonds between the carboxylate and coordinated amino groups. Close contact between Cu(II) and the aromatic ring of pba (3.18 Å) is considered to contribute to formation of the layer structure. Similar structures and bonding modes have been observed for mbs and pma.[111]

An inorganic dianion, SO_4^{2-}, formed through hydrogen bonds with the arg guanidinium groups a 1D infinite $\{-[Cu(L\text{-}argH)_2]^{2+}-SO_4^{2-}-\}_n$ chain with a right-handed single-helical structure ascribable to the *cis*-configuration of the $Cu(L\text{-}argH)_2$ complex, due to the amino group–sulfate oxygen hydrogen bonds, which further contributed to formation of a double-stranded single helix.[112]

These results are summarized in Scheme 2, which shows that self-organization of molecules and thus the structure of a supramolecule may be controlled by a combination of factors such as the geometry around the metal center, structure and chirality of ligands, and selection of counterions.

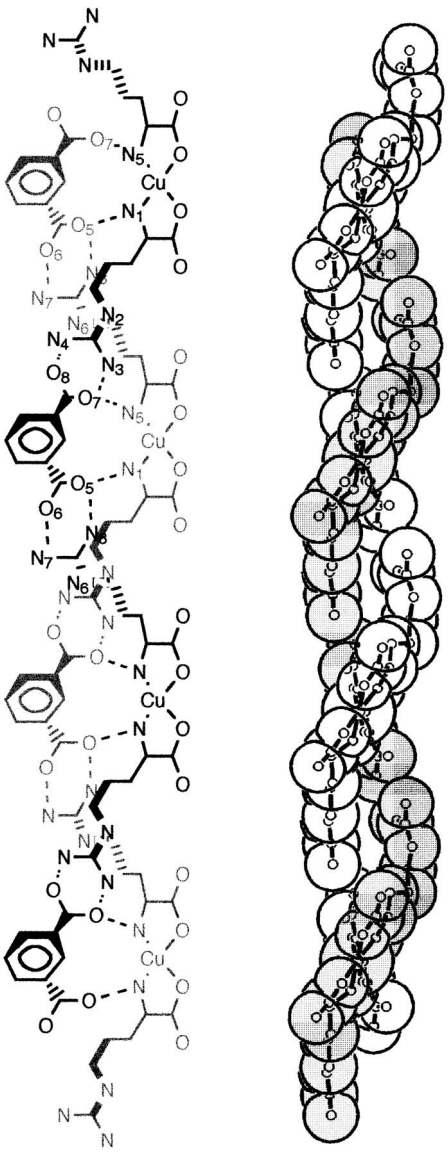
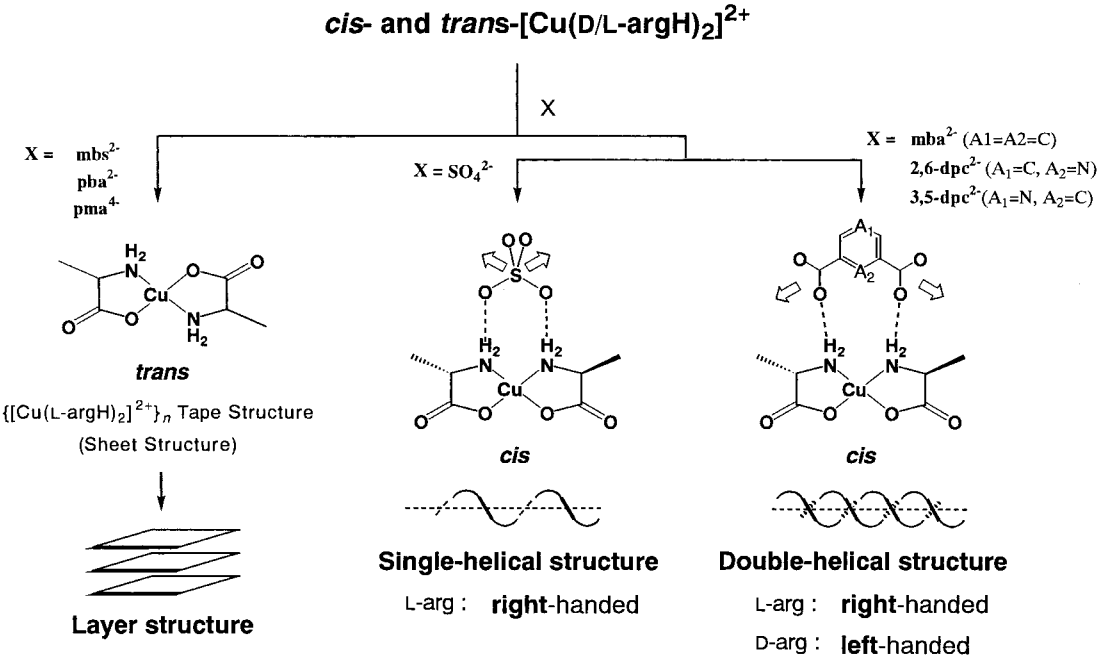


