Structure-Stability Relationship in Ternary Copper(II) Complexes Involving Aromatic Amines and Tyrosine or Related Amino Acids. Intramolecular Aromatic Ring Stacking and Its Regulation through Tyrosine Phosphorylation

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Abstract: Stabilization due to the intramolecular noncovalent bonding between the side chains of an L-amino acid (AA) and a diamine (DA) within a ternary Cu(II) complex has been evaluated from the constant K for the following equilibrium:

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

where en and ala are ethylenediamine and L-alanine, respectively, and most of the relevant stability constants have been determined potentiometrically at 25 °C and I = 0.1 M (KNO_3). The complexes Cu(DA)(AA) are stabilized relative to Cu(en)(ala) in the order en ≃ *N,N'*-dibenzylethylenediamine < 1,2-diaminobenzene < histamine ≃ 2-aminomethylpyridine < 2,2'-bipyridine (bpy) < 1,10-phenanthroline for DA and ala ≃ L-valine < L-phenylalanine < L-tyrosine (tyr) < L-tryptophan (trp) < 5-hydroxy-L-tryptophan (htrp) for AA. The significantly positive log K values (0.23-2.22) imply the stacking interaction between the aromatic rings of AA and DA, which is supported by the circular dichroism (CD) spectral magnitude enhancement and the solvent dependence of the spectral patterns. Additional evidence for the stacking was obtained from the rotamer populations calculated from the ^{1}H NMR spectra of related ternary Pd(II) systems with DA = bpy or en and AA = L-tyrosyl-L-glutamate. Stacking involving appreciable charge transfer (CT) was demonstrated for the systems without Cu(II), (protonated 1,10-phenanthroline) (protonated htrp, trp, or tyr), by the CT bands, and by the stability order (tyr < trp < htrp) which coincides with the order of the log K values. The ternary systems with *O*-phospho-L-tyrosine (ptyr) and DA containing pyridine or imidazole rings have negative log K values and show weak solvent dependence of the CD patterns in neutral solution where the phospho ester moiety is fully deprotonated. The results indicate that the aromatic ring stacking is regulated by the phosphorylation of the phenol OH.

Noncovalent interactions are vital in the processes of biological recognition of molecules and subsequent specific reactions.[1] The nucleic base-base, hormone-receptor, enzyme-substrate, and antigen-antibody interactions are among the most important in biological systems, and many of them are achieved by the specific side-chain groups of proteins involved.[2]

Endogenous analgesic peptides (opioid peptides) such as endorphin and enkephalin isolated from the brain invariably have a tyrosyl residue at the N-terminus,[3] which is essential for their analgesic activity[4] probably through control of the transmission of pain.[5] The receptor site has been reported to contain an imidazole group,[6] which prompted us to investigate the interaction between the tyrosine phenol and the histidine imidazole moiety in ternary copper(II) complexes with the expectation that opioid peptides may be bound to the receptor through the interactions with its histidine and other amino acid residues.[7] The phenol moiety of tyrosine consists of a hydrophilic OH group and a hydrophobic benzene ring, and thus shows a bilateral mode of interactions depending on ionization of the OH group or its esters. Phosphorylation and sulfation of the tyrosine OH group currently attract much attention because of their significance in the control of biological processes such as cell growth.[8] Sulfation of the tyrosyl residue of opioid peptides is known to greatly diminish their analgesic activity,[9] suggesting that the hydrophilicity of the phenol sulfate may regulate the physiological activity by disturbing the hydrophobic interaction between the phenol moiety and the receptor site.

In view of the possible importance of the hydrophobic interaction involving a phenol moiety, quantitative evaluation of the extent of such interactions would offer a key to the mechanism of the opioid peptide activity and similar biological phenomena. An efficient approach to this goal is to detect the thermodynamic stabilization and spectral behavior due to noncovalent bonds within ternary metal complexes, where the central metal ion may serve as a positive center, a probe giving various spectroscopic signals, and a template mimicking the three-dimensional environment at the receptor site. The use of metal ions such as Cu(II) may be especially justified, because Cu(II) is believed to be associated with the opioid peptide activity as an agonist[10] and the analgesic action of morphine.[11] Its content in the brain which is abnormally high among various tissues[12] is reported to affect the brain function.[13]

Ligand-ligand interactions in ternary metal complexes as biological models have been studied in detail for amino acids and nucleotides by various methods. We have previously revealed the existence of electrostatic interactions between the charged side chains of a protonated basic amino acid and an acidic amino acid

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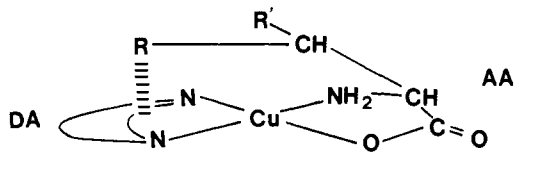
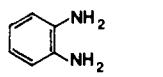
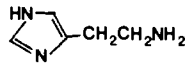
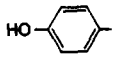
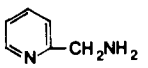
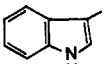
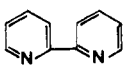
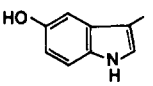
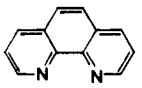
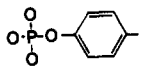
Chart I



DA		AA R	AA R′	
$NH_2CH_2CH_2NH_2$	en	H	H	ala
$C_6H_5CH_2NHCH_2CH_2NHCH_2C_6H_5$	dben	CH_3	CH_3	val
	dabe		H	phe
	hista		H	tyr
	ampy		H	trp
	bpy		H	htrp
	phen		H	ptyr

both coordinated to copper(II) or palladium(II), on the basis of the circular dichroism (CD) spectral magnitude anomaly arising from the interactions and the rotamer populations calculated from the NMR spectra.[14] Sigel and his collaborators have studied ternary metal complexes involving aromatic amines and nucleotides,[15] amino acids,[16] or carboxylates with aliphatic and aromatic groups,[17,18] unambiguously revealed the intramolecular hydrophobic interactions, and evaluated the equilibria between the stacked and unstacked species.[16-20] From careful examinations of the stability constants of a number of ternary amino acid complexes, they concluded considerable aromatic ring stacking in the complexes with such ligand pairs as histidine–tryptophan,[16] which was later confirmed by our potentiometric and CD spectral studies.[7] Hydrophobic interactions between aromatic amines and long aliphatic chains of amino acids or alkylcarboxylates have also been demonstrated for various ternary complexes.[16-18,19a,20] Noncovalent bonding in ternary palladium(II)–peptide–amine complexes has been studied by ^1H NMR spectroscopic methods by Martin et al., who disclosed the hydrophobic ligand–ligand interactions between the side chains of coordinated glycyl-L-phenylalanine and a monodentate amine with a varying length aliphatic chain with or without an attached aromatic ring and determined the stability order of the interactions.[21]

In order to get an insight into noncovalent interactions involving the tyrosine phenol and other side chain aromatic rings of amino acids, we have made a detailed study of the model ternary copper(II) systems comprising bidentate amines (DA) and amino acids (AA) (Chart I) by potentiometric and spectroscopic methods. We report here the structure–stability relationship of aromatic ring stacking in ternary copper(II) complexes and the effect of phosphorylation of the phenol OH group on the stacking, which is a novel example of regulation of the "stacked form ⇌ unstacked form" equilibrium and may serve as a chemical model of the biological processes involving tyrosine phosphorylation.[8,22]

Experimental Section

Materials.[23] Histamine dihydrochloride, 5-hydroxy-L-tryptophan, *O*-phospho-L-serine, and L-tyrosyl-L-glutamic acid were purchased from Sigma, 1,10-phenanthroline hydrochloride monohydrate from Merck, and 2-aminomethylpyridine and *O*-methyl-L-tyrosine from Aldrich. L-Alanine, L-valine, L-phenylalanine, L-tyrosine, L-tryptophan, L-glutamic acid, L-leucine, ethylenediamine, 2,2′-bipyridine, and 1,2-diaminobenzene were purchased from Nakarai. *O*-Phospho-L-tyrosine was prepared according to the literature,[24] and ethylenediamine and 2-aminomethylpyridine were distilled in vacuo and used as the hydrochlorides. All reagents used were of the highest grade available.

Preparation of Cu(phen)(L-AA). The ternary complexes Cu(phen)(L-AA) were prepared according to the following procedures. (a) $Cu(ClO_4)_2\cdot 6H_2O$ (5 mmol) and phen (5 mmol) dissolved in aqueous methanol were mixed with L-AA (5 mmol) and NaOH (5 mmol) in water. The mixture was heated to complete dissolution, and the crystals which separated on standing at room temperature were collected and recrystallized from aqueous methanol to give the following analytically pure complexes: $[Cu(phen)(L\text{-ala})]ClO_4\cdot H_2O$; $[Cu(phen)(L\text{-leu})]ClO_4\cdot H_2O$; $[Cu(phen)(L\text{-phe})]ClO_4\cdot 2H_2O$; $[Cu(phen)(L\text{-trp})]ClO_4\cdot 3H_2O$. (b) For the complex with AA = tyr, $Cu(ClO_4)_2\cdot 6H_2O$, phen, and L-tyr·HCl (5 mmol, respectively) were dissolved in aqueous methanol, and an aqueous solution of NaOH (10 mmol) was added to the mixture. The crystals of $[Cu(phen)(L\text{-tyr})]ClO_4\cdot 3H_2O$ were obtained as described in (a).

Spectral Measurements. CD spectra were measured with a JASCO J-20 or a J-500C spectropolarimeter in quartz cells with path lengths of 0.2–2 cm. Absorption spectra were recorded on a Union Giken SM-401 or a Hitachi 330 recording spectrophotometer. Samples were prepared either by dissolving the isolated Cu(II) complexes or by mixing the stock solutions of Cu(II) and ligands in the desired ratios at various pH. Sample concentrations were 0.2–2 mM with respect to Cu(II), and their pH values were checked with a Horiba M-7II or an M-8 pH meter with a Toko CE103C combination microelectrode. ^1H NMR spectra were measured for palladium(II) complexes at 25 °C by a JEOL FX-100 NMR spectrometer.

pH Titrations. Potentiometric titrations were carried out at 25 °C and I = 0.1 M (KNO_3) as described previously[25] for solutions involving Cu(II), DA, and AA in the molar ratio of 1:0:2 or 1:1:1. The stability constants, β_{pqrs}, defined by eq 1 were calculated by the method of nonlinear least-squares with the computer program MINIQUAD[26] with the aid of a FACOM M-170F computer at the Kanazawa University Computation Center (charges are omitted for simplicity):

$$pCu + q(DA) + r(AA) + sH \overset{\beta_{pqrs}}{\rightleftharpoons} Cu_p(DA)_q(AA)_rH_s$$

$$\beta_{pqrs} = \frac{[Cu_p(DA)_q(AA)_rH_s]}{[Cu]^p[DA]^q[AA]^r[H]^s} \quad (1)$$

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(23) Following abbreviations were used, and unless otherwise specified, those for amino acids refer to deprotonated L-enantiomers: tyr and tyrOH, tyrosinate with undissociated OH; $tyrO^-$, tyr with dissociated OH; mtyr, *O*-methyltyrosinate; H_2tyrOH, tyrosinium ion; H_2trp, tryptophanium ion; htrp and htrpOH, 5-hydroxytryptophanate with undissociated OH; $htrpO^-$, htrp with dissociated OH; $H_2htrpOH$, 5-hydroxytryptophanium ion; ptyr, *O*-phosphotyrosinate; Hptyr, ptyr with monoprotonated phosphate; H_3ptyr, ptyr with protonated amino and carboxyl groups and monoprotonated phosphate; pser, *O*-phosphoserinate; Hpser, pser with monoprotonated phosphate; tyr-glu, tyrosylglutamate; ala, alaninate; val, valinate; leu, leucinate; phe, phenylalaninate; en, ethylenediamine; bpy, 2,2′-bipyridine; ampy, 2-aminomethylpyridine; phen, 1,10-phenanthroline; hista, histamine; dben, *N*,*N*′-dibenzylethylenediamine; dabe, 1,2-diaminobenzene.

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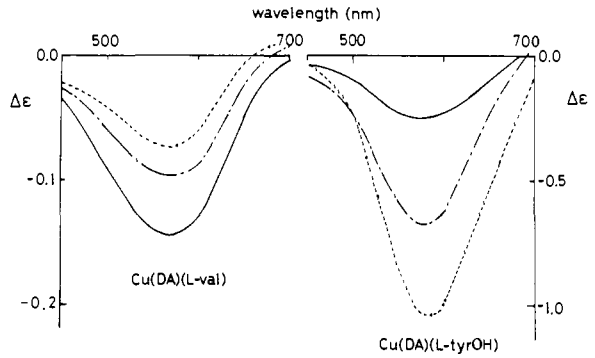

Figure 1. CD spectra of Cu(DA)(L-val) and Cu(DA)(L-tyrOH) in the d-d region at pH 6–6.5. DA: en, —; ampy, -·-; bpy, ---.

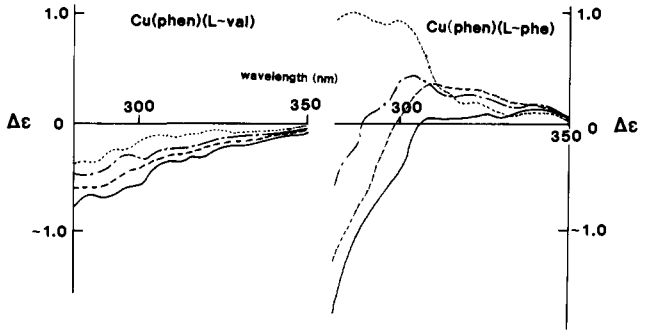

Figure 2. Solvent dependences of CD spectra of Cu(phen)(L-val) and Cu(phen)(L-phe) in the region 280–350 nm. Solvents: water, ---; methanol, -·-; 80 v/v % dioxane–10 v/v % methanol–10 v/v % water, --; 90 v/v % dioxane–10 v/v % methanol, —.

where p, q, r, and s are the numbers of Cu(II), DA, AA, and proton (H), respectively, in the complex $Cu_p(DA)_q(AA)_rH_s$. The ratio of the hydrogen ion concentration obtained from the pH meter reading, pH_M, to the analytical concentration, [H], was determined to be 0.855 under the conditions used. The apparent ion product of water $pK_w' = pH_M - \log [OH]$, where [OH] is the analytical hydroxide ion concentration, was 13.96. The pK_a values and stability constants of the binary complexes and the ternary complexes Cu(dben)(L-AA) and Cu(en)(L-AA) except AA = ptyr and pser were taken from the literature.[16,27-32]

Results

Absorption and CD Spectral Properties. The ternary Cu(DA)(L-AA) systems in aqueous solution exhibit a d–d absorption peak at 580–616 nm at neutral pH (Table I). Dissociation of the tyrosine phenol OH group slightly shifts the peak to shorter wavelengths, while phosphorylation shifts it to longer wavelengths. The corresponding CD spectra have a negative maximum at 510–600 nm and in some cases an additional positive peak at >700 nm. Figure 1 shows that for the systems with tyr and DA = ampy, bpy, or phen, the magnitudes of the negative peak are much stronger than those for the en-containing systems and Cu(DA)(L-val). The magnitude enhancement is in the order of L-AA, phe < $tyrO^-$ < tyrOH < trp, and proportional to the number of aromatic rings in DA, which suggests distortion and increased rigidness of the side-chain conformation due to the interaction between coordinated DA and AA around Cu(II). The enhanced magnitudes for the Cu(phen)(L-AA) systems (AA = phe, tyrOH, or trp) in water are greatly reduced in less polar solvents, further indicating that they are due to the aromatic ring-stacking interaction which is weakened in hydrophobic environments.[33]

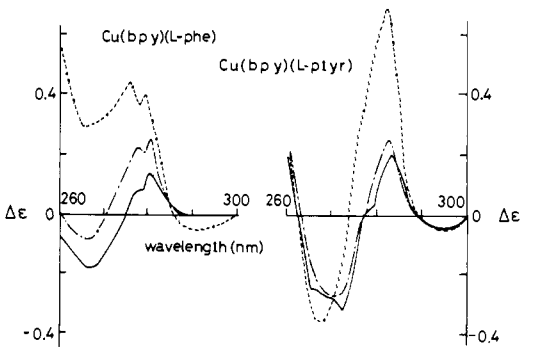

Figure 3. Solvent dependences of CD spectra of Cu(bpy)(L-ptyr) and Cu(bpy)(L-phe) in the region 260–300 nm. Solvents: water, ---; methanol, -·-; 50 v/v % dioxane–methanol, —.

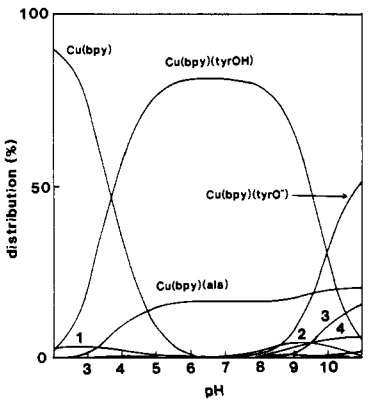

Figure 4. Species distributions as a function of pH in the quaternary Cu(bpy)(ala)(tyr) system.[35] Concentrations: Cu(II), 1 mM; bpy, 1 mM; ala, 1 mM; tyr, 1 mM. Curves for minor species: 1, $Cu(bpy)_2$; 2, Cu(ala)(tyrOH); 3, Cu(ala)($tyrO^-$); 4, $Cu(ala)_2$. The other minor species are omitted from the figure.

Similar solvent dependence of the CD spectral pattern was observed for Cu(phen)(L-phe) at 280–350 nm where the effect of coordinated phen only is reflected (Figure 2). On the other hand, the ternary systems with aromatic DA's and ptyr exhibit only half as strong a peak as that for the systems with tyrOH in place of ptyr and weak solvent dependence (Figure 3).

Stability Constants as a Measure of Stacking Interactions. Stabilization of the ternary systems Cu(DA)(AA) due to aromatic DA and AA may be evaluated by considering the following hypothetical equilibrium:[7]

$$Cu(DA)(ala) + Cu(en)(AA) \overset{K}{\rightleftharpoons} Cu(DA)(AA) + Cu(en)(ala) \quad (2)$$

where all the species have the same coordinating atoms and ligand–ligand interactions, or, more specifically, aromatic ring stacking interactions, are possible only in Cu(DA)(AA). The equilibrium constant $\log K$ is given by the stability constants of the relevant ternary species according to eq 3:[34]

$$\log K = \log \beta_{Cu(DA)(AA)} + \log \beta_{Cu(en)(ala)} - \log \beta_{Cu(DA)(ala)} - \log \beta_{Cu(en)(AA)} \quad (3)$$

where the values $\beta_{Cu(Da)(AA)}$, etc., correspond to the stability constants β_{1110} or β_{1111} (Tables II and III).

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(33) For ternary complexes with phen and arylalkanecarboxylates, Sigel et al.[18b,20] recently observed stronger hydrophobic interactions in 40–50 v/v % aqueous dioxane as compared with those in water. Because the interactions are weakened in pure dioxane and other much less polar solvents, their results suggest that the maximum degrees of hydrophobic interactions in certain systems occur in media of intermediate polarity. The CD spectral magnitudes for the present ternary systems decreased gradually with the increase of dioxane content (Figures 1 and 2).
(34) $\log K$ is also expressed by using $\Delta \log K$ proposed by Sigel[15] as a measure of favorable ternary complex formation.

Table I. Absorption and CD Spectra of Cu(DA)(L-AA) Systems[a]

	DA																							
	en					ampy					bpy					phen					phen[b]				
		absorption		CD			absorption		CD			absorption		CD			absorption		CD			absorption		CD	
AA	pH	λ_{max}	ϵ	λ_{max}	$\Delta\epsilon$	pH	λ_{max}	ϵ	λ_{max}	$\Delta\epsilon$	pH	λ_{max}	ϵ	λ_{max}	$\Delta\epsilon$	pH	λ_{max}	ϵ	λ_{max}	$\Delta\epsilon$	λ_{max}	ϵ	λ_{max}	$\Delta\epsilon$
ala	6.8	593	52	600	−0.03	6.4	600	59	610	−0.01	6.9	600	58	600	0.02	7.1			680	0.02	607	81	546	−0.03
																							692	0.04
val	6.6	593	54	570	−0.10	6.2	595	61	570	−0.06	6.7	598	59	570	−0.05	7.4	614	58	585	−0.08	605	71	562	−0.14
																			720	0.02			700	0.06
leu																7.3	616	58	690	0.02	605	69	560	−0.07
																							708	0.05
phe	6.5	580	55	570	−0.23	5.9	590	64	580	−0.44	6.7	605	72	580	−0.91	6.8	610	77	596	−0.92	609	78	582	−0.56
																			>800	0.11			774	0.11
tryOH	6.5	580	53	570	−0.25	5.6	590	65	580	−0.68	6.3	605	74	580	−1.04	6.8	600	65	597	−1.14	608	89	583	−0.71
																			800	0.12			773	0.11
$tryO^-$	10.1	570	57	570	−0.36	9.6	590	57	570	−0.64	9.5	600	65	580	−0.97									
trp	6.1	580	52	570	−0.32	5.5	595	62	570	−0.80	5.7	600	67	580	−1.15	6.5	611	74	602	−1.22	609	78	588	−0.91
																			>800	0.16			752	0.10
ptyr	7.1	582	55	665	−0.24	7.1	594	65	574	−0.32	7.3	610	71	589	−0.70	7.1	626	76	597	−0.57				
				740	0.02				743	0.05				700	0.13				790	0.07				
ptyr[c]		588	62	668	−0.20		594	69	580	−0.17		610	68	584	−0.45		626	76	589	−0.32				
				747	0.04				745	0.05				760	0.10				758	0.05				
pser						6.9	600	64	500	0.01	7.6	606	58	550	0.07	7.1	625	62	555	0.03				
									610	−0.08									725	0.03				
pser[c]							600	67	600	−0.07		620	64	540	0.01		638	71	555	0.06				
														638	−0.03				755	0.05				
														795	0.02									

[a] The systems except those with DA = phen and/or AA = ptyr or pser were measured in the range 400–700 nm. [b] Measured in 80 v/v % dioxane–10 v/v % methanol–10 v/v % water. The CD spectral data (AA, λ_{max} ($\Delta\epsilon$)) in 80 v/v % dioxane–20 v/v % methanol are as follows: ala, 535 (−0.04), 680 (0.02); val, 562 (−0.14), 685 (0.04); leu, 560 (−0.08), 695 (0.03); phe, 578 (−0.51), 760 (0.09); tyrOH, 575 (−0.60), 755 (0.09); trp, 580 (−0.67), 752 (0.10). [c] Measured in methanol.

Table II. Stability Constants log β_{pqrs} for Proton–Ligand and Cu(II)–Ligand Complexes at 25 °C and I = 0.1 M (KNO_3)[a]

species	L-AA									
pqrs	ala[b]	val[c]	glu[c]	phe[c]	tyr[d]	mtyr[e]	trp[c]	htrp[e]	ptyr	pser
1011			12.73		17.99			18.606	14.218 (2)	14.901 (3)
1010	8.33	8.049	8.545	7.931	10.64	7.742	8.020		9.327 (2)	9.756 (4)
1022			25.18		34.90			36.760		
1021			20.57		25.47			26.258		
1020	15.27	14.913	15.222	14.834	15.36	14.663	15.562	15.259	14.940 (4)	15.869 (4)
0011	9.82	9.573	9.746	9.194	10.142	9.068	9.312	10.764	9.540 (2)	9.999 (1)
0012	12.16		13.988	11.452	19.170	11.159		20.128	15.228 (3)	15.784 (2)
0013			16.27		21.051			22.559	17.166 (6)	17.945 (3)

species	DA						
pqrs	en[c]	ampy[f]	bpy[c]	phen[b]	hista[g]	dben[e]	dabe[h]
1100	10.523	9.72	8.10	9.25	9.67	7.963	4.55
1200	19.505	17.47	13.44	16.00	16.41	12.754	7.72
0101	9.976	8.70	4.503	4.95	9.92	8.938	4.63
0102	17.148	10.75			16.06	14.941	

[a] Values in parentheses denote estimated standard deviations. [b] Reference 16. [c] Reference 27. [d] Reference 31. [e] Reference 32. [f] Reference 28. [g] Reference 29. [h] Reference 30.

Table III. Stability Constants log β_{pqrs} for Ternary Cu(II) Complexes at 25 °C and I = 0.1 M (KNO_3)[a]

		DA						
L-AA	species *pqrs*	phen	bpy	ampy	hista	dabe	dben	en
ala	1110	17.131 (1)	16.116 (2)	17.344 (0)	17.321 (1)	12.008 (1)	15.853[b]	17.949[b]
val	1110	16.987 (2)	15.911 (1)	17.240 (1)	17.162 (1)	11.955 (1)	15.695[b]	17.726[b]
glu	1111		20.812 (2)					23.267[c]
	1110		16.455 (1)					18.317[c]
phe	1110	17.570 (2)	16.513 (3)	17.360 (1)	17.375 (1)	11.936 (1)	15.487[b]	17.746[b]
tyr	1111	28.001 (3)	26.838 (4)	27.715 (2)	27.651 (2)	21.969 (4)	26.616[b]	27.772[b]
	1110		16.879 (5)	18.234 (2)	17.944 (3)		16.164[b]	18.462[b]
mtyr	1110		16.766 (2)				15.325[b]	17.580[b]
trp	1110	18.647 (7)	17.436 (1)	17.951 (3)	18.054 (1)	12.201 (2)	16.113[b]	18.078[b]
htrp	1111	30.052 (6)	28.619 (3)	28.873 (2)	28.901 (4)	23.149 (2)	26.750[b]	28.655[b]
	1110			18.650 (2)	18.572 (7)		16.424[b]	18.585[b]
ptyr	1111	23.675 (2)	22.530 (4)		23.467 (3)			24.141 (4)
	1110	17.654 (5)	16.515 (6)		17.715 (2)			18.492 (2)
pser	1111	23.783 (4)	22.655 (2)					24.621 (4)
	1110	18.015 (4)	16.870 (2)					18.955 (3)

[a] Values in parentheses denote estimated standard deviations. [b] Reference 32. [c] Reference 7.

Table IV. log K Values for Cu(DA)(L-AA) Systems[a]

		DA						
AA	species *pqrs*	phen	bpy	ampy	hista	dabe	dben	en
ala	1110	0.00	0.00	0.00	0.00	0.00	0.00	0.00
val	1110	0.08	0.02	0.12	0.06	0.17	0.07	0.00
glu	1110		−0.03					0.00
phe	1110	0.64	0.60	0.22	0.26	0.13	−0.16	0.00
tryOH	1111	1.05	0.90	0.55	0.51	0.14	−0.06	0.00
$tyrO^-$	1110		0.25	0.38	0.11		−0.20	0.00
mtyr	1110		1.02				−0.16	0.00
trp	1110	1.39	1.19	0.48	0.60	0.06	0.13	0.00
htrpOH	1111	2.22	1.80	0.82	0.87	0.44	0.19	0.00
$htrpO^-$	1110			0.67	0.62		−0.07	0.00

[a] Calculated according to eq 3 with Cu(DA)(L-ala) and Cu(en)(L-AA) as standards.

Table V. log K and log $\beta_{1111}/\beta_{1110}$ Values for Cu(DA)(L-AA) Systems[a]

	log K				log $\beta_{1111}/\beta_{1110}$	
DA	Hptyr	ptyr	Hpser	pser	ptyr	pser
phen	0.35	−0.02	−0.02	−0.12	6.02	5.77
bpy	0.22	−0.14	−0.13	−0.25	6.02	5.79
hista	−0.05	−0.15	0.02	−0.10	5.75	5.79
en	0.00	0.00	0.00	0.00	5.65	5.67

[a] Calculated according to eq 3 with Cu(DA)(L-ala) and Cu(en)(AA) as standards.

The log K values as a parameter giving the stabilization within the ternary complex are comparable to the $\Delta\Delta$ log K_M values defined by Sigel et al.,[16,19] but the former reflect the stabilization due to specific combination of certain DA and AA among similar ligands. We chose as the standards the systems with DA = en and AA = ala and calculated the log K values for various Cu(DA)(AA) systems from the stability constants according to eq 3. Table IV shows that the systems with aromatic DA and AA have large positive log K values as compared with those for the systems with aliphatic DA or AA. Favorable combination of bpy with tyr around Cu(II) is evident from Figure 4, which illustrates the calculated percentage species distributions in a quaternary Cu(bpy)(ala)(tyr) system:[35] as compared with Cu(bpy)(ala) (17%), Cu(bpy)(tyr) is formed preferentially (82%) in a wide pH range. While the Cu(DA)(AA) systems with DA = dabe or dben and AA = val, phe, tyrOH, or trp have log K values less than 0.17, the systems with DA = phen, bpy, ampy, or hista and AA = phe, tyrOH, mtyr, trp, or htrpOH have values ranging from 0.22 to 2.22, the orders being htrpOH > trp > tyrOH > phe for AA and phen > bpy > ampy ≃ hista for DA. The order of AA, trp > tyr > phe, agrees with that concluded from the calculated $\Delta\Delta$ log K_M and K_I values by Fischer and Sigel[16] and that found in our previous study.[7] The enhanced log K values are reasonably proportional to the area of the aromatic rings of DA and AA, supporting the stacking between them. The log K increments due to the component rings[36] and the OH group are calculated to be 0.1–0.3 and 0.2–0.3 log unit, respectively.

Comparison of the values for Cu(DA)(tyrOH) and Cu(DA)($tyrO^-$) (DA = bpy, ampy, or hista) reveals that deprotonation of the phenol OH group decreases the ternary complex stability in spite of the neutralization of the total charge attained as the result. On the other hand, phosphorylation of the OH group of tyr has a greater effect on the stability: for the systems with a monoprotonated phospho ester group, Cu(DA)(Hptyr), the log K values are comparable with those for Cu(DA)($tyrO^-$), but the fully deprotonated phospho ester group lowers them close to the stabilities of Cu(DA)(pser) where no stacking interaction is possible (Table V). These points will be discussed in more detail. The differences log β_{1111} − log β_{1110} (5.7–6.0) are equal to the pK_a values of the phospho ester group, indicating that the phosphate moiety of ptyr and pser is not involved in the metal binding.

Discussion

Aromatic Ring Stacking with Charge Transfer in the Ternary Systems Cu(DA)(AA). The enhanced log K values and increased

(35) The species distributions were calculated from the stability constants listed in Tables II and III and ref 7 by considering the following complex species $Cu_p(bpy)_q(ala)_r(tyr)_s(H)_t$ expressed as *pqrst* (no quaternary complexes were considered): 11100; 11011; 11010; 10111; 10110; 11000; 12000; 10100; 10200; 10011; 10010; 10022; 10021; 10020; 01001; 00101; 00102; 00011; 00012; 00013.

(36) The component rings refer to pyridine, imidazole, benzene, or pyrrole rings of DA and AA.

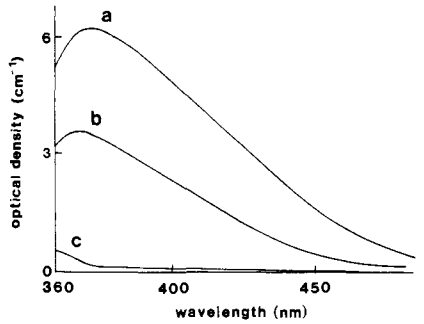


Figure 5. Difference spectra indicating charge transfer between phen and the aromatic ring of AA in (Hphen)(L-AA) systems. Curves: a, (Hphen)(H_2htrp); b, (Hphen)(H_2trp); c, (Hphen)(H_2tyrOH). Conditions: [phen] = [L-AA] = 0.02 M; I = 0.4 M (HCl); 23 °C.

Table VI. Stability Constants log K' and CT Spectral Properties of CT Complexes

complex	solvent	log K'	CT band[a] λ_{max}	CT band[a] ϵ
(Hphen)(H_2htrpOH)	H_2O	1.7	370	820
	60% (v/v) dioxane–H_2O	0.4		
(Hphen)(H_2trp)	H_2O	1.4	360	640
	60% (v/v) dioxane–H_2O	−0.2		
(Hphen)(H_2tyrOH)	H_2O	1.0	<360	
(Hphen)(H_3ptyr)	H_2O	0.3	<360	

[a] Obtained from the difference spectra measured at 23 °C and I = 0.4 M (HCl).

asymmetry of coordinated amino acid side chains as can be seen from the CD spectral magnitudes clearly indicate that there is an attractive interaction between DA and AA when both have aromatic rings. The free energy change accompanying the complex formation defined by eq 2 is calculated from the relation $\Delta G° = -2.303RT \log K$ to be −1.3 kJ mol^{-1} for Cu(hista)(phe) and Cu(ampy)(phe) and as large as −12.7 kJ mol^{-1} for Cu(phen)(htrpOH). This large stabilization points to considerable electron density difference between the stacked rings, which would give rise to CT absorption bands usually in the near ultraviolet region. For the ternary systems involving Cu(II), an aromatic amine, and a nucleotide, a CT band has been reported to appear in this region in the difference spectra.[37] Although the Cu(DA)(AA) systems with aromatic rings failed to give unambiguous CT peaks, the systems involving protonated phen (Hphen) and tyrOH, trp, or htrpOH exhibited a single well-resolved CT peak near 360 nm in the difference spectra (Figure 5), showing that there is a CT transition between Hphen and AA and that the proton behaves just as Cu(II) as a positive center making phen electron deficient. This is supported by the fact that the CT peak disappears in alkaline solution owing to deprotonation, and in line with the self-stacking of phen in the monoprotonated dimeric species H(phen)$_2$ concluded from ^{1}H NMR studies.[38,39] Similar CT bands have been observed for the systems with indoles and oxidized nicotinamide-adenine dinucleotide having a positive charge.[40] Since the OH group in htrpOH increases the CT band intensity with the maximum wavelength unchanged, it probably serves as an electron donor and makes the aromatic ring interaction more effective. The stability constants, K', of the CT complexes defined by eq 4 were calculated from the concentration dependence of the CT band intensities (Table VI):

$$\text{Hphen} + \text{AA} \overset{K'}{\rightleftharpoons} \text{(Hphen)(AA)} \qquad (4)$$

where AA denotes a protonated amino acid, H_2trp, H_2htrpOH, H_2tyrOH, or H_3ptyr. The stability constants are smaller in 60

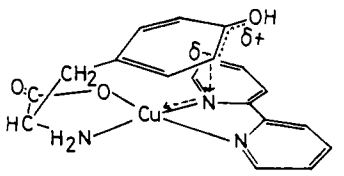


Figure 6. Proposed structure of Cu(bpy)(L-tyrOH) showing the electron flow.

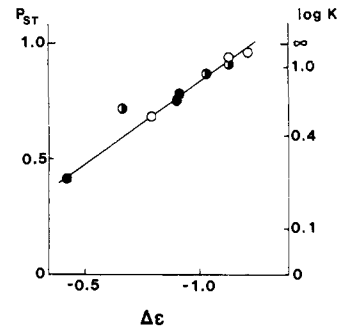


Figure 7. Relationship between populations of stacked species and CD magnitudes exhibited by the Cu(aromatic DA)(aromatic L-AA) systems in the d–d region. Aromatic L-AA: trp, ○; tyrOH, ◑; phe, ●.

v/v % dioxane–water, indicating that the CT complexes are less stable in a less polar solvent.[33,41] Interestingly the stability sequence of (Hphen)(AA) determined by the log K' values corresponds well with that of the ternary systems Cu(phen)(AA) (htrpOH > trp > tyrOH > ptyr) involving Cu(II) in place of the proton attached to phen.

Space-filling models of Cu(bpy)(tyrOH) suggest that in the stacked structure the carbons ortho to the OH group of tyrOH are placed above one of the Cu(II)-coordinated nitrogens of bpy (Figure 6). This conformation would allow the electron flow from the phenol moiety to the electron-deficient bpy. The OH group tends to increase the ring electron density,[42] which makes the CT interaction stronger[18b] and gives rise to preference for the stacking involving phenol rings. On the other hand, stacking between rings with similar electron densities, i.e., those without directly attached electron positive or negative groups, should have a weaker stabilizing effect. Comparison of the log K values for the systems with DA = ampy and hista indicates that the stacking with the imidazole ring makes nearly the same contribution to the complex stability as that by the pyridine ring. The low values (−0.16 to 0.19) for Cu(dben)(AA) and Cu(dabe)(AA) except Cu(dabe)(htrpOH) (log K = 0.44), which appears to be favored to some extent by the interaction, are ascribed to the lack of CT and poor ring overlapping. According to a recent study on the ternary Cu(II) and Ni(II) complexes involving phen or bpy as DA and tyr, phe, or 3,4-dihydroxyphenylalanine as AA, the preferential formation of ternary Cu(II) complexes has been interpreted as due to the Cu(II)–aromatic ring interaction and not to the aromatic ring stacking on the basis of the constructed models and lack of CT bands.[43] However, the solvent dependences of the CD spectra in the 280–350 nm region (Figure 2), where the bands due to phen and possible CT between stacked rings are observed, clearly show that the electronic transition involving phen is affected by the dioxane content which reduces the solvent polarity and

(37) Naumann, C. F.; Sigel, H. *J. Am. Chem. Soc.* **1974**, *96*, 2750–2756.
(38) Mitchell, P. R. *J. Am. Chem. Soc.* **1980**, *102*, 1180–1181.
(39) Mitchell, P. R. *J. Chem. Soc., Dalton Trans.* **1980**, 1079–1086.
(40) Alivisatos, S. G. A.; Ungar, F.; Jibril, A.; Mourkides, G. A. *Biochim. Biophys. Acta* **1961**, *51*, 361–372.
(41) During the revision of the manuscript, a study of protonation of phen in water and various dioxane–water mixtures has been published (Ishiguro, S.; Wada, H.; Ohtaki, H. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 932–937). It shows that Hphen and H(phen)$_2$ species explain the titration curve at pH 2–7 and that formation of H(phen)$_2$ is negligible in media with the mole fraction of dioxane greater than 0.1 (ca. 34 v/v %). The species H(phen)$_2$ corresponding to (Hphen)(AA) in eq 4 has the log K_2 values (= log K' in eq 4) of 1.69 at 25 °C and I = 0.3 (NaCl) which is remarkably close to the value of 1.7 for (Hphen)(H_2htrpOH) in water, suggesting that the 5-hydroxyindole moiety in fully protonated htrp stacks with Hphen nearly as effectively as phen.
(42) Hammett, L. P. "Physical Organic Chemistry", 2nd ed.; McGraw-Hill: New York, 1970.
(43) Patel, V. K.; Bhattacharya, P. K. *J. Inorg. Biochem.* **1984**, *21*, 169–177.

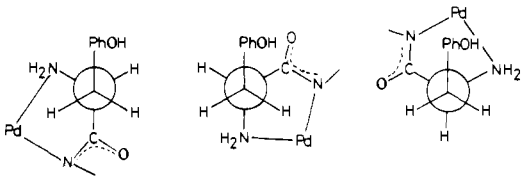
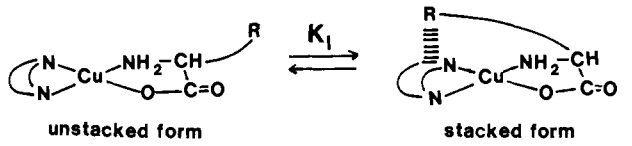


Figure 8. Staggered rotamers viewed through the C_α–C_β bond in the tyr moiety of the Pd(tyr·glu) complex.

hence the ring stacking. Our previous studies[32,44] have indicated that the stabilization due to the Cu(II)–aromatic ring interaction is much smaller than that due to the stacking, which is in accordance with the conclusion by Sigel et al.[20] on ternary Cu(II) complexes and the stability sequence of ligand–ligand and metal–ligand interactions in ternary Pd(II)–dipeptide–amine complexes determined by the ^{1}H NMR spectroscopic method by Kim and Martin.[21] The latter investigators evaluated the $-\Delta G°$ values for the Pd(II) aromatic ring interaction to be 0.3–1.5 kJ mol^{-1} which is smaller than the values of 1.3–12.7 kJ mol^{-1} for the present Cu(DA)(AA) complexes.

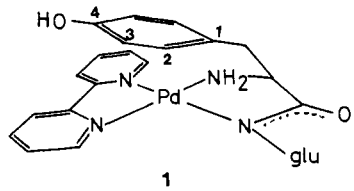
The fractional populations P_{ST} of stacked complex species in solution may be evaluated from the log K values according to eq 5.[16,19,45]



$$K_I = 10^{\log K} - 1$$
$$P_{ST} = 1 - 10^{-\log K} \quad (5)$$

where $K_1 \geq 0$ and hence log $K \geq 0$. Figure 7 demonstrates that the CD magnitudes in the d–d region for the Cu(DA)(L-AA) systems, where both DA and AA are aromatic, correlate well with the log K_1 and accordingly the P_{ST} values, supporting that the side chain conformation of AA becomes fixed in the ternary systems owing to aromatic ring stacking.

Additional Evidence for Aromatic Ring Stacking. Indirect but convincing evidence for the proposed stacking is furnished by the ^{1}H NMR study of Pd(II) complexes with analogous coordination structures and aromatic rings. By assuming that both Cu(DA)(AA) and Pd(DA)(tyr·glu), where DA denotes bpy or en, have the same planar structure, we may expect that the stacking between bpy and the aromatic side chain as shown by structure **1** corresponds to the increase of the fractional population (P_{III})



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of the staggered rotamer III (Figure 8) at the α-carbon of the tyr moiety, because only rotamer III makes the stacking possible and causes upfield shifts of the phenol ring proton signals due to the ring current effect of bpy. Figure 9 shows that the C3 proton signal for Pd(bpy)(tyr·glu) is shifted upfield as compared with that for Pd(en)(tyr·glu). With Pd(en)(tyr·glu) as standard the shift difference and the P_{III} value calculated according to Martin[46] are 0.66 ppm and 0.79, respectively, and decrease to 0.37 ppm and 0.43 upon dissociation of the OH group, which is in line with the aromatic ring stacking in Pd(DA)(tyr·glu) and hence also in

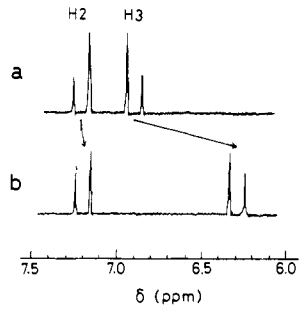

Figure 9. ^{1}H NMR spectra of Pd(en)(tyr·glu) (a) and Pd(bpy)(tyr·glu) (b) showing the aromatic proton signals.

Cu(DA)(AA) with similar modes of coordination.

Inhibition of Aromatic Ring Stacking by Phosphorylation of the Phenol OH Group. As shown in Table V, the ternary systems with phosphorylated tyr, ptyr, have negative log K values close to those for pser when the phospho ester group is fully deprotonated, indicating the absence of aromatic ring stacking. Weak magnitudes and slight solvent dependence of their CD spectra support that there is no appreciable conformational changes indicative of the stacking upon formation of Cu(DA)(ptyr). The differences of log K values between Cu(DA)(tyrOH) and Cu(DA)(ptyr) are 1.07, 1.04, and 0.66 for DA = phen, bpy, and hista, respectively, and satisfactorily large for blocking effective stacking. This inhibition of stacking mainly results from the presence of the dinegative phospho ester group making the aromatic side chain hydrophilic as seen from the hydrogen bonds through the phosphate moiety of pser[47] and *O*-sulfotyrosine[48] in the solid state and from the NMR spectra of an *O*-sulfotyrosine-containing peptide in solution.[49] Alternatively, the influence of the phospho ester group may be viewed as arising from the steric hindrance due to the hydration shell around this group.[50] The log K difference (0.37) between Cu(phen)(Hptyr) and Cu(phen)(ptyr) indicates that the charges of the phospho ester group have a stepwise negative effect on the stability gained by stacking. Similar destabilization is observed for the systems with deprotonated tyrosine, tyrO$^-$ (Table IV), but the effect is much greater with the phospho ester group.

These results indicate that the phospho ester group almost completely inhibits aromatic ring stacking at neutral–alkaline pH by the hydration of this group and partly by canceling the contribution of 0.3 to log K by the electron-donating OH group.

Concluding Remarks on Stacking in Ternary Copper(II) Complexes and Its Possible Biological Significance. The present study reveals that stacking occurs between the aromatic rings incorporating coordinated nitrogens and the side-chain aromatic rings of coordinated amino acids. Remarkable stabilization of the ternary complexes due to stacking has been detected for the Cu(DA)(AA) systems with phen and bpy as DA and trp and htrpOH as AA. The systems with tyrOH and htrpOH are more stabilized than those with phe and trp, respectively, indicating the effect of the phenol OH group. As evidenced by the CT transition in (Hphen)(AA) where AA refers to H_2trp, H_2htrpOH, or H_2tyrOH, the transition probably occurs from AA to DA in the Cu(II)-containing ternary systems.[18b]

The present study as well as previous studies[7,16] established that the coordinated imidazole ring of histidine and hista can be involved in ring stacking with the aromatic side chains of amino acids. Formation of stable Cu(hista)(tyrOH) may imply that the opioid peptide receptor site, where an imidazole ring has been reported to be involved,[6] effectively binds through stacking the tyrosine phenol moiety of enkephalin and other opioid peptides when the imidazole nitrogen is protonated or metal coordinated.

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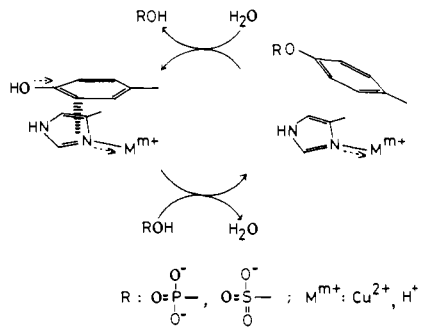

Figure 10. Proposed on-off regulation of stacking by phosphorylation or sulfation at the phenol moiety of tyr.

The importance of hydrophobic interactions in the opioid activity suggested from experiments in vitro[51] lends further support to this view.

A significant outcome of the present investigation is that the ring stacking is modulated by deprotonation of the OH group and most remarkably by its phosphorylation. The stability differences between the systems with nonphosphorylated and phosphorylated tyrosine have been determined to be 0.66-1.07 log units for various DA's, which means that phosphorylation results in nearly tenfold excess of the unstacked species over the stacked one. Because of the high pK_a value, dissociation of the tyrosine phenol OH group is difficult under physiological conditions, and accordingly the possibility of regulating the stacking in vivo by this process may be low without the aid of metal ions. Phosphorylation and sulfation by relevant kinases,[52] however, quite effectively convert the OH group to highly polar groups and may be a plausible biological choice for facile on-off switching of the stacking interaction at physiological pH (Figure 10). It is noteworthy in this context that sulfation of the tyrosyl OH group of opioid peptides greatly decreases their analgesic activity.[9] In addition to possible significance in the opioid peptide binding, stacking interactions can be an important step in enzymatic reactions such as transphosphorylation[53] and biological electron transfer.[54] Tyrosinase, a copper-containing monooxygenase that converts monophenols to diphenols,[55] has been inferred to have coordinated imidazoles at the binuclear copper site;[56] phenols as substrates may be fixed through stacking with the imidazoles as well as through the proposed direct bonding with the copper.[55b,57] Interestingly it is not benzene but phenol that is oxygenated by this enzyme.

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Polarized X-ray Absorption Edge Spectroscopy of Single-Crystal Copper(II) Complexes

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Abstract: Polarized X-ray absorption Cu K-edge spectra (monitored as the Cu Kα excitation fluorescence signal) have been measured on oriented single crystals of a series of square-planar Cu(II) complexes, including single-ligand species of the type CuN_4 and $CuCl_4$ and trans-mixed-ligand species of the type CuN_2Cl_2 (N = imidazole or pyridine ligands). For all complexes studied, the polarized edge spectra reveal two prominent features at ~8986 and ~8993 eV for polarizations perpendicular to the equatorial ligand plane. The positions of these sharp features change by less than 2 eV over the range of complexes studied. The spectra obtained with polarization along the in-plane Cu-ligand bonds reveal higher-energy broad or split principal maxima located between 8994 and 9001 eV. Unlike the lower-energy sharp resonance peaks, the positions of these principal maxima are strongly influenced by in-plane ligand differences and exhibit an inverse relationship to the Cu-ligand distance along the direction of polarization. Multiple-scattered-wave Xα calculations have been successful at qualitatively reproducing the prominent features of the square-planar Cu(II) polarized spectra, including the two sharp lower-energy out-of-plane-polarized features and the bond distance dependence of the in-plane polarized principal maxima. On the basis of the results of calculations and the observed orientational dependence of the edge features, the sharp resonances at 8986 and 8993 eV are assigned to a Cu 1s to $4p_z$ bound-to-bound transition and a 1s to localized continuum resonance, respectively. The higher-energy broad principal maxima observed with in-plane polarization are assigned to more delocalized continuum shape resonances.

Absorption spectra in the X-ray region are characterized by abrupt increases in the absorption coefficient, giving rise to sharp discontinuities (absorption edges). These absorption edges, resulting from the removal of a core electron, occur at characteristic threshold energies specific to a particular absorbing atom. The spectra are generally divided into two main regions: (a) the low-energy edge and near-edge region and (b) the higher-energy extended X-ray absorption fine structure (EXAFS) region, although the distinction between the regions is somewhat arbitrary.

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