compounds: reaction of cystine and AuSTm,[30] reaction of cysteine and AuSTm,[18] and spontaneous decomposition of $(C_6H_5)_3PAuSCy$.[31] The factors causing the very low solubility of AuSCy at neutral pH are unknown, but they apparently drive many reactions involving gold and cysteine to completion.

Given the large values for the equilibrium formation constant of $Au(CN)_2^-$ in the literature (4×10^{28} or 10^{39}),[20,21] the presence of substantial amounts of $RSAuCN^-$ at equilibrium in the presence of 2 mol of cyanide was somewhat unexpected. The estimated formation constants for AuSCy and $Au(SCy)_2^-$, however, demonstrate substantial thermodynamic stability for these species. Stability constants for other gold(I) thiolates are presumably of similar magnitude, and hence, formation of $RSAuCN^-$ by thiol displacement of CN^- is thermodynamically feasible, but not favorable.

Graham et al.[9] examined the reactions of gold thioglucose and $AuSTm^{2-}$ with cyanide using UV spectral changes and chemical analysis of HCN and concluded that the reaction is dominated by formation of $Au(CN)_2^-$. While this conclusion holds at CN^-/Au ratios approaching or exceeding 2.0, the probes used here, which are more sensitive to the molecular species involved, demonstrate that the mixed-ligand complexes such as $TmSAuCN^{3-}$ are important at lower CN^-/Au ratios. The formation of $RSAuCN^-$ in equilibrium with $Au(CN)_2^-$ explains their observation of volatile cyanide, HCN, before the CN^-/Au ratio reaches 2.0 (Figure 6 of ref 9).

In the blood, the concentration of protein-bound gold species such as the serum albumin complex AlbSAuSR (RSH = GtSH, CySH, TmSH, etc.) is likely to exceed the cyanide concentration, so that $CN^-/Au \leq 1.0$. Under these conditions AlBSAuCN and $RSAuCN^-$, etc., may be the major species present and contribute to the transport of gold across cell membranes into red blood cells.

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Registry No. $Au(CN)_2^-$, 14950-87-9; CN^-, 57-12-5.

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^1H NMR Study of Aromatic Ring Stacking in Ternary Palladium(II) Complexes Involving Aromatic Diamines and Dipeptides with N-Terminal Aromatic Amino Acid Residues

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A ^1H NMR spectroscopic study on intramolecular aromatic ring stacking has been made for the ternary palladium(II) complexes Pd(L)(DA), where L refers to dipeptides with the N-terminal aromatic amino acids tyrosylglutamate, tyrosylglycinate (Tyr-Gly), tryptophylglutamate (Trp-Glu), tryptophylglycinate (Trp-Gly), phenylalanylglutamate, or phenylalanylglycinate and DA refers to 2,2′-bipyridine (bpy), 4,7-diphenyl-1,10-phenanthroline-4′,4″-disulfonate (bphen), or ethylenediamine (en). The ternary complexes have a planar N_4 coordination structure, and the fractional populations of the three staggered rotamers calculated from the coupling constants indicated that the rotamer capable of intramolecular ring stacking is favored in Pd(L)(DA) (DA = bpy or bphen) as compared with Pd(L)(en), which does not involve the stacking. Upfield shifts $\Delta\delta$ of the ring proton signals of L due to proximal aromatic rings of DA (0.66–1.27 ppm for H-3 of the tyrosine phenol ring) have substantiated the stacking interaction. From the observed $\Delta\delta$ values and the values for complete stacking, the stability, log K, of the stacking in Pd(L)(DA), which is related to the constant K_{ST} for the "unstacked form $\rightleftharpoons$ stacked form" equilibrium by $K = K_{ST} + 1$, has been evaluated to be high for L = Trp-Gly (1.73), Trp-Glu (0.95), and Tyr-Gly (0.95) at 25 °C, the orders being indole > phenol > benzene > phenolate as expressed by the side chain aromatic ring of L and bphen > bpy. Temperature dependences of log K values have revealed that the enthalpy change contributes to the stacking interaction. Dissociation of the tyrosine phenol OH group of L weakens the aromatic ring stacking, with a log K decrease of 0.26–0.73, indicating that the stacking is regulated by introducing a charged substituent.

Introduction

Ligand–ligand interactions in mixed-ligand–metal complexes as a source of ligand discrimination that leads to specificity or selectivity of ternary complex formation have attracted much attention in recent years for their relevance to biological reactions involving metal ions at the active sites.[1] Ligand specificity arising from the interactions in ternary complexes may be regarded as a prototype of the substrate specificity of metalloenzymes and may offer a key to elucidating metal ion mediated specific interactions between biomolecules. It may also give information on the processes of biological recognition both in the absence and the presence of metal ions,[2] because metal ions serve as a probe giving various signals, and the coordination structure may mimic the stereochemistry at the recognition site.

We have been interested in the copper(II) complex formation by tyrosine-containing peptides[3] as models for endogenous peptides with morphine-like analgesic activity (opioid peptides) such as enkephalin and endorphin, all of which have the essential N-terminal tyrosyl residue with a phenol group capable of binding with copper(II)[3,4-9] and stacking with aromatic rings.[10-12] The

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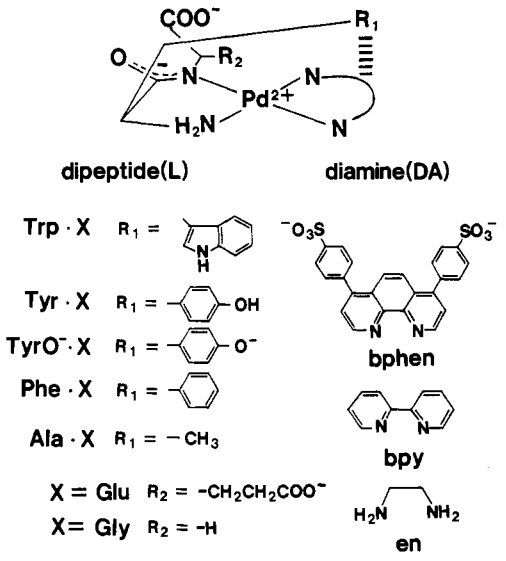
Chart I



reactions with copper(II) seem to be particularly important, because it is believed to be involved in the opioid peptide activity,[13] and the copper content in brain is abnormally high among various tissues.[14] Considering that the receptor site of opioid peptides has been reported to have a histidine imidazole group,[15] it is tempting to infer that the opioid peptides may be bound to the receptor partly through the stacking interaction between this imidazole and the phenol group of N-terminal tyrosine, possibly in the presence of a Lewis acid such as proton and copper ion; participation of transition-metal ions in the neuronal activity is supported by recent studies showing that zinc(II) is both taken up and released upon electrical stimulation of hippocampal tissue.[16] An important characteristic of the phenol ring is that it has a hydrophilic OH group attached to the hydrophobic benzene ring, and accordingly hydrophobic interactions involving the phenol moiety could be modulated by the charge introduced by deprotonation, sulfation, or phosphorylation of the OH group.

Stability enhancement and circular dichroism (CD) spectral magnitude anomalies have been detected for the ternary copper(II) complexes containing aromatic diamines such as 2,2′-bipyridine (bpy) and 1,10-phenanthroline and aromatic amino acids such as tyrosine and tryptophan.[12] It was concluded that stacking takes place between the aromatic rings of coordinated ligands, in agreement with the earlier conclusions on related systems,[10,11] and phosphorylation of the phenol OH group was found to decrease the ternary complex stability and thus effectively inhibit the stacking interaction. The ^{1}H NMR spectrum of the ternary palