DALTON PERSPECTIVE

Metal–amino acid chemistry. Weak interactions and related functions of side chain groups

Osamu Yamauchi,*[a] Akira Odani[b] and Masako Takani[c]

[a] *Faculty of Engineering, Kansai University, Yamate-cho, Suita, Osaka 564-8680, Japan. E-mail: osamuy@ipcku.kansai-u.ac.jp*
[b] *Research Center for Materials Science, Nagoya University, Chikusa-ku, Nagoya 464-8602, Japan*
[c] *Faculty of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan*

Received 6th March 2002, Accepted 23rd July 2002
First published as an Advance Article on the web 19th August 2002

α-Amino acids are highly functional small molecules whose side chain groups are like prototypes of metal coordination and weak interactions in proteins. This perspective focuses on non-covalent or weak interactions of the side chain groups of amino acids, such as the charged groups of arginine and aspartic acid and the aromatic rings of tyrosine and tryptophan, in metal complexes in solution and in the solid state. The structure and function of small complexes exhibiting weak interactions and their biological relevance are described.

Introduction

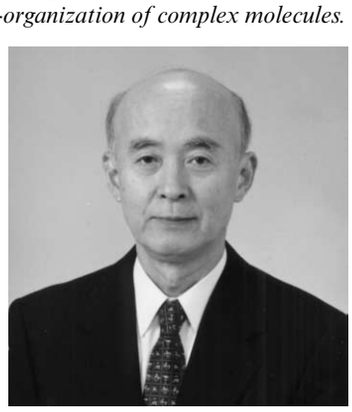
α-Amino acids as protein constituents are small molecules with various functional groups. They are excellent metal complexing agents forming chelates through the amino and carboxylato groups.[1–3] In addition they often have a side chain with a metal binding group, such as the imidazole group of histidine (his), the side chain carboxylato group of aspartate (asp) and glutamate (glu), and the phenol ring of tyrosine (tyr), which serve as the metal binding sites in proteins.[1–4]

Amino acid complexes of copper(II) and zinc(II) are known to be important for metal ion transport in blood. A ternary Cu(II) complex, Cu(his)(thr) (thr = threonine), was isolated from human blood serum by Sarkar *et al.*,[5] and tracer studies indicated that ternary Cu(II)–amino acid complexes composed of his and asparagine (asn), glutamine (gln), or thr are preferentially formed in blood plasma.[6] Computer simulation of the

Osamu Yamauchi was born in 1936 in Nagoya. He received his doctor's degree in pharmaceutical sciences from Kyoto University and was appointed Associate Professor at Osaka University in 1967. In 1980 he was promoted to Professor at the Faculty of Pharmaceutical Sciences, Kanazawa University, and moved to the Department of Chemistry, Nagoya University, in 1987. He retired from Nagoya University in 2000 and is now Professor at Kansai University and Professor Emeritus of Nagoya University. His research interests include solution equilibria and weak interactions involving metal–amino acid and related complexes, peptide–metalloprotein adduct formation, chemical models of metalloenzyme active sites and self-organization of complex molecules.

Akira Odani was born in Kyoto in 1953. He received his doctor's degree in pharmaceutical sciences from Osaka University in 1981. He joined Professor Osamu Yamauchi's group at Kanazawa University as Assistant Professor in 1981 and moved to the Department of Chemistry, Nagoya University, in 1987. He was promoted to Associate Professor in 1996 and is now an Associate Professor of the Research Center for Materials Science, Nagoya University. His research interests include the solution chemistry of metal–biomolecule complexes, weak interactions in ternary metal complexes involving amino acids and other biomolecules, metal-phytate chemistry and the reactivities of platinum complexes.

Masako Takani was born in Komatsu outside Kanazawa and received her doctor's degree in pharmaceutical sciences from the University of Tokyo in 1972. She worked on natural products chemistry with Professor Kotaro Takahashi of Kanazawa University and was promoted to Lecturer in 1975. She joined Professor Osamu Yamauchi's group in 1980 and is now an Associate Professor of the Faculty of Pharmaceutical Sciences, Kanazawa University. Her research interests include natural products chemistry, metal–lichen chemistry, metal complexes of indole-containing ligands and weak interactions in biomolecule–platinum(II) complex systems.



Osamu Yamauchi

Akira Odani

Masako Takani

DOI: 10.1039/b202385g

species distributions in model systems involving several metal ions and amino acids as well as other small ligands supported the preferred formation of his-containing ternary complexes.[7–9] The strong chelating properties of his and D-penicillamine (= β,β-dimethylcysteine) have been used for the treatment of genetic disorders of copper transport; Menkes and Wilson diseases, respectively: On the basis of the chemical and biological studies of copper transport in blood serum, Sarkar *et al.* successfully developed a copper(II)–his formulation for the treatment of Menkes disease.[10]

The biological activity of the modified side chain groups of some amino acid residues located close to the metal center, such as trihydroxyphenylalanine (topa) quinone of copper amine oxidase[11–13] formed by post-translational modification and the tyr phenoxyl radical of galactose oxidase,[13–15] have been disclosed to be essential for the functions of metalloenzymes.

Of particular significance in bioinorganic chemistry are hydrogen bonds, aromatic–aromatic interactions, and other non-covalent interactions (weak interactions) involving the amino acid side chain groups, which are important for biological molecular recognition, reactivity of enzymes, and stabilization of protein structures[16–18] especially in the presence of metal ions. In addition to these interactions, cation–π,[19–21] CH–π,[22] and NH–π[23,24] interactions have recently attracted much attention due to their relevance to biological functions. The charged or polar side chain groups of arginine (arg), lysine (lys), and other residues of the finger tips of zinc finger proteins play an important role in the specific interaction with DNA.[25,26] The substrate specificity of metalloenzymes owes much to weak interactions between the substrate molecule and the enzyme active site and steric factors affecting them. Fig. 1 illustrates

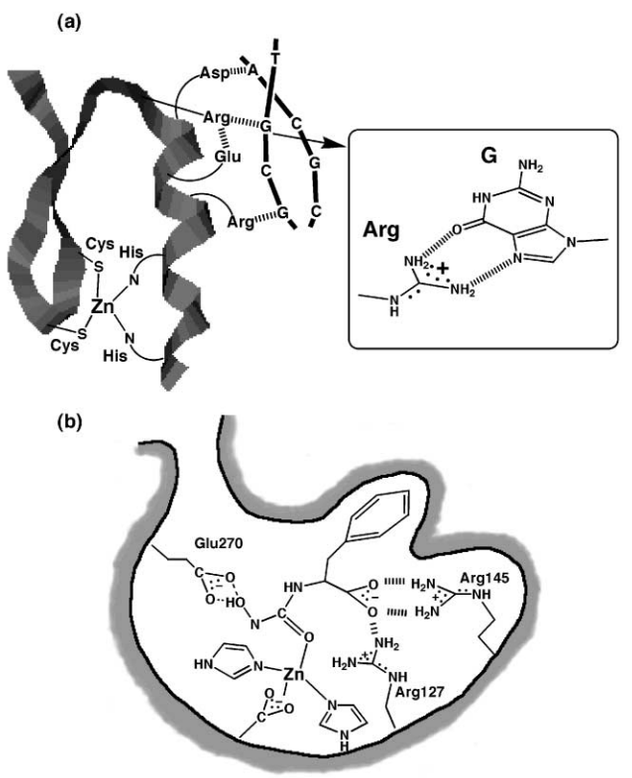


Fig. 1 Weak interactions in zinc finger protein–DNA[27] and carboxypeptidase A–inhibitor peptide[29] bindings.

typical examples of weak interactions around the metal center in zinc finger protein–DNA[27] and carboxypeptidase A–inhibitor peptide bindings.[28,29] Ligand–ligand interactions in metal complexes containing two different ligands such as amino acids, *i.e.* ternary metal complexes, may be regarded as a prototype of enzyme–substrate and other intermolecular interactions.[30–32] Structural, spectroscopic, and thermodynamic studies have been performed for such models and are expected to provide insights into small molecule–protein and protein–protein recognitions at the molecular level, and by incorporating the essential part of amino acid side chains into ligands we may be able to develop various specific chemical systems.

The chemistry of metal–amino acid complexes has long been studied in detail, and the structures and solution equilibria are well established.[1–3,33] This short perspective is intended to focus on intra- and inter-molecular interactions involving the side chain groups of amino acids and their derivatives in complexes such as MAB (M = Cu(II), Pd(II), *etc.*; A and B = amino acids, peptides, *etc.*), their effects on structures and properties, and possible relevance to biological systems.

Abbreviations

Abbreviations used in the text are as follows. Amino acids and peptides refer to the L-enantiomers unless otherwise stated. ala, alanine; ampy, 2-aminomethylpyridine; arg, arginine; arg·asp, arginylaspartate; arg·glu, arginylglutamate; asn, asparagine; asn· ser·phe·arg·tyr-NH_2, asparaginylserylphenylalanylarginyltyrosineamide; asp, aspartate; bphen, 4,7-diphenyl-1,10-phenanthroline-4′,4″-disulfonate; bpy, 2,2′-bipyridine; cit, citrulline; cys, cysteine; $cySO_3H$, cysteinesulfonate; cyt *c*, cytochrome *c*; cyt *f*, cytochrome *f*; dopa, 3,4-dihydroxyphenylalanine; edma, ethylenediamine-*N*,*N*′-diacetate; en, ethylenediamine; gln, glutamine; glu, glutamate; gly, glycine; gly·gly, glycylglycine; gly·tyr, glycyltyrosine; his, histidine; *cyclo*-his·his, *cyclo*-histidylhistidine; hisOMe, histidine methylester; hista, histamine; htrp, 5-hydroxytryptophan; I_2tyr, 3,5-diiodotyrosine; I_2tyrO, 3,5-diiodotyrosine with deprotonated phenol OH; leu, leucine; lys, lysine; lys·asp, lysylaspartic acid; mba, benzene-2,4-dicarboxylate; MI, 2-methylindole; mphe, methyl-substituted phenylalanine; NH_2phe, *p*-aminophenylalanine; orn, ornithine; PC, plastocyanin; phe, phenylalanine; phen, 1,10-phenanthroline; Pser, phosphoserine; Ptyr, phosphotyrosine; ser, serine; thr, threonine; trp, tryptophan; trp·gly, tryptophylglycine; tyr, tyrosine; tyrO, tyrosine with deprotonated phenol OH; tyr·gly, tyrosylglycine.

Mixed ligand complex formation

Amino acids normally coordinate to metal ions through the amino and carboxylato groups, and when the side chain has a functional group, it may provide an additional metal binding site or undergo various weak interactions with the other interacting groups. The central metal ion of mixed ligand complexes serves as a template for intermolecular interactions between coordinated ligands, exerts electronic effects on the coordinated molecules, and provides spectroscopic and thermodynamic information on the coordination structures, side chain conformations, and energy of interactions. Ligand–ligand interactions may be classified into (i) through-metal electronic and (ii) through-space non-covalent interactions.[31,32,34] Among the through-space interactions relevant to amino acid side chains R (Fig. 2) are hydrogen bonds involving polar or oppositely charged side groups and aromatic ring stacking interactions between a side chain aromatic ring and a coordinated aromatic ligand such as bpy.[32]

Attractive ligand–ligand interactions favor the formation of mixed ligand complexes, giving rise to ligand selectivity or a preferred combination of ligands around a metal ion.[31,32] Mixed ligand complex formation in ternary systems is defined by the following equation (charges are omitted for clarity):

$$pM + qA + rB + sH \underset{}{\overset{\beta_{pqrs}}{\rightleftharpoons}} M_pA_qB_rH_s \qquad (1)$$

$$\beta_{pqrs} = [M_pA_qB_rH_s]/[M]^p[A]^q[B]^r[H]^s$$

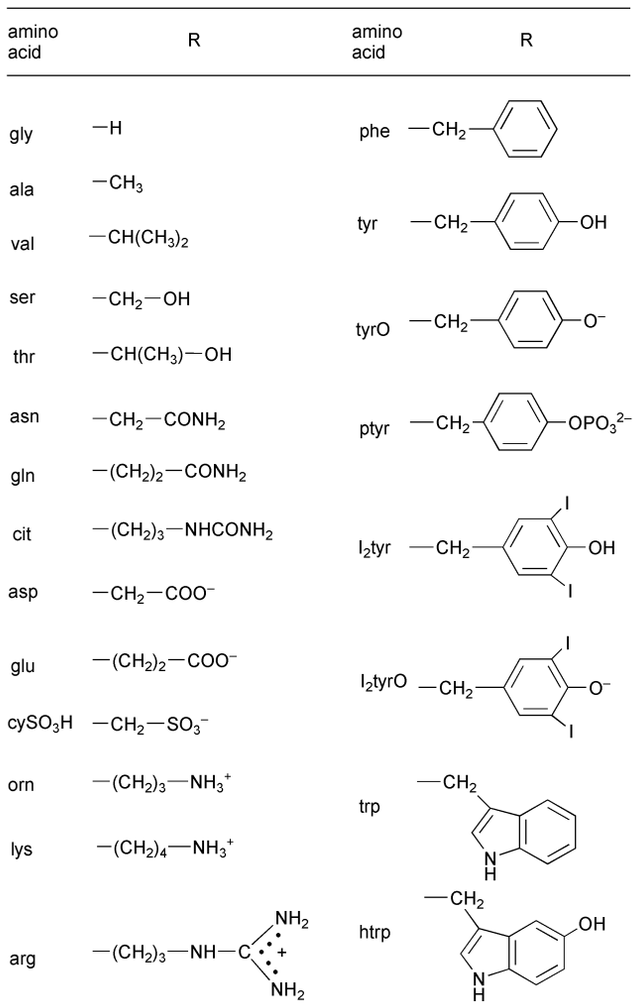


Fig. 2 Amino acid side chains, R, with interacting groups.

where β_{pqrs} and H refer to the overall stability constant and proton, respectively, and p, q, r, and s are the stoichiometric numbers of M, A, B, and H in complexes, respectively. Ligand–ligand interactions have been considered mostly for the ternary systems involving complex species MA, MA_2, MB, MB_2, MAB, and their protonated or hydroxo species. Complex MAB is stabilized relative to the parent binary complexes, MA_2 and MB_2, due to the statistical effect,[35] and weak interactions between the side chain groups of A and B further stabilize MAB.

There are various ways of evaluating stabilization of ternary complexes due to ligand–ligand interactions. One of them is to calculate the equilibrium constant K for a hypothetical equilibrium describing formation of the complex species with ligand–ligand interactions, MAB, from species of comparable structures but without such interactions, MAB′ and MA′B:[36,37]

$$\mathrm{MAB'} + \mathrm{MA'B} \overset{K}{\rightleftharpoons} \mathrm{MAB} + \mathrm{MA'B'} \qquad (2)$$

where A′ and B′ have similar coordinating groups but are devoid of interacting groups and therefore ligand–ligand interactions are possible only in MAB (Fig. 3). The log K value is given by the difference between the sums of the log overall stability constants of the complexes of the right hand side and of the left hand side:

$$\log K = (\log \beta_{\mathbf{MAB}} + \log \beta_{\mathbf{MA'B'}}) - (\log \beta_{\mathbf{MAB'}} + \log \beta_{\mathbf{MA'B}}) \qquad (3)$$

where $\log \beta_{\mathbf{MAB}}$ *etc.* denote the log β_{1110} values for respective complexes. When MAB is stabilized relative to the other

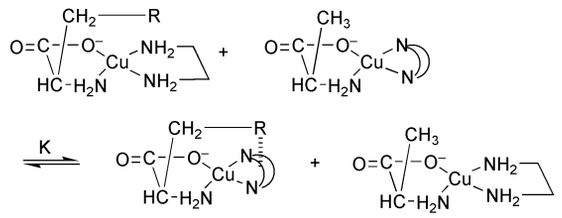


Fig. 3 Schematic presentation of the hypothetical equilibrium showing formation of the complex species with ligand–ligand interactions.

species, equilibrium (2) shifts to the right hand side, giving a log K value greater than 0. MAB itself is in equilibrium with the interacting form MAB_{on} and the non-interacting form MAB_{off} (Fig. 4), and the fractional population of MAB_{on}, P_{on}, is defined as follows:[38]

$$P_{on} = [\mathrm{MAB_{on}}]/([\mathrm{MAB_{on}} + [\mathrm{MAB_{off}}]) \qquad (4)$$

The log K value [eqn. (3)] is shown to be related to P_{on} by the following relationship:[37]

$$P_{on} = 1 - 1/K \qquad (5)$$

Therefore, it is possible to calculate the population of the intramolecularly interacting species from the log K value.

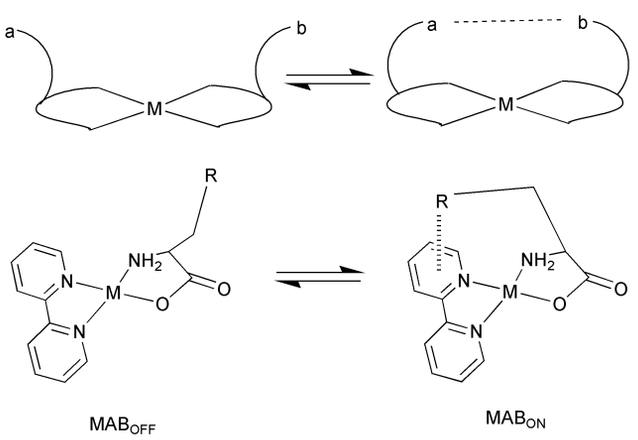


Fig. 4 Equilibrium between the interacting and non-interacting forms, MAB_{on} and MAB_{off}, respectively.

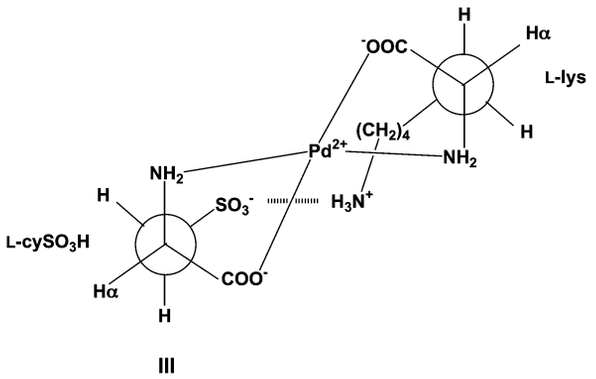
Circular dichroism and ^{1}H NMR spectra

Ligand–ligand interactions in MAB cause the side chains of coordinated A and B to be fixed in certain conformations, which may be reflected on the circular dichroism (CD) spectrum of the complex. Cu(II), Ni(II), Pd(II), and other metal complexes of optically active amino acids and peptides exhibit CD spectra in the d–d region due to the vicinal effect. The CD magnitudes ($\Delta\varepsilon$) for Cu(II)– and Ni(II)–peptide complexes have been reported to be an additive function of the magnitudes for the component amino acid complexes.[39,40] We found that this additivity holds also for ternary complexes MAB with similar donor sets and that the magnitude $\Delta\varepsilon_{calcd}$ can be estimated from the values for the binary complexes by eqn. (6):[30,41–43]

$$\Delta\varepsilon_{calcd} = (\Delta\varepsilon_{\mathrm{M(L\text{-}A)_2}} + \Delta\varepsilon_{\mathrm{M(L\text{-}B)_2}})/2 \qquad (6)$$

where $\Delta\varepsilon_{\mathrm{M(L\text{-}A)_2}}$ and $\Delta\varepsilon_{\mathrm{M(L\text{-}B)_2}}$ are the CD magnitudes observed for the optically active binary complexes at an appropriate wavelength. When there are side chain–side chain interactions or metal ion–side chain interactions, the observed magnitude of $\Delta\varepsilon$ deviates from $\Delta\varepsilon_{calcd}$, and the relative magnitude, $\Delta\varepsilon/\Delta\varepsilon_{calcd}$, serves as an indication of weak interactions.[32,44,45]

The ^{1}H NMR spectra of coordinated α-amino acids are known to give the populations of three staggered rotational isomers around the C_α–C_β bond and are thus expected to provide substantial information on side chain conformational changes due to ligand–ligand interactions. Rotamer populations can be estimated from the coupling constants J.[46,47] The population of the rotamer favoring side chain–side chain interactions (Fig. 5) increases relative to the other rotamers in



III

Fig. 5 Rotamer favoring ligand–ligand interactions as exemplified for Pd(L-cySO_3H)(L-lys).[44]

systems such as Pd(cySO_3H)(lys), where hydrogen bonds were concluded to be formed between the cySO_3H sulfonate and lys ammonium groups.[44] ^{1}H NMR spectra were used for calculating P_{on} for stacked species from the chemical shifts for solutions with fast chemical exchange.[48] Stabilization of ternary complexes is estimated by the P_{on} value obtained by ^{1}H NMR measurements, where the observed spectral parameters are weighted averages of those for the 'MAB_{on}' and 'MAB_{off}' species (Fig. 4); for the Pd(da)(dipeptide) systems with aromatic ring stacking (da = bpy, phen, *etc.*; dipeptide = tyr·gly, trp·gly, *etc.* coordinated as bidentate ligands through the amino and deprotonated peptide nitrogens), P_{on} was calculated from the observed upfield shifts, $\Delta\delta$, of the aromatic proton signals due to the ring current effect and the shifts for 100% stacking, $\Delta\delta_{calcd}$, which were estimated from the Johnson–Bovey diagram[49] (Fig. 6), by the relationship $P_{on} = \Delta\delta/\Delta\delta_{calcd}$.[50] The

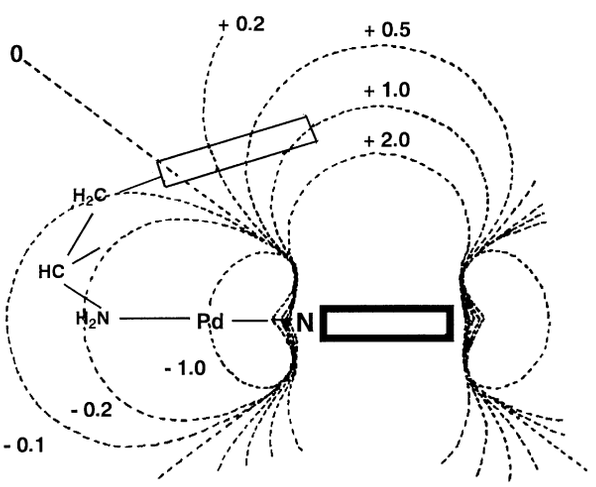


Fig. 6 Johnson–Bovey diagram drawn for Pd(bpy)(tyr·gly).[49,50]

P_{on} values for Pd(bpy)(tyr·gly) and Pd(bphen)(trp·gly) were found to be 0.73 and 0.98, respectively, indicating that the side chain aromatic ring in each complex is mostly stacked with da. This was confirmed by the crystal structure analysis of Pd(bpy)(tyr·gly).[51] In this connection, a downfield shift due to the aromatic ring was detected for the ε-methylene group of lys in Pd(I_2tyrO)(lys), which was interpreted according to the Johnson–Bovey diagram to support the intramolecular lys ε-ammonium···I_2tyrO hydrogen bond.[52]

Hydrogen bonds between ligand side chain groups

Hydrogen bonds are directed along the line connecting two electronegative atoms separated by 2.7–3.5 Å and are therefore rather specific and have been utilized for the construction of self-organized structures.[53,54] Polar or charged amino acid side chain groups, such as hydroxyl, amide, guanidinium, and ω-ammonium groups, are involved in hydrogen bonds with strongly electronegative atoms, and the peptide groups, –CO–NH–, in proteins are hydrogen bonded to each other.[17,18] The arg···asp (or glu) hydrogen bonds are well established in proteins and in their salts in the solid state.[55–57]

Hydrogen bonds near the metal center of a metalloenzyme were first revealed by the X-ray crystal structure analysis of a zinc enzyme reaction intermediate, carboxypeptidase A–gly·tyr adduct, where the guanidinium group of Arg145 binds with the carboxylato group of a model substrate, gly·tyr.[28,58,59] Cu,Zn-superoxide dismutase (Cu,Zn-SOD) binds a superoxide ion by coordination to the Cu center and a hydrogen bond with the neighboring arg guanidinium group.[60,61] Arg itself as a substrate of nitric oxide synthase is bound close to the heme site by hydrogen bonds.[62]

The side chain groups of ternary metal complexes involving these and related amino acid pairs within the complex molecule may form intramolecular hydrogen bonds. Ternary Cu(II) systems involving an acidic and a basic amino acid, Cu(A)(B) (A = asp or glu; B = protonated forms of arg, lys, or orn), exhibited a strongly enhanced CD magnitude in the d–d region as compared with that estimated from the magnitude additivity [eqn. (6)].[32,42,43] For example, a 1 : 1 : 1 Cu(L-asp)(L-arg) system with oppositely charged side chains exhibited a relative magnitude of 1.4 in water, whereas 1 : 1 : 1 Cu(L-asp)(L-ala) which is devoid of one of the interacting groups showed a normal magnitude ($\Delta\varepsilon/\Delta\varepsilon_{calcd} \approx 1$).[42] The anomaly has been ascribed to the electrostatic interactions or hydrogen bonds between the oppositely charged side chain groups of A and B (Fig. 4), which increase the asymmetry of the α-carbons by fixing the side chain conformations. The stability constants for the Cu(L- or D-A)(L-B) systems (A = ala, asp, or glu; B = lys or orn) at 25 °C [I = 0.1 M (KNO_3)] showed that the stability difference, $\log \beta_{1111} - \log \beta_{1110}$, which corresponds to the deprotonation of the side chain ammonium group of B, was greater for A = asp and glu than for A = ala by 0.6–0.9 log units, indicating that the side chain–side chain interactions as depicted in Fig. 3 stabilize the protonated species 1111.[63] Stability increases due to electrostatic interactions have been detected for systems with various A and B.[45,64] X-Ray structural analysis of Cu(edma)(L-arg) showed that two intermolecular hydrogen bonds are formed between the arg guanidinium group of a complex molecule and the axially coordinated edma carboxylate moiety of a neighboring molecule (Fig. 7).[64] The result suggests that the intermolecular bonding may be necessary for the crystal growth but that in dilute solution there could be intramolecular hydrogen bonds between these groups. The stability enhancements in the systems containing phosphorylated tyrosine (Ptyr) or serine (Pser), Cu(Ptyr or Pser)(arg or lys), show that Ptyr and Pser with two negative charges are better hydrogen bond acceptors than tyr, tyrO, and ser.[45] The arg guanidinium group interacts more effectively with a carboxylato group than the lys ammonium group does.

Pettit and Brookes detected an increase of the $\log \beta_{1111}$ values for Cu(L-his)(L-B) as compared with those for Cu(D-his)(L-B) (B = protonated arg, lys, or orn) and concluded this to be due to electrostatic interactions between the his carboxylate and the positively charged side chain of B in Cu(L-his)(L-B),[65] on the assumption that the complexes have the *cis* configuration with respect to the coordinated amino groups (Fig. 8) as revealed

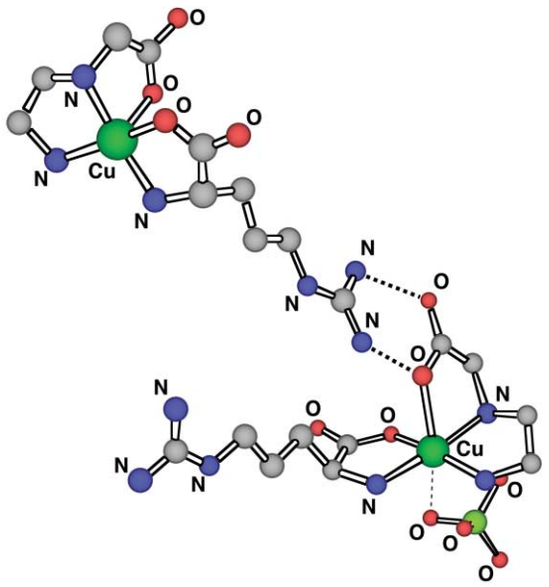


Fig. 7 Structure of [Cu(edma)(L-arg)]ClO_4 showing intermolecular carboxylate–guanidinium hydrogen bonds.[64]

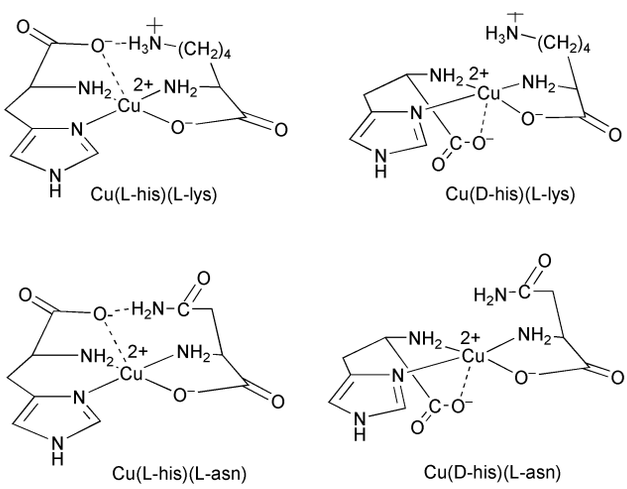


Fig. 8 Mode of interactions in Cu(L/D-his)(L-A or -B).

for Cu(his)(thr),[66] Cu(his)(asn),[67] and Cu(his)(ser)[68] by X-ray analysis. It was shown that the L-enantiomer of his is preferentially incorporated into complexes Cu(his)(L-A) isolated from the systems Cu(II)–DL-his–L-A (A = asn or cit).[69,70] These results indicate that the polar side chains of A form hydrogen bonds with the his carboxylato group under favorable conditions. Comparison of the ^{1}H NMR spectra of Pd(hisOMe)(A) with those of Pd(his)(A) showed that the his carboxylato group is necessary for interactions with the side chain group of A.[71]

Since peptides can accumulate various charged or polar amino acid side chains, metal–peptide complexes are expected to form intramolecular hydrogen bonds. Binary Cu(II) complexes of dipeptides composed of an acidic and a basic amino acid, *e.g.*, arg·asp, lys·asp, and arg·glu, were found to have stability constants for $Cu_pL_qH_r$, log β_{110} and log β_{11-1} (L = dipeptide), in the order X·asp > X·glu > X·gly (X = arg or lys), suggesting that the electrostatic interactions or hydrogen bonds between the charged side chains of X and asp or glu contribute to the stability of the resulting complexes CuL and $Cu(LH_{-1})$ (LH_{-1}, L deprotonated from the peptide bond) (Fig. 9).[72] Cu(arg·aspH_{-1}) in the solid state has been disclosed to have the guanidinium group of the arg residue hydrogen-bonded to the coordinated β-carboxylate group of the asp residue of a neighboring complex molecule, which supports the idea that the peptide side chains of the same complex molecule may interact

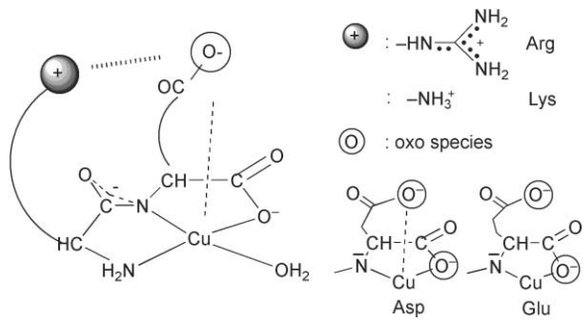


Fig. 9 Possible interactions in Cu(II)–dipeptide complexes with charged side chains.[72]

with each other through similar hydrogen bonds in dilute solution.

Kozlowski *et al.* observed an extreme stability enhancement in the Cu(II) complex of a pentapeptide amide (asn·ser·phe·arg·tyr-NH_2) with a 4N donor set and suggested that this resulted from a collapse of the non-bonding side chains, which shielded the coordination plane from the bulk of the solution.[73,74] From inspection of the CD spectra, they proposed the following view on this complex: asn and phe side chains provide a fence around the Cu(II) ion, and an additional stabilization is provided by the hydrogen bonding between the polar atoms of the N-terminal and C-terminal residues; the arg and tyr side chains form a secondary fence over the first one and exert a greater effect on the complex stability.

Interactions between a protein and a peptide with charged side chains, such as lysine peptides and aspartic acid peptides, may mimic protein–protein interactions. The molecular recognition characters of plastocyanin (PC) and cytochrome *c* (cyt *c*) or cytochrome *f* (cyt *f*) have been studied by using positively charged lysine peptides or negatively charged aspartic acid peptides as protein models and competitive inhibitors of binding.[75–78] The inhibitory effects of lysine peptides on the PC–cyt *f* (or *c*) binding indicated that the electrostatic interactions between the oppositely charged patches are important for the adduct formation and functions of these proteins. Interestingly, binding with the peptides caused a slight structural change and redox potential increase of the Cu center to make it adapted for accepting electrons.

Stacking interactions in solution

Stacking of the side chain aromatic rings of aromatic amino acids is important in biological systems for determining and stabilizing the tertiary structure of proteins.[17,79] Close contacts between aromatic rings are observed, for example, for the active sites of galactose oxidase[80] and cytochrome *c* oxidase,[81,82] and constitute the electron transfer pathway proposed for the cytochrome *c* peroxidase–cytochrome *c* couple.[83] Stacking is also important for biological molecular recognition as shown for the nucleobase–aromatic amino acid residue interaction involving tRNA.[84,85] While stacked nucleobase pairs in DNA are separated by 3.4 Å, the stacked phenylalanine rings in proteins are separared by 4.5–7 Å and tilted or offset to acquire effective binding, and no complete face-to-face stacking was observed.[86] Hunter and Sanders studied the π–π interactions in porphyrins and explained them by electrostatic interactions.[87,88] They successfully predicted the stacking geometry in the porphyrin systems and phenylalanine–phenylalanine interactions in proteins,[89] which may not be explained by the classical hydrophobic effect or the entropy effect alone. The stacking geometry has been further investigated recently.[90]

Phe, tyr, trp, and his have an aromatic side chain group. The aromatic rings of tyr and his residues in proteins are established to be important metal binding sites, and the indole moiety of trp is the largest ring that has the highest hydrophobicity[91] and

electron density. Sigel *et al*. carried out extensive studies on intramolecular hydrophobic or aromatic ring stacking interactions in ternary systems involving M(bpy, *etc*.) and nucleotides, amino acids, or alkane- (or arylalkane-) carboxylates.[92–96] They found that the tendency of the aromatic groups of amino acids to form stacked structures was in the order indole > phenyl > imidazole.[97] While the percentage of the closed form (Fig. 4) for leu were calculated to be 0–26% from the stability constants determined for M(bpy or phen)(leu) [M = Co(II), Ni(II), or Cu(II)], the values for mixed aromatic amino acid complexes were found to be much higher, *e.g*. 35% for Cu(tyr)-(phe) and 72% for Cu(his)(trp), showing that the intramolecular interaction is most favored in the systems where aromatic ring stacking is possible.[98] The ternary systems Cu(L/D-his)(L-AA) (AA = phe, tyr, trp) indeed exhibited anomalous CD spectral magnitudes, and by considering the following equilibrium [eqn. (7)] we found that Cu(L/D-his)-(L-AA) complexes have larger log *K* values as compared with those for AA = ala or val:[99]

$$\text{Cu(L/D-his)(L-ala)} + \text{Cu(en)(L-AA)} \overset{K}{\rightleftharpoons} \text{Cu(L/D-his)(L-AA)} + \text{Cu(en)(L-ala)} \quad (7)$$

The stacking in the active complexes Cu(L-his)(L-AA) was less effective than in the *meso* complexes Cu(D-his)(L-AA), which was in agreement with previous observations.[62,94–96] This may be interpreted as due to the axial coordination by the carboxylate group, which interferes with the stacking in the active form but not in the *meso* form if we assume the *cis*-configuration with respect to the amino groups (Fig. 8).

Thermodynamic measurements offer valuable data for clarifying the factors for stabilization due to weak interactions. The observed stereoselectivity is supported by calorimetric studies on his-containing ternary Cu(II) complexes with aromatic amino acids (AA), Cu(II)–his–AA,[100] which showed that formation of the *meso* complexes are more exothermic than that of the active complexes (AA = phe or trp) with the $-\Delta H$ difference of 5.94 kJ mol^{-1} for AA = trp at 25 °C and I = 0.1 M (KNO_3). On the other hand, the active complexes were found to be enthalpically more favored in Cu(II)–*cyclo*-his·his–AA.[101] Both results were ascribed to the stacking interaction between the coordinated imidazole and the side chain aromatic ring. In this connection an interesting enantioselective binding of trp was reported to be attained by a Cu(II)–hista moiety tethered through the amino group to β-cyclodextrin, whose cavity is concluded to interact favorably with the indole ring of D-trp coordinated to Cu(II) on the basis of the *cis* configuration of the Cu(II) site with respect to the amino groups and the anomalously enhanced CD mangitude for D-trp.[102–105]

The stacking interactions in the ternary systems Cu(da)(AA) (da = bpy, phen, *etc*.; AA = phe, tyr, trp, *etc*.) have been studied by CD spectra and potentiometric titrations.[36] The CD magnitudes for Cu(da)(tyr) in water drastically increased with da in the order en < ampy < bpy and decreased in less polar solvents (Fig. 10). The magnitude enhancement was also found to be in the order phe < tyr < trp and proportional to the number of aromatic rings in da. The log *K* values calculated according to an equation similar to eqn. (7) for various systems from the stability constants determined at 25 °C and I = 0.1 M (KNO_3) clearly indicated that stacking interactions exist between da and the side chain of AA and that the extent of stacking depends on the size of the aromatic rings involved and the electron density difference between them. With Cu(en)(ala) as standard, the values for Cu(da)(phe) increased from 0.22–0.26 for da = hista and ampy to 0.60–0.64 for bpy and phen, while those for Cu(da)(htrp) ranged from 0.82 for ampy to 2.22 for phen, the latter of which corresponds to a ΔG value of -12.7 kJ mol^{-1}. The OH group introduced into the aromatic ring of AA increased the log *K* values, and the stability sequence of

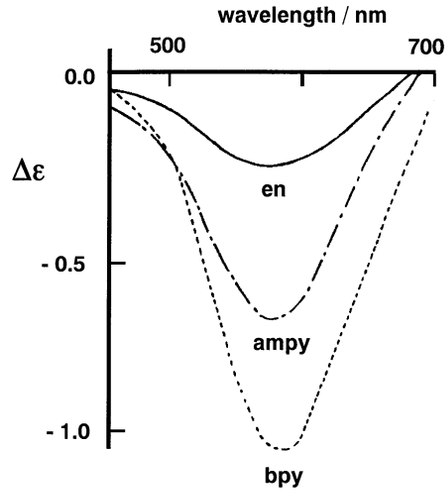


Fig. 10 CD spectra for Cu(da)(tyr). da = en, ampy, or bpy.[36]

stacking in Cu(da)(L-AA) has been revealed to be in the following order of AA:[36,106]

$$\text{htrp} \sim \text{I}_2\text{tyr} > \text{trp} > \text{I}_2\text{tyrO} > \text{tyr} > \text{phe} > \text{tyrO} \gg \text{Ptyr}$$

The iodine atoms in the aromatic ring have a profound effect on the stacking; 3,5-diiodotyrosinate (I_2tyr) was found to undergo stacking with da with log *K* values larger than those for AA = tyr, the value for Cu(phen)(I_2tyr) (2.18) being comparable with that for Cu(phen)(htrp) (2.22).[106] Deprotonated I_2tyr (I_2tyrO) still gave a log *K* value as high as 1.38, which is large enough to support stacking. Such a stabilizing effect may be correlated with the function of the essential iodine atoms incorporated into the growth hormone thyroxine (*vide infra*). It is remarkable that while the fractional populations of stacked species, P_{on}, obtained from the log *K* values [eqn. (5)] are larger than 0.9 for Cu(phen)(tyr, trp, or htrp) and Cu(bpy)(trp or htrp), the value for Cu(da)(Ptyr) is 0,[36] indicating that the stacking interaction is cleaved by phosphorylation of the tyr OH group probably due to strong hydration and, as described in the previous section, may be converted to the electrostatic interaction with arg *etc*.[45]

Stacking of an aromatic ring with metal-coordinated aromatic ligands was found to affect the electron density of the metal ion. Platinum(II) complexes with planar aromatic ligands have been known as DNA intercalators.[107] They form adducts with mononucleotides such as AMP and GMP in dilute solution with log formation constants of 2–3.[108] In addition to the ^{1}H NMR upfield shifts of the aromatic rings, the ^{195}Pt NMR exhibited downfield shifts upon adduct formation, indicating that the electron density of Pt(II) was decreased by aromatic ring stacking.[109] This could be ascribed to the delocalization of the electrons of Pt(II) over stacked aromatic rings and suggests that the reactivity of the central metal ion may be controlled by vicinal aromatic rings through electronic effects.

Intramolecular stacking in the solid state

Intramolecular stacking interactions involving amino acids and peptides concluded for ternary complexes in solution have been substantiated for a number of cases by X-ray crystal structure determinations. Cu(phen)(trp),[110,111] Cu(phen)(AA) (AA = phe, tyr),[112] Cu(bpy)(trp) (Fig. 11),[113] Cu(hista)(phe) (Fig. 12a), and Cu(hista)(tyr) (Fig. 12b)[114] were disclosed to have their side chain aromatic rings tilted over the metal coordination plane to undergo face-to-face stacking with da with distances of *ca.* 3.5 Å. These figures show that the α-carbon moiety of AA is considerably distorted from the plane probably to accommodate the steric requirement for the intramolecular stacking.

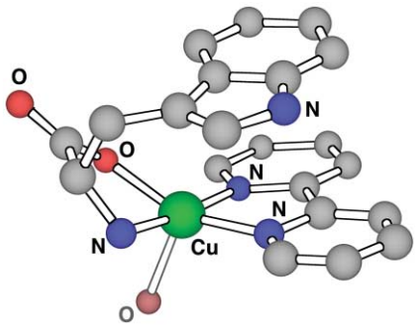


Fig. 11 Structure of $[Cu(bpy)(trp)(H_2O)]^+$.[113]

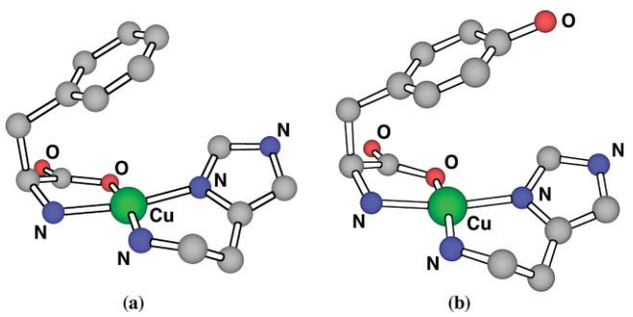


Fig. 12 Structures of $[Cu(hista)(phe)]^+$ (a) and $[Cu(hista)(tyr)]^+$ (b).[114]

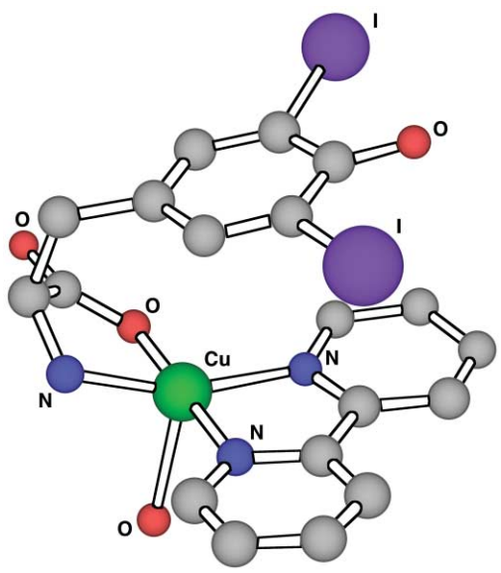


Fig. 13 Structure of $[Cu(bpy)(I_2tyrO)]$ showing stacking of the deprotonated diiodophenol moiety.[106]

In line with the large log *K* values the diiodophenol moiety in $Cu(bpy)(I_2tyr)$,[106] $Cu(bpy)(I_2tyrO)$ (Fig. 13),[106] and $Cu(hista)(I_2tyrO)$[115] in the solid state is stacked with da irrespective of the deprotonation of the OH group. The structures also showed that the iodine atoms are in contact with the aromatic ring and the carbonyl oxygen atom of the coordinated carboxylate moiety of a neighboring complex molecule, which shows the versatility of the soft iodine atom in non-covalent interactions with various groups. The structures of the ternary Cu(II) complexes with bpy and *p*-halogen-substituted phe derivatives disclosed that the *p*-iodophenyl ring but not the *p*-Cl- and *p*-Br-substituted rings is stacked with bpy,[116] while *p*-aminophenylalanine (NH_2phe) in $Cu(bpy)(NH_2phe)$ and phe in Cu(phen)(phe) were found to assume a stacked form and both stacked and unstacked forms, respectively.[112] These results indicate that stacking of phe seems to be intermediate in strength and may be affected by axial coordination and/or conditions of crystallization. A recent study on $[Cu(da)(L\text{-phe})(H_2O)]ClO_4$ (da = phen or bpy) showed that the complex with da = bpy has a stacked structure in the solid state;[117] interconversion between the stacked and unstacked forms was indicated for both complexes in solution at temperatures above 323 K, and the energy difference between the two conformations was calculated by the density functional method. Masuda *et al.*[118] reported that the stacking of phe is assisted by CH–π interactions; they observed that *m*- and *p*-methyl substituted phenylalanines (AA = *m*- and *p*-mphe, respectively) in Pd(phen)(AA) exhibited ^{1}H NMR upfield shifts of the methyl protons, while there were no such shifts for Pd(phen)(*o*-mphe). This indicates that the side chains of *m*- and *p*-mphe are stacked with phen in the Pd(II) complexes in aqueous solution. X-Ray structural analysis of Cu(phen)(*p*-mphe) and Cu(phen)(*o*-mphe) established that the side chain aromatic ring is stacked with phen in the former but not in the latter. While Pd(bpy)(tyr·gly) has a planar structure with four nitrogen atoms and the side chain phenol moiety bent over the plane,[51] Cu(phen)(gly·gly),[119] and Cu(phen)(tyr·gly)[51] have a square-pyramidal structure, where phen occupies one equatorial and one axial position and the dipeptides coordinate equatorially with the phenol moiety of tyr·gly located approximately perpendicular to the Cu(II) plane to be stacked with phen. In a ternary Cu(II) complex containing gly·tyr and a bidentate ligand, the tyr phenol moiety is located close to a neighboring methyl group to be involved in a CH–π-type interaction with the coordinated pyridine ring (Fig. 14), and this has been found to increase the stability of the complex.[120]

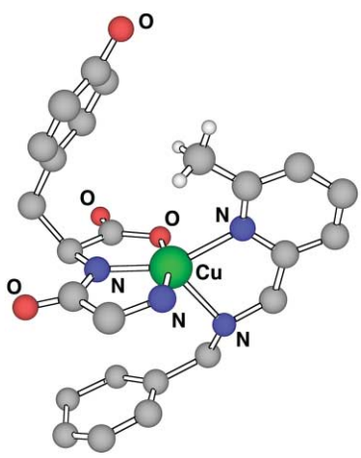


Fig. 14 A CH–π-type interaction in a gly·tyr-containing Cu(II) complex.[120]

As already seen from the above description and the stability constants as well as from other sources of information, the imidazole ring is an efficient ligand and stacking partner that is comparable with pyridine.[121] It is interesting to note in this connection that the Cu-coordinated imidazole ring of His90 at the active site of a new plastocyanin from the fern, *Dryopteris crassirhizoma*, stacks with the phenyl ring of an adjacent phe residue (Fig. 15)[122] in the same manner as revealed for the model complex Cu(hista)(phe) (Fig. 12a).[114] The structure of this plastocyanin serves as the first example of face-to-face stacking involving a coordinated imidazole ring in proteins. The $Cu–N_{His90}$ bond in the fern plasctocyanin (corresponding to the $Cu–N_{His87}$ bond of poplar plastocyanin[123]) is stabilized against reduction at pH 4.5 where the corresponding bond in higher plant plastocyanins dissociates, and the redox potential is higher than that of most other plastocyanins by *ca.* 20 mV. This is what we may expect from the effects of intramolecular stacking on the electron density of the metal center as seen from the ^{195}Pt NMR shifts[109] and stabilization of Cu(hista)(AA) by stacking.[36]

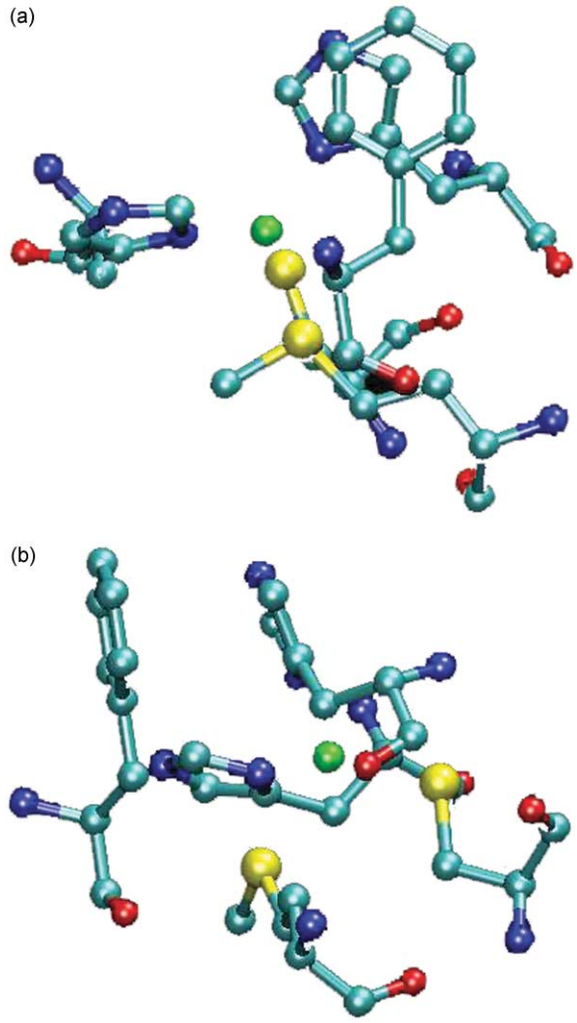


Fig. 15 Active site structure of *Dryopteris crassirhizoma* plastocyanin;[122] (a) top view, (b) side view. Figures prepared by VMD[139] were kindly supplied by Dr Takamitsu Kohzuma (Ibaraki University).

Structure and function of complexes due to weak interactions

Arg, tyr, and trp are very intriguing in the sense that their side chains have a unique group that distinguishes them from the other amino acids in the mode of interactions and functions in biological systems:

(1) The guanidinium group of arg has been known to be important in hydrogen bond formation with and recognition of a carboxylate, phosphate, and other groups in proteins as revealed for the carboxypeptidase A–gly·tyr[28,59] and Cu,Zn-SOD–superoxide binding,[60,61] and in chemical systems,[124] and recently the guandinium–π interaction[21] has also attracted attention for its biological importance.

(2) As compared with phenylalanine, tyr has a phenol moiety, which makes it drastically different or *talented* owing to a number of reactions it undergoes, such as metal binding, hydrogen bonding, phenoxyl radical and quinone formations, and phosphorylation. The OH group enhances aromatic ring stacking ability, and opioid peptides and thyroid hormones have an essential tyr OH for their activity. As shown earlier, the OH group, when phosphorylated, becomes capable of forming electrostatic or hydrogen bonds with positively charged groups such as the guanidinium group, which could imply that phosphorylation of tyr residues in proteins may convert stacking into hydrogen bonding and thus trigger the structural change of proteins (Fig. 16).[36,45] X-Ray structural analysis revealed that the phosphotyrosine-binding site in the SH2 domain of a tyrosine kinase binds with the phosphotyrosyl group through various weak interactions involving arg guanidinium–phosphate hydrogen bonds and the NH–π interaction.[125] The interaction between the peptide NH and an aromatic side chain group was recently studied by molecular dynamics simulations for a simple peptide.[126]

(3) The indole ring of trp is notable for its electron-rich character and hydrophobicity. It stacks most efficiently with electron-deficient aromatic rings, which may be related to the strong physiological activities of indole-containing compounds such as ergot alkaloids and hallucinogens.[127]

Since metal ions coordinate these amino acids in a fixed geometry which can be favorable for intermolecular interactions and affect their conformation, electron density, *etc*., we may expect a variety of possibilities regarding structures and functions of metal–amino acid complexes.

Ligand–ligand interactions around the central metal ion in isolated complex molecules may be converted to interactions between neighboring molecules under certain conditions. Such intermolecular interactions may be controlled by combination of interacting groups and their steric requirements, and under favorable conditions interactions between complex molecules or between a metal complex and an uncoordinated molecule may lead to an organized structure by self-organization. The crystal structure of a binary complex, $[Cu(L\text{-arg})_2](NO_3)_2 \cdot 3H_2O$, has a three-dimensional hydrogen bonding network involving the arg molecules, nitrate ions, and water molecules, and the Cu(II) coordination plane has two amino groups in the *cis*-positions as a result of the hydrogen bonds with the oxygen atoms of a nitrate ion (Fig. 17).[64] When the nitrate ions were replaced with an aromatic *meta*-dicarboxylate, such as benzene-2,4-dicarboxylate (mba) and pyridine-2,6-dicarboxylate, $[Cu(L\text{-arg})_2](mba)$ *etc*. were obtained as crystals, where the carboxylate groups of mba *etc*. keep the *cis*-configuration of the Cu(II) coordination plane and bridge the $[Cu(L\text{-arg})_2]^{2+}$ ions *via* hydrogen bonds with the amino and guanidinium moieties, respectively.[128] This one-dimensional (1D) infinite chain formation ($\{-[Cu(L\text{-arg})_2]^{2+}-mba^{2-}-\}_n$ *etc*.) attained by the dicarboxylate-dependent *cis*-geometry gave a right-handed double helical structure as a result of self-organization (Fig. 18). Interestingly a left-handed double helix with the same bonding mode was obtained when D-arg was used in place of L-arg. With different dianions different organized structures

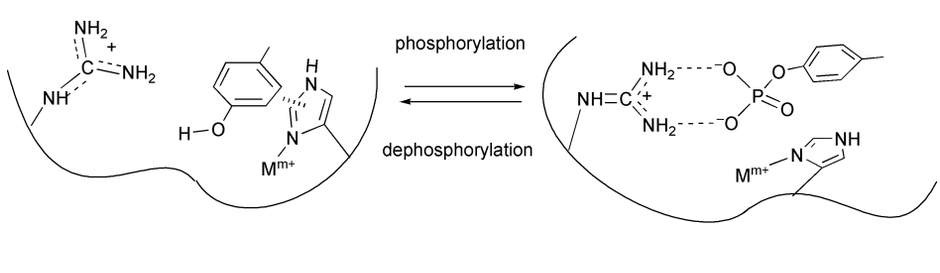


Fig. 16 Possible interconversion between stacking and hydrogen bonding with conformational change due to tyrosine phosphorylation.[32,36,45] Reproduced from ref. 32 with modification.

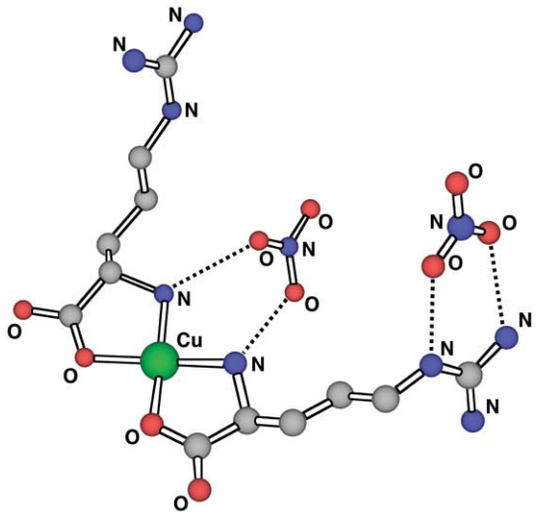


Fig. 17 Structure of $[Cu(arg)_2](NO_3)_2$ showing the guanidinium···nitrate hydrogen bonds.[64]

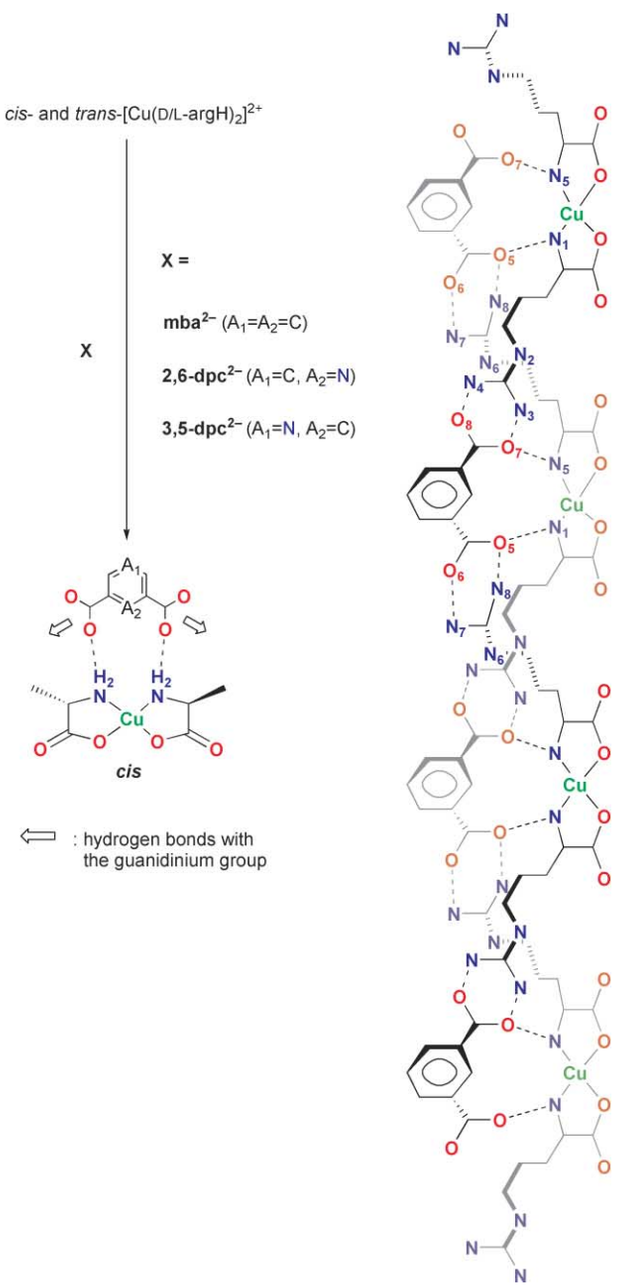


Fig. 18 Self-organization of a $[Cu(arg)_2]^{2+}$–aromatic dicarboxylate system.[128]

were obtained.[129,130] These organized structures are considered to be determined by the chirality of arg, the *cis–trans* geometry of the Cu(II) coordination structure, and the directions of the hydrogen bonds between the guanidinium and dianionic groups.

Intramolecular stacking can fix the conformation of molecules and thus assist stereoselective reactions, molecular recognition, *etc*. Stacking of metal-coordinated aromatic amino acids, tyr or trp, has been successfully applied to Cu(II)- and Ni(II)-catalyzed Diels–Alder reactions in water to attain enantioselectivity, where the side chain aromatic ring of tyr or trp stacked with the coordinated pyridine ring of a dienophile to restrict the reaction with a diene to only one side of the dienophile.[131] As described earlier, an aromatic ring close to the metal center lowers the electron density of the metal center. This may further affect the reactivity of the metal ion and/or the aromatic ring. The indole ring of trp in proteins is not known to be involved in metal binding, but Pd(II) was disclosed to bind with the nitrogen atom of indole in the 3*H*-indole form in methanol as shown for $[PdCl_2(MI)_2]$ (MI = 2-methylindole).[132] It was also found to similarly bind with the nitrogen atom of the 3*H*-indole ring of indole-3-acetate and at the same time form a dimer through a *spiro*-type chelate ring by binding with the carboxylate oxygen atom and the deprotonated indole C-3 atom (Fig. 19).[133] The results suggest that the indole ring may have

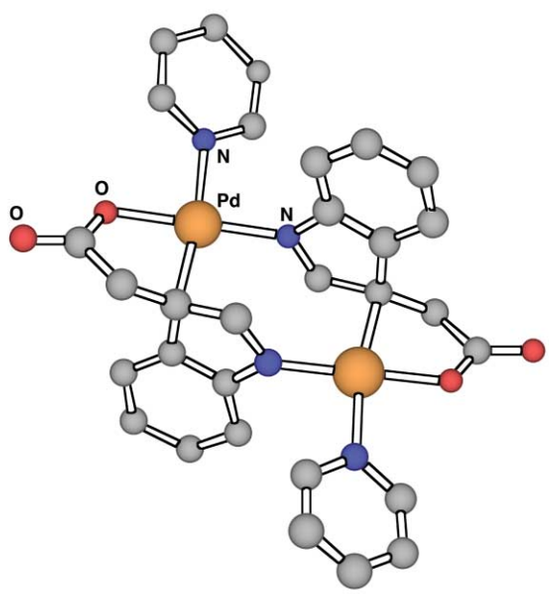


Fig. 19 Structure of $[\{Pd(indoleacetate)(pyridine)\}_2]$.[133]

been located close to Pd(II), resulting in the Pd(II)–C-3 bonding. In this connection, Kostic *et al*. investigated the Pt(II) and Pd(II) binding with the indole ring[134] and regioselective hydrolysis of peptides involving methionine, his, or trp residues by Pd(II) and Pt(II) complexes as artificial metallopeptidases.[135–137] The complexes have been concluded to bind with the side chain groups, *i.e*. the methionine sulfur, his imidazole, and trp indole, and selectively hydrolyze the adjacent peptide bonds; for trp-containing peptides they hydrolyzed the C-terminal peptide bond of the trp residue owing to the metal–indole binding in a manner as described above.

Concluding remarks

Depending on the side chain structures, amino acids exhibit a variety of functions, where metal binding ability is among the most important. Ligand–ligand interactions in metal–amino acid and –peptide complexes have been substantiated by various methods and shown to affect the properties of the complexes. Weak interactions involving the side chain groups are

closely connected with complex formation, which is favored by attractive intramolecular ligand–ligand interactions. At the same time the metal ion assists them both by fixing interacting molecules and by exerting electronic effects. The proximity of an aromatic ring such as an indole ring at the metal site was shown to affect the reactivity of some Cu(I) complexes with O_2,[138] which may suggest certain electronic effects of the ring on the metal center, stabilization of reaction intermediates, and desolvation of the metal coordination sphere. Basic studies on the properties of metal–amino acid complexes may open up further insights into bioinorganic reaction mechanisms.

Acknowledgements

We wish to express our sincere thanks to all the collaborators and students for their contribution and enthusiasm. We gratefully acknowledge the helpful suggestions by Professors Helmut Sigel (University of Basel), R. Bruce Martin (University of Virginia), Motoharu Tanaka (Nagoya University), Giuseppe Arena (University of Catania), and Tamas Kiss (University of Szeged). We thank Dr Takamitsu Kohzuma (Ibaraki University) for kindly supplying the graphics for fern plastocyanin (including the cover figure, prepared using Molscript).[140] Financial support by the Ministry of Education, Culture, Sports, Science and Technology of Japan is gratefully acknowledged.

References

1 H. C. Freeman, in *Inorganic Biochemistry*, vol. 1, ed. G. L. Eichhorn, Elsevier, Amsterdam, 1973, ch. 4.
2 S. H. Laurie, in *Comprehensive Coordination Chemistry*, vol. 2, eds. G. Wilkinson, R. D. Gillard and J. A. McCleverty, Pergamon Press, Oxford, 1987, pp. 739–776.
3 S. H. Laurie, in *Handbook of Metal-Ligand Interactions in Biological Fluids: Bioinorganic Chemistry*, vol. 1, ed. G. Berthon, Marcel Dekker, New York, 1995, pp. 603–619.
4 R. B. Martin, *Met. Ions Biol. Syst.*, 1979, **9**, 1.
5 B. Sarkar and T. P. A. Kruck, in *The Biochemistry of Copper*, eds. J. Peisach, P. Aisen and W. E. Blumberg, Academic Press, New York, 1966, p. 183.
6 P. Z. Neumann and A. Sass-Kortsak, *J. Clin. Invest.*, 1967, **46**, 646.
7 P. M. May, P. W. Linder and D. R. Williams, *J. Chem. Soc., Dalton Trans.*, 1977, 588.
8 G. Berthon, B. Hacht, M.-J. Blais and P. M. May, *Inorg. Chim. Acta*, 1986, **125**, 219.
9 V. Brumas, N. Alliey and G. Berthon, *J. Inorg. Biochem.*, 1993, **52**, 287.
10 B. Sarkar, *Chem. Rev.*, 1999, **99**, 2535.
11 P. F. Knowles and D. M. Dooley, *Met. Ions Biol. Syst.*, 1994, **30**, 361.
12 J. P. Klinman, *Chem. Rev.*, 1996, **96**, 2541.
13 J. P. Klinman, *J. Biol. Inorg. Chem.*, 2001, **6**, 1.
14 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*, 1991, **350**, 87.
15 J. W. Whittaker, *Met. Ions. Biol. Syst.*, 1994, **30**, 315.
16 C. Branden and J. Tooze, *Introduction to Protein Structure*, Garland, New York, 1991.
17 S. K. Burley and G. A. Petsko, *Adv. Protein Chem.*, 1988, **39**, 125.
18 R. E. Babine and S. L. Bender, *Chem. Rev.*, 1997, **97**, 1359.
19 D. A. Dougherty, *Science*, 1996, **271**, 163.
20 J. C. Ma and D. A. Dougherty, *Chem. Rev.*, 1997, **97**, 1303.
21 J. P. Gallivan and D. A. Dougherty, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 9459.
22 M. Nishio, M. Hirota and Y. Umezawa, *The CH–π Interactions*, Wiley-VCH, New York, 1998.
23 M. Perutz, *Philos. Trans. R. Soc. Lond. A*, 1993, **345**, 105.
24 For recent studies on small complexes, see for example: (*a*) S. D. Zaric, D. M. Popovic and E.-W. Knapp, *Chem. Eur. J.*, 2000, **6**, 3935; (*b*) H. Kumita, T. Kato, K. Jitsukawa, H. Einaga and H. Masuda, *Inorg. Chem.*, 2001, **40**, 3936.
25 N. P. Pavletich and C. O. Pabo, *Science*, 1991, **252**, 809.
26 B. F. Luisi, W. X. Xu, Z. Otwinoski, L. P. Freedman, K. R. Yamamoto and P. B. Sigler, *Nature*, 1991, **352**, 497.
27 J. C. Miller and C. O. Pabo, *J. Mol. Biol.*, 2001, **313**, 309.
28 F. A. Quiocho and W. N. Lipscomb, *Adv. Protein Chem.*, 1971, **25**, 1.
29 J. H. Cho, D. H. Kim, S. J. Chung, N.-C. Ha, B.-H. Oh and K. Y. Choi, *Bioorg. Med. Chem.*, 2002, **10**, 2015.
30 O. Yamauchi, Y. Nakao and A. Nakahara, *Bull. Chem. Soc. Jpn.*, 1975, **48**, 2572.
31 H. Sigel, *Angew. Chem., Int. Ed. Engl.*, 1975, **14**, 394.
32 O. Yamauchi, A. Odani and S. Hirota, *Bull. Chem. Soc. Jpn.*, 2001, **74**, 1525.
33 T. Kiss, in *Biocoordination Chemistry: Coordination Equilibria in Biologically Active Systems*, ed. K. Burger, Ellis Horwood, Chichester, 1990, pp. 56–134.
34 O. Yamauchi, in *Handbook of Metal–Ligand Interactions in Biological Fluids: Bioinorganic Chemistry*, vol. 1, ed. G. Berthon, Marcel Dekker, New York, 1995, pp. 583–596.
35 R. DeWitt and J. I. Watters, *J. Am. Chem. Soc.*, 1954, **76**, 3810.
36 O. Yamauchi and A. Odani, *J. Am. Chem. Soc.*, 1985, **107**, 5938.
37 O. Yamauchi and A. Odani, *Nippon Kagaku Kaishi*, 1988, 369.
38 R. B. Martin and H. Sigel, *Comments Inorg. Chem.*, 1988, **6**, 285.
39 J. M. Tsangaris and R. B. Martin, *J. Am. Chem. Soc.*, 1970, **92**, 4255.
40 R. B. Martin, *Met. Ions Biol. Syst.*, 1974, **1**, 129.
41 T. Sakurai, O. Yamauchi and A. Nakahara, *Bull. Chem. Soc. Jpn.*, 1976, **49**, 169.
42 T. Sakurai, O. Yamauchi and A. Nakahara, *Bull. Chem. Soc. Jpn.*, 1976, **49**, 1579.
43 O. Yamauchi, *J. Mol. Catal.*, 1984, **23**, 255.
44 O. Yamauchi and A. Odani, *J. Am. Chem. Soc.*, 1981, **103**, 391.
45 O. Yamauchi, A. Odani and H. Masuda, *Inorg. Chim. Acta*, 1992, **198–200**, 749.
46 K. G. R. Pachler, *Spectrochim. Acta*, 1964, **20**, 581.
47 J. J. Feeney, *Magn. Reson.*, 1976, **21**, 473.
48 S. H. Kim and R. B. Martin, *J. Am. Chem. Soc.*, 1984, **106**, 1707.
49 F. R. Bovey, *Nuclear Magnetic Resonance Spectroscopy*, Academic Press, New York, 1969.
50 A. Odani, S. Deguchi and O. Yamauchi, *Inorg. Chem.*, 1986, **25**, 62.
51 T. Sugimori, K. Shibakawa, H. Masuda, A. Odani and O. Yamauchi, *Inorg. Chem.*, 1993, **32**, 4951.
52 F. Zhang, T. Yajima, A. Odani and O. Yamauchi, *Inorg. Chim. Acta*, 1998, **278**, 136.
53 J.-M. Lehn, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 1304.
54 J.-M. Lehn, *Supramolecular Chemistry: Concepts and Perspectives*, VCH, Weinheim, 1995.
55 T. N. Bhat and M. Vijayan, *Acta Crystallogr., Sect. B*, 1977, **33**, 1754.
56 D. M. Salunke and M. Vijayan, *Acta Crystallogr., Sect. B*, 1982, **38**, 1329.
57 D. M. Salunke and M. Vijayan, *Int. J. Peptide Protein Res.*, 1981, **18**, 348.
58 D. W. Christianson and W. N. Lipscomb, *Acc. Chem. Res.*, 1989, **22**, 62.
59 W. N. Lipscomb and N. Sträter, *Chem. Rev.*, 1996, **96**, 2375.
60 J. A. Tainer, E. D. Getzoff, J. S. Richardson and D. C. Richardson, *Nature*, 1983, **306**, 284.
61 E. D. Getzoff, J. A. Tainer, P. K. Weiner, P. A. Kollman, J. S. Richardson and D. C. Richardson, *Nature*, 1983, **306**, 287.
62 B. R. Crane, A. S. Arvai, R. Gachhui, C. Wu, D. K. Ghosh, E. D. Getzoff, D. S. Stuehr and J. A. Tainer, *Science*, 1997, **278**, 425.
63 T. Sakurai, O. Yamauchi and A. Nakahara, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 3203.
64 H. Masuda, A. Odani, T. Yamazaki, T. Yajima and O. Yamauchi, *Inorg. Chem.*, 1993, **32**, 1111.
65 G. Brookes and L. D. Pettit, *J. Chem. Soc., Dalton Trans.*, 1977, 1918.
66 H. C. Freeman, J. M. Guss, M. J. Healy, R.-P. Martin, C. E. Nockolds and B. Sarkar, *Chem. Commun.*, 1969, 225.
67 T. Ono, H. Shimanouchi, Y. Sasada, T. Sakurai, O. Yamauchi and A. Nakahara, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2229.
68 Y. Sasada, A. Takenaka and T. Furuya, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1745.
69 O. Yamauchi, T. Sakurai and A. Nakahara, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 1776.
70 O. Yamauchi, T. Sakurai and A. Nakahara, *J. Am. Chem. Soc.*, 1979, **101**, 4164.
71 A. Odani and O. Yamauchi, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3773.
72 W. J. Puspita, A. Odani and O. Yamauchi, *J. Inorg. Biochem.*, 1999, **73**, 203.
73 W. Bal, M. Dyba, F. Kasprzykowski, H. Kozlowski, R. Latajka, L. Lankiewicz, Z. Mackiewicz and L. D. Pettit, *Inorg. Chim. Acta*, 1998, **283**, 1.
74 H. Kozlowski, W. Bal, M. Dyba and T. Kowalik-Jankowska, *Coord. Chem. Rev.*, 1999, **184**, 319.
75 S. Hirota, K. Hayamizu, M. Endo, T. Hibino, T. Takabe, T. Kohzuma and O. Yamauchi, *J. Am. Chem. Soc.*, 1998, **120**, 8177.
76 S. Hirota, M. Endo, K. Hayamizu, T. Tsukazaki, T. Takabe, T. Kohzuma and O. Yamauchi, *J. Am. Chem. Soc.*, 1999, **121**, 849.

77 S. Hirota and O. Yamauchi, *Chem. Rec.*, 2001, **1**, 290.
78 S. Hirota and O. Yamauchi, *Eur. J. Inorg. Chem.*, 2002, 17.
79 S. K. Burley and G. A. Petsko, *Science*, 1985, **229**, 23.
80 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*, 1991, **350**, 87.
81 T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono and S. Yoshikawa, *Science*, 1995, **269**, 1069.
82 S. Iwata, C. Ostermeier, B. Ludwig and H. Michel, *Nature*, 1995, **376**, 660.
83 H. Pelletier and J. Kraut, *Science*, 1992, **258**, 1748.
84 U. Heinemann and W. Saenger, *Nature*, 1982, **299**, 27.
85 W. Saenger, *Principles of Nucleic Acid Structure*, Springer, Heidelberg, 1984.
86 J. Singh and J. M. Thornton, *FEBS Lett.*, 1985, **191**, 1.
87 C. A. Hunter and J. K. M. Sanders, *J. Am. Chem. Soc.*, 1990, **112**, 5525.
88 C. A. Hunter, *Chem. Soc. Rev.*, 1994, 101.
89 C. A. Hunter, J. Singh and J. M. Thornton, *J. Mol. Biol.*, 1991, **218**, 837.
90 C. Janiak, *J. Chem. Soc., Dalton Trans.*, 2000, 3885.
91 Y. Nozaki and C. Tanford, *J. Biol. Chem.*, 1971, **246**, 2211.
92 H. Sigel, in *Coordination Chemistry-20*, ed. D. Banerjea, Pergamon Press, Oxford, 1980, pp. 27–45.
93 H. Sigel, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 389.
94 H. Sigel, *Pure Appl. Chem.*, 1989, **61**, 923.
95 H. Sigel and B. Song, *Met. Ions Biol. Syst.*, 1996, **32**, 135.
96 O. Yamauchi, A. Odani, H. Masuda and H. Sigel, *Met. Ions Biol. Syst.*, 1996, **32**, 207.
97 R. Malini-Balakrishnan, K. H. Scheller, U. K. Haring, R. Tribolet and H. Sigel, *Inorg. Chem.*, 1985, **24**, 2067.
98 B. E. Fischer and H. Sigel, *J. Am. Chem. Soc.*, 1980, **102**, 2998.
99 O. Yamauchi and A. Odani, *Inorg. Chim. Acta*, 1985, **100**, 165.
100 G. Borghesani, F. Pulidori, M. Remelli, R. Purrello and E. Rizzarelli, *J. Chem. Soc., Dalton Trans.*, 1990, 2095.
101 G. Arena, R. Bonomo, L. Casella, M. Gullotti, G. Impellizzeri, G. Maccarrone and E. Rizzarelli, *J. Chem. Soc., Dalton Trans.*, 1991, 3203.
102 G. Impellizzeri, G. Maccarrone, E. Rizzarelli, G. Vecchio, R. Corradini and R. Marchelli, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1348.
103 R. Corradini, A. Dossena, G. Impellizzeri, G. Maccarrone, R. Marchelli, E. Rizzarelli, G. Sartor and G. Vecchio, *J. Am. Chem. Soc.*, 1994, **116**, 10267.
104 L. D. Pettit, in *Handbook of Metal–Ligand Interactions in Biological Fluids: Bioinorganic Chemistry*, vol. 1, ed. G. Berthon, Marcel Dekker, New York, 1995, pp. 620–626.
105 E. Rizzarelli and G. Vecchio, *Coord. Chem. Rev.*, 1999, **188**, 343.
106 F. Zhang, A. Odani, H. Masuda and O. Yamauchi, *Inorg. Chem.*, 1996, **35**, 7148.
107 J. K. Barton and S. J. Lippard, in *Metal Ions in Biology*, vol. 1, ed. T. G. Spiro, Wiley-Interscience, New York, 1980, pp. 31–113.
108 A. Odani, R. Shimata, H. Masuda and O. Yamauchi, *Inorg. Chem.*, 1991, **30**, 2133.
109 A. Odani, T. Sekiguchi, H. Okada, S. Ishiguro and O. Yamauchi, *Bull. Chem. Soc. Jpn.*, 1995, **68**, 2093.
110 K. Aoki and H. Yamazaki, *J. Chem. Soc., Dalton Trans.*, 1987, 2017.
111 H. Masuda, O. Matsumoto, A. Odani and O. Yamauchi, *Nippon Kagaku Kaishi*, 1988, 783.
112 T. Sugimori, H. Masuda, N. Ohata, K. Koiwai, A. Odani and O. Yamauchi, *Inorg. Chem.*, 1997, **36**, 576.
113 H. Masuda, T. Sugimori, A. Odani and O. Yamauchi, *Inorg. Chim. Acta*, 1991, **180**, 73.
114 O. Yamauchi, A. Odani, T. Kohzuma, H. Masuda, K. Toriumi and K. Saito, *Inorg. Chem.*, 1989, **28**, 4066.
115 F. Zhang, T. Yajima, H. Masuda, A. Odani and O. Yamauchi, *Inorg. Chem.*, 1997, **36**, 5777.
116 T. Sugimori, H. Masuda and O. Yamauchi, *Bull. Chem. Soc. Jpn.*, 1994, **67**, 131.
117 P. S. Subramanian, E. Suresh, P. Dastidar, S. Waghmode and D. Srinivas, *Inorg. Chem.*, 2001, **40**, 4291.
118 M. Mizutani, S. Tomosue, H. Kinoshita, K. Jitsukawa, H. Masuda and H. Einaga, *Bull. Chem. Soc. Jpn.*, 1999, **72**, 981.
119 M. C. Lim, E. Sinn and R. B. Martin, *Inorg. Chem.*, 1976, **15**, 807.
120 T. Yajima, M. Okajima, A. Odani and O. Yamauchi, *Inorg. Chim. Acta, in press.*
121 H. Sigel, R. Tribolet and O. Yamauchi, *Comments Inorg. Chem.*, 1990, **9**, 305.
122 T. Kohzuma, T. Inoue, F. Yoshizaki, Y. Sasakawa, K. Onodera, S. Nagotomo, T. Kitagawa, S. Uzawa, Y. Isobe, Y. Sugimura, M. Gotowda and Y. Kai, *J. Biol. Chem.*, 1999, **274**, 11817.
123 J. M. Guss, P. R. Harrowell, M. Murata, V. A. Norris and H. C. Freeman, *J. Mol. Biol.*, 1986, **192**, 361.
124 J. Suh and S.-J. Moon, *Inorg. Chem.*, 2001, **40**, 4890.
125 G. Waksman, D. Kominos, S. C. Robertson, N. Pant, D. Baltimore, R. B. Birge, D. Cowburn, H. Hanafusa, B. J. Mayer, M. Overduin, M. D. Resh, C. B. Rios, L. Silverman and J. Kuriyan, *Nature*, 1992, **358**, 646.
126 G. Toth, R. F. Murphy and S. Lovas, *J. Am. Chem. Soc.*, 2001, **123**, 11782.
127 F. Hucho, *Neurochemistry: Fundamentals and Concepts*, VCH, Weinheim, 1986.
128 N. Ohata, H. Masuda and O. Yamauchi, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 531.
129 N. Ohata, H. Masuda and O. Yamauchi, *Inorg. Chim. Acta*, 1999, **286**, 37.
130 N. Ohata, H. Masuda and O. Yamauchi, *Inorg. Chim. Acta*, 2000, **300–302**, 749.
131 S. Otto and J. B. F. N. Engberts, *J. Am. Chem. Soc.*, 1999, **121**, 6798.
132 O. Yamauchi, M. Takani, K. Toyoda and H. Masuda, *Inorg. Chem.*, 1990, **29**, 1856.
133 M. Takani, H. Masuda and O. Yamauchi, *Inorg. Chim. Acta*, 1995, **235**, 367.
134 N. V. Kaminskaia, G. M. Ullmann, D. B. Fulton and N. M. Kostic, *Inorg. Chem.*, 2000, **39**, 5004.
135 N. V. Kaminskaia, T. W. Johnson and N. M. Kostic, *J. Am. Chem. Soc.*, 1999, **121**, 8663.
136 N. V. Kaminskaia and N. M. Kostic, *Inorg. Chem.*, 2001, **40**, 2368.
137 N. M. Milovi and N. M. Kostic, *Met. Ions Biol. Syst.*, 2001, **38**, 145.
138 Y. Shimazaki, T. Nogami, F. Tani, A. Odani and O. Yamauchi, *Angew. Chem., Int. Ed.*, 2001, **40**, 3859.
139 W. Humphrey, A. Dalke and K. Schulten, *J. Molec. Graphics*, 1996, **14**, 33.
140 P. J. Kraulis, *J. Appl. Crystallogr.*, 1991, **24**, 946.