Fig. 19. Infinite double-helical structure of $\{-[Cu(L\text{-}argH)_2]^{2+}-mba^{2-}-\}_n$.[110]

Concluding Remarks

The importance of weak interactions is apparent from the structures and functions of highly organized and controlled biological systems. Although the energy of one interaction alone is small, e.g., ΔH^0 = ca. -20 kJ mol^{-1} for the Pt(II) complex–NMP interactions (Table 5), multiple interactions can be as strong as a covalent bond and suitable for molecular recognition and specific binding, such as in enzyme–substrate and antigen–antibody complexes. Because of the weakness of interactions, they are flexible, take place as soon as the interacting molecules come close together, and easily dissociate depending on the conditions, which is a great advantage for quick biological response.



Scheme 2. Counteranion-controlled self-organization of $[Cu(argH)_2]^{2+}$.[110–112]

From the studies on model systems incorporating interacting molecules, it is possible to collect information on weak interactions and their structural dependences, which are rather difficult to follow in systems with proteins and complex molecules. Findings on model systems may point to the possibilities in biological systems and offer clues to biological reaction mechanisms. As shown earlier, the complex Cu(bpy)(tyr), which is stabilized by intramolecular stacking with the log K value of 0.90 (Table 3), is no more stabilized when the tyr phenol OH group is phosphorylated to the phosphoester, $-OPO_3^{2-}$. However, the negatively charged phosphoester moiety now shows the affinity for a positively charged group such as the arg guanidinium and the lys ammonio group, resulting in the stabilization of Cu(argH)(ptyr) and Cu(lysH)(ptyr) (Table 2). These findings indicate that phosphorylation of tyr may convert the aromatic ring stacking to electrostatic interactions between the phosphoester and guanidinium groups, on the basis of which we suggested a possible scheme of protein conformational changes due to the change in the interaction mode from stacking to electrostatic bonding, as shown in Fig. 20.[31,46]

The stacking involving the his imidazole ring has been shown to occur in Cu(hista)(phe) and Cu(hista)(tyr) in solution[45,46] and in the solid state,[53] suggesting its possibility in proteins. Kohzuma et al.[113] recently isolated a new plastocyanin from the fern, *Dryopteris crassirhizoma*, and discovered by X-ray analysis of the molecular structure that the active site Cu has a his ligand (His 90) which is stacked with the phenyl ring of an adjacent phe residue in a manner very similar to that disclosed in Cu(hista)(phe) (Fig. 8).[53] This is the first example of stacking between a coordinated imidazole ring and a nearby aromatic ring in proteins, and the Cu site exhibits a high redox potential as compared with the other known plastocyanins, probably as a result of the electron density decrease due to stacking.

The ideas of weak interactions in small complexes have been shown to be applicable to certain metalloprotein-containing systems such as plastocyanin–lys peptide and plastocyanin–cytochrome *c* systems and have provided valuable infor-

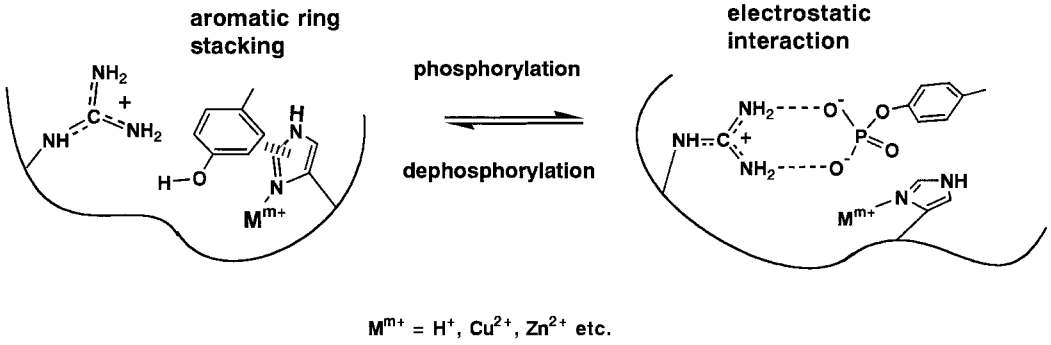


Fig. 20. Possible conformational change due to phosphorylation of the stacked tyr phenol OH group.[31,46]

mation on so far undetected phenomena, such as the subtle structural change of the plastocyanin Cu site upon interaction with lys peptides and cytochrome *c*. This may contribute to our understanding of protein–protein binding and its effect on the functions of proteins. Apart from biological significance, aromatic ring stacking, which is strong in aqueous media, may be effective for fixing the structure or conformation of organic molecules in synthetic chemistry. A stacking-enhanced enantioselectivity has recently been reported for Cu(II)- and Ni(II)-catalyzed Diels–Alder reactions in water using complexes involving an aromatic amino acid, tyr or trp, as ligand, which stacks with the coordinated pyridine ring of a dienophile, protecting one side of this from the attack by the diene.[114]

It is by nature difficult to determine the mode and energy of weak interactions, but such interactions are ubiquitous and their effects have been recognized and reported explicitly or implicitly for selectivity and effeciency of reactions. They will be essential for understanding biological molecular recognition and specificity and will serve as attractive forces for construction of organized reaction systems from component molecules, which will open up a new field of chemistry.

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References

1 For reviews, see for example: a) B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, "Molecular Biology of the Cell," Garland Publishing, New York (1994), pp. 89–138; b) E. Frieden, *J. Chem. Educ.*, **52**, 754 (1975); c) H. A. Scheraga, *Acc. Chem. Res.*, **12**, 7 (1979); d) S. K. Burley and G. A. Petsko, *Science*, **229**, 23 (1985); e) S. K. Burley and G. A. Petsko, *Adv. Protein Chem.*, **39**, 125 (1988); f) "Molecular Recognition," ed by S. H. Gellman, *Chem. Rev.*, **97**(5) (1997).

2 a) C. Branden and J. Tooze, "Introduction to Protein Structure," Garland Publishing, 2nd ed, New York (1998); b) T. E. Creighton, "Proteins: Structures and Molecular Properties," 2nd ed, Freeman, New York (1993); c) W. Saenger, "Principles of Nucleic Acid Structure," Springer, Heidelberg (1984).

3 D. A. Dougherty, *Science*, **271**, 163 (1996); J. C. Ma and D. A. Dougherty, *Chem. Rev.*, **97**, 1303 (1997); J. P. Gallivan and D. A. Dougherty, *Proc. Natl. Acad. Sci. U. S. A.*, **96**, 9459 (1999).

4 M. Nishio, M. Hirota, and Y. Umezawa, "The CH-π Interactions," Wiley-VCH, New York (1998).

5 J. Miller, A. D. McLachlan, and A. Klug, *EMBO J.*, **4**, 1609 (1985); R. S. Brown, C. Sander, and P. Argos, *FEBS Lett.*, **186**, 271 (1985); J. M. Berg, *Met. Ions Biol. Syst.*, **25**, 235 (1989); A. Klug, *Nature*, **365**, 486 (1993).

6 N. P. Pavletich and C. O. Pabo, *Science*, **252**, 809 (1991); B. F. Luisi, W. X. Xu, Z. Otwinoski, L. P. Freedman, K. R. Yamamoto, and P. B. Sigler, *Nature*, **352**, 497 (1991).

7 a) B. Sarkar and T. P. A. Kruck, "The Biochemistry of Copper," ed by J. Peisach, P. Aisen, and W. E. Blumberg, Academic Press, New York (1966), p. 183; b) P. Z. Neumann and A. Sass-Kortsak, *J. Clin. Invest.*, **46**, 646 (1967).

8 O. Yamauchi, T. Sakurai, and A. Nakahara, *J. Am. Chem. Soc.*, **101**, 4164 (1979).

9 a) H. Sigel, *Angew. Chem., Int. Ed. Engl.*, **14**, 395 (1975); b) H. Sigel, in "Advances in Solution Chemistry," ed by I. Bertini, L. Lunazzi, and A. Dei, Plenum Press, New York (1981), p. 149; c) O. Yamauchi, *J. Mol. Catal.*, **23**, 255 (1983).

10 R. Griesser and H. Sigel, *Inorg. Chem.*, **10**, 2229 (1971), and papers cited therein; O. Yamauchi, H. Benno, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, **46**, 3458 (1973).

11 a) M. Tanaka, *J. Inorg. Nucl. Chem.*, **35**, 965 (1973); b) M. Tanaka, *J. Inorg. Nucl. Chem.*, **36**, 151 (1974).

12 M. Tabata and M. Tanaka, *Inorg. Chem.*, **27**, 3190 (1988).

13 O. Yamauchi, T. Takaba, and T. Sakurai, *Bull. Chem. Soc. Jpn.*, **53**, 106 (1980).

14 For reviews, see for example: J.-M. Lehn, *Angew. Chem., Int. Ed. Engl.*, **29**, 1304 (1990); J.-M. Lehn, "Supramolecular Chemistry: Concepts and Perspectives," VCH, Weinheim (1995); G. M. Whitesides, E. E. Simanek, J. P. Mathias, C. T. Seto, D. N. Chin, M. Mammen, and D. M. Gordon, *Acc. Chem. Res.*, **28**, 37 (1995); "Comprehensive Supramolecular Chemistry," ed by J. L. Atwood, J. E. D. Davis, D. D. MacNicol, F. Vögtle, and J.-M. Lehn, Pergamon, Oxford (1996), Vol. 9; M. C. T. Fyfe and J. F. Stoddart, *Acc. Chem. Res.*, **30**, 397 (1997); "Transition Metals in Supramolecular Chemistry," Perspectives in Supramolecular Chemistry, ed by J.-P. Sauvage, Wiley, New York (1999), Vol. 5; M. Munakata, L. P. Wu, and T. Kuroda-Sowa, *Adv. Inorg. Chem.*, **46**, 173 (1999); M. Fujita, *Acc. Chem. Res.*, **32**, 53 (1999); M. Fujita, *Struct. Bond.*, **96**, 177 (2000).

15 W. N. Lipscomb, G. N. Reeke, Jr., J. A. Hartsuck, F. A. Quiocho, and P. H. Bethge, *Phil. Trans. Roy. Soc.*, **B257**, 177 (1970); F. A. Quiocho and W. N. Lipscomb, *Adv. Protein Chem.*, **25**, 1 (1971); W. N. Lipscomb and N. Sträter, *Chem. Rev.*, **96**, 2375 (1996).

16 O. Yamauchi, Y. Nakao, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, **48**, 2572 (1975).

17 T. Sakurai, O. Yamauchi, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, **49**, 169 (1976).

18 W. J. Puspita, A. Odani, and O. Yamauchi, *J. Inorg. Biochem.*, **73**, 203 (1999).

19 H. C. Freeman, J. M. Guss, M. J. Healy, R.-P. Martin, C. E. Nockolds, and B. Sarkar, *J. Chem. Soc., Chem. Commun.*, **1969**, 225.

20 T. Ono, H. Shimanouchi, Y. Sasada, T. Sakurai, O. Yamauchi, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, **52**, 2229 (1979).

21 Y. Sasada, A. Takenaka, and T. Furuya, *Bull. Chem. Soc. Jpn.*, **56**, 1745 (1983).
22 T. Sakurai, O. Yamauchi, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, **49**, 1579 (1976).
23 J. M. Tsangaris and R. B. Martin, *J. Am. Chem. Soc.*, **92**, 4255 (1970); R. B. Martin, *Met. Ions Biol. Syst.*, **1**, 129 (1974).
24 O. Yamauchi and A. Odani, *J. Am. Chem. Soc.*, **103**, 391 (1981).
25 A. Odani and O. Yamauchi, *Bull. Chem. Soc. Jpn.*, **54**, 3773 (1981).
26 K. G. R. Pachler, *Spectrochim. Acta*, **20**, 581 (1964); J. Feeney, *J. Magn. Reson.*, **21**, 473 (1976).
27 G. Brookes and L. D. Pettit, *J. Chem. Soc., Dalton Trans.*, **1977**, 1918.
28 T. Sakurai, O. Yamauchi, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, **51**, 3203 (1978).
29 R. Tribolet, H. Sigel, and K. Trefzer, *Inorg. Chim. Acta*, **79**, 278 (1983).
30 a) T. Sakurai, O. Yamauchi, and A. Nakahara, *J. Chem. Soc., Chem. Commun.*, **1976**, 553; b) O. Yamauchi, T. Sakurai, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, **50**, 1776 (1977).
31 O. Yamauchi, A. Odani, and H. Masuda, *Inorg. Chim. Acta*, **198-200**, 749 (1992).
32 H. Masuda, A. Odani, T. Yamazaki, T. Yajima, and O. Yamauchi, *Inorg. Chem.*, **32**, 1111 (1993).
33 F. Zhang, T. Yajima, A. Odani, and O. Yamauchi, *Inorg. Chim. Acta*, **278**, 136 (1998).
34 W. J. Pupita, M. Suzuki, D. Inoue, A. Odani, O. Yamauchi, and T. Kiss, *Inorg. React. Mech.*, **2**, 137 (2000).
35 W. Bal, M. Dyba, F. Kasprzykowski, H. Kozlowski, R. Latajka, L. Lankiewicz, Z. Mackiewicz, and L. D. Pettit, *Inorg. Chim. Acta*, **283**, 1 (1998).
36 H. Kozlowski, W. Bal, M. Dyba, and T. Kowalik-Jankowska, *Coord. Chem. Rev.*, **184**, 319 (1999).
37 N. Ito, S. E. V. Phillips, C. Stevens, Z. B. Ogel, M. J. McPherson, J. N. Keen, K. D. S. Yadav, and P. F. Knowles, *Nature*, **350**, 87 (1991); N. Itoh, S. E. V. Phillips, K. D. S. Yadav, and P. F. Knowles, *J. Mol. Biol.*, **238**, 794 (1994).
38 T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, and S. Yoshikawa, *Science*, **269**, 1069 (1995); S. Yoshikawa, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, E. Yamashita, N. Inoue, M. Yao, M. J. Fei, C. P. Libeu, T. Mizushima, H. Yamaguchi, T. Tomizaki, and T. Tsukihara, *Science*, **280**, 1723 (1998); S. Iwata, C. Ostermeier, B. Ludwig, and H. Michel, *Nature*, **376**, 660 (1995).
39 H. Pelletier and J. Kraut, *Science*, **258**, 1748 (1992).
40 C. A. Hunter and J. K. M. Sanders, *J. Am. Chem. Soc.*, **112**, 5525 (1990); C. A. Hunter, *Angew. Chem., Int. Ed. Engl.*, **32**, 1584 (1993).
41 O. Yamauchi, in "Handbook of Metal–Ligand Interactions in Biological Fluids," ed by G. Berthon, Dekker, New York (1995), Vol. 1, p. 583.
42 H. Sigel, in "Coordination Chemistry-20," ed by D. Banerjea, Pergamon, Oxford (1980), p. 27; H. Sigel, *Pure Appl. Chem.*, **61**, 923 (1989); H. Sigel, *Coord. Chem. Rev.*, **144**, 287 (1995).
43 R. B. Gómez-Coca, L. E. Kapinos, A. Holy, R. A. Vilaplana, F. Gonzáles-Vilchez, and H. Sigel, *J. Inorg. Biochem.*, **84**, 39 (2001), and references cited therein.
44 B. E. Fischer and H. Sigel, *J. Am. Chem. Soc.*, **102**, 2998 (1980).
45 H. Sigel, R. Tribolet, and O. Yamauchi, *Comments Inorg. Chem.*, **9**, 305 (1990).
46 O. Yamauchi and A. Odani, *J. Am. Chem. Soc.*, **107**, 5938 (1985).
47 a) O. Yamauchi and A. Odani, *Inorg. Chim. Acta*, **100**, 165 (1985); b) O. Yamauchi and A. Odani, *Nippon Kagaku Kaishi*, **1988**, 369.
48 T. Sugimori, H. Masuda, N. Ohata, K. Koiwai, A. Odani, and O. Yamauchi, *Inorg. Chem.*, **36**, 576 (1997).
49 F. Zhang, A. Odani, H. Masuda, and O. Yamauchi, *Inorg. Chem.*, **35**, 7148 (1996).
50 F. Zhang, T. Yajima, H. Masuda, A. Odani, and O. Yamauchi, *Inorg. Chem.*, **36**, 5777 (1997).
51 H. Masuda, T. Sugimori, A. Odani, and O. Yamauchi, *Inorg. Chim. Acta*, **180**, 73 (1991).
52 a) K. Aoki and H. Yamazaki, *J. Chem. Soc., Dalton Trans.*, **1987**, 2017; b) H. Masuda, O. Matsumoto, A. Odani, and O. Yamauchi, *Nippon Kagaku Kaishi*, **1988**, 783.
53 O. Yamauchi, A. Odani, T. Kohzuma, H. Masuda, K. Toriumi, and K. Saito, *Inorg. Chem.*, **28**, 4066 (1989).
54 T. Sugimori, K. Shibakawa, H. Masuda, A. Odani, and O. Yamauchi, *Inorg. Chem.*, **32**, 4951 (1993); corrections, *Inorg. Chem.*, **33**, 3848 (1994).
55 a) M. C. Lim, E. Sinn, and R. B. Martin, *Inorg. Chem.*, **15**, 807 (1976); b) C. J. Simmons, M. Lundeen, and K. Seff, *Inorg. Chem.*, **17**, 1429 (1978).
56 R. B. Martin and H. Sigel, *Comments Inorg. Chem.*, **6**, 285 (1988).
57 C. F. Naumann and H. Sigel, *J. Am. Chem. Soc.*, **96**, 2750 (1974).
58 T. Sugimori, H. Masuda, and O. Yamauchi, *Bull. Chem. Soc. Jpn.*, **67**, 131 (1994).
59 A. Odani, S. Deguchi, and O. Yamauchi, *Inorg. Chem.*, **25**, 62 (1986).
60 C. E. Johnson, Jr. and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).
61 D. van der Helm and C. E. Tatsch, *Acta Crystallogr., Sect. B*, **B28**, 2307 (1972).
62 M. B. Hursthouse, S. A. A. Jayaweera, H. Milburn, and A. Quick, *J. Chem. Soc., Dalton Trans.*, **1975**, 2569.
63 R. B. Martin, *Met. Ions Biol. Syst.*, **9**, 1 (1979).
64 G. Arena, R. Cali, V. Cucinotta, S. Musumeci, E. Rizzarelli, and S. Sammartano, *J. Chem. Soc., Dalton Trans.*, **1983**, 1271; G. Borghesani, F. Pulidori, M. Remelli, R. Purrello, and E. Rizzarelli, *J. Chem. Soc., Dalton Trans.*, **1990**, 2095.
65 H. Masuda, N. Fukushima, T. Sugimori, and O. Yamauchi, *J. Inorg. Biochem.*, **51**, 158 (1993).
66 O. Yamauchi, K. Tsujide, and A. Odani, *J. Am. Chem. Soc.*, **107**, 659 (1985).
67 R. J. W. Hefford and L. D. Pettit, *J. Chem. Soc., Dalton Trans.*, **1981**, 1331.
68 P. I. Vestues and R. B. Martin, *J. Am. Chem. Soc.*, **102**, 7906 (1980).
69 A. Odani and O. Yamauchi, *Inorg. Chim. Acta*, **93**, 13 (1984).
70 O. Yamauchi, M. Takani, K. Toyoda, and H. Masuda, *Inorg. Chem.*, **29**, 1856 (1990).
71 a) M. Takani, H. Masuda, and O. Yamauchi, *Inorg. Chim. Acta*, **235**, 367 (1995); b) N. V. Kaminskaia, G. M. Ullmann, D. B. Fulton, and N. M. Kostic, *Inorg. Chem.*, **39**, 5004 (2000).
72 Y. Shimazaki, H. Yokoyama, and O. Yamauchi, *Angew. Chem., Int. Ed. Engl.*, **38**, 2401 (1999).

73 W. S. Striejewske and R. R. Conry, *Chem. Commun.*, **1998**, 555; R. R. Conry, W. S. Striejewske, and A. A. Tipton, *Inorg. Chem.*, **38**, 2833 (1999).

74 S. D. Zaric, D. M. Popovic, and E.-W, Knapp, *Chem. Eur. J.*, **6**, 3935 (2000).

75 J. K. Barton and S. J. Lippard, in "Metal Ions in Biology," ed by T. G. Spiro, Wiley-Interscience, New York (1980), Vol. 1, p. 31; A. M. Pyle and J. K. Barton, *Prog. Inorg. Chem.*, **38**, 413 (1990); J. K. Barton, in "Bioinorganic Chemistry," ed by I. Bertini, H. B. Gray, S. J. Lippard, and J. S. Valentine, University Science Books, Mill Valley (1994), p. 455; B. Nordén, P. Lincoln, B. Åkerman, and E. Tuite, *Met. Ions Biol. Syst.*, **33**, 177 (1996).

76 O. Yamauchi, A. Odani, H. Masuda, and H. Sigel, *Met. Ions Biol. Syst.*, **32**, 207 (1996).

77 O. Yamauchi, A. Odani, R. Shimata, and Y. Kosaka, *Inorg. Chem.*, **25**, 3337 (1986).

78 A. Odani, R. Shimata, H. Masuda, and O. Yamauchi, *Inorg. Chem.*, **30**, 2133 (1991).

79 A. Odani, T. Sekiguchi, H. Okada, S. Ishiguro, and O. Yamauchi, *Bull. Chem. Soc. Jpn.*, **68**, 2093 (1995).

80 H. Masuda and O. Yamauchi, *Inorg. Chim. Acta*, **136**, L29 (1987).

81 O. Yamauchi, A. Odani, R. Shimata, and S. Ishiguro, *Recl. Trav. Chim. Pays-Bas*, **106**, 196 (1987).

82 A. Odani, H. Masuda, O. Yamauchi, and S. Ishiguro, *Inorg. Chem.*, **30**, 4484 (1991).

83 G. Arena, R. Cali, V. Cucinotta, S. Musumeci, E. Rizzarelli, and S. Sammartano, *J. Chem. Soc., Dalton Trans.*, **1984**, 1651.

84 L. Lomozik, A. Odani, and O. Yamauchi, *Inorg. Chim. Acta*, **219**, 107 (1994).

85 a) P. M. Colman, H. C. Freeman, J. M. Guss, M. Murata, V. A. Norris, J. A. M. Ramshaw, and M. P. Venkatappa, *Nature*, **272**, 319 (1978). b) H. C. Freeman, in "Coordination Chemistry-21," ed by J. L. Laurent, Pergamon Press, Oxford (1981), pp. 29–51. c) J. M. Guss, P. R. Harrowell, M. Murata, V. A. Norris, and H. C. Freeman, *J. Mol. Biol.*, **192**, 361 (1986). d) H. Sugawara, T. Inoue, C. Li, M. Gotowda, T. Hibino, T. Takabe, and Y. Kai, *J. Biochem.*, **125**, 899 (1999).

86 S. E. Martinez, D. Huang, A. Szczepaniak, W. A. Cramer, and J. L. Smith, *Structure*, **2**, 95 (1994).

87 See for example: B. H. Lee, T. Hibino, T. Takabe, P. J. Weisbeek, and T. Takabe, *J. Biochem.*, **117**, 1209 (1995); R. V. Roberts, H. C. Freeman, A. J. Olson, J. A. Tainer, and E. D. Getzoff, *J. Biol. Chem.*, **266**, 13431 (1991); E. L. Gross, *Photosynth. Res.*, **37**, 103 (1993); L. Qin and N. M. Kostic, *Biochemistry*, **31**, 5145 (1992).

88 S. Hirota and O. Yamauchi, *Chem. Rec.*, **1**, in press (2001).

89 S. Hirota, K. Hayamizu, M. Endo, T. Hibino, T. Takabe, T. Kohzuma, and O. Yamauchi, *J. Am. Chem. Soc.*, **120**, 8177 (1998).

90 S. Hirota, M. Endo, K. Hayamizu, T. Tsukazaki, T. Takabe, T. Kohzuma, and O. Yamauchi, *J. Am. Chem. Soc.*, **121**, 849 (1999).

91 S. K. Chapman, C. V. Knox, and A. G. Sykes, *J. Chem. Soc., Dalton Trans.*, **1984**, 2775.

92 D. Beoku-Betts, S. K. Chapman, C. V. Knox, and A. G. Sykes, *Inorg. Chem.*, **24**, 1677 (1985).

93 S. K. Chapman, A. D. Watson, and A. G. Sykes, *J. Chem. Soc., Dalton Trans.*, **1983**, 2543.

94 S. K. Chapman, I. Sanemasa, and A. G. Sykes, *J. Chem. Soc., Dalton Trans.*, **1983**, 2549.

95 S. Hirota, M. Endo, T. Tsukazaki, T. Takabe, and O. Yamauchi, *J. Biol. Inorg. Chem.*, **3**, 563 (1998).

96 E. I. Solomon, M. J. Baldwin, and M. D. Lowery, *Chem. Rev.*, **92**, 521 (1992).

97 S. Hirota, K. Hayamizu, T. Okuno, M. Koshi, H. Iwasaki, T. Kondo, T. Hibino, T. Takabe, T. Kohzuma, and O. Yamauchi, *Biochemistry*, **39**, 6357 (2000).

98 S. Hirota, K. Hayamizu, T. Takebe, and O. Yamauchi, *Inorg. React. Mech.*, **2**, 113 (2000).

99 M. M. Crnogorac, C. Shen, S. Young, Ö. Hansson, and N. M. Kostic, *Biochemistry*, **35**, 16465 (1996).

100 M. M. Ivkovic-Jensen and N. M. Kostic, *Biochemistry*, **35**, 15095 (1996).

101 L. Qin and N. M. Kostic, *Biochemistry*, **32**, 6073 (1993).

102 G. M. Ullmann and N. M. Kostic, *J. Am. Chem. Soc.*, **117**, 4766 (1995).

103 G. M. Ullmann, E.-W. Knapp, and N. M. Kostic, *J. Am. Chem. Soc.*, **119**, 42 (1997).

104 S. Hirota, T. Tsukazaki, and O. Yamauchi, *Biochem. Biophys. Res. Commun.*, **268**, 395 (2000).

105 S. He, S. Modi, D. S. Bendall, and J. C. Gray, *EMBO J.*, **10**, 4011 (1991).

106 S. Modi, M. Nordling, L. G. Lundberg, Ö. Hansson, and D. S. Bendall, *Biochim. Biophys. Acta*, **1102**, 85 (1992).

107 S. Modi, S. He, J. C. Gray, and D. S. Bendall, *Biochim. Biophys. Acta*, **1101**, 64 (1992).

108 N. Ohata, H. Masuda, and O. Yamauchi, *Kobunshi Ronbunshu*, **57**, 167 (2000).

109 D. M. Salunke and M. Vijayan, *Int. J. Peptide Protein Res.*, **18**, 348 (1981).

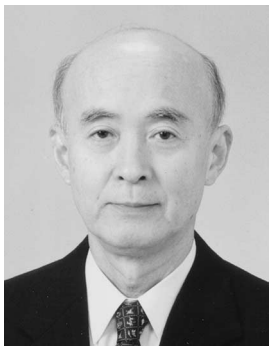
110 N. Ohata, H. Masuda, and O. Yamauchi, *Angew. Chem., Int. Ed. Engl.*, **35**, 531 (1996).

111 N. Ohata, H. Masuda, and O. Yamauchi, *Inorg. Chim. Acta*, **286**, 37 (1999).

112 N. Ohata, H. Masuda, and O. Yamauchi, *Inorg. Chim. Acta*, **300-302**, 749 (2000).

113 T. Kohzuma, T. Inoue, F. Yoshizaki, Y. Sasakawa, K. Onodera, S. Nagatomo, T. Kitagawa, S. Uzawa, Y. Isobe, Y. Sugimura, M. Gotowda, and Y. Kai, *J. Biol. Chem.*, **274**, 11817 (1999).

114 S. Otto and J. B. F. N. Engberts, *J. Am. Chem. Soc.*, **121**, 6798 (1999).



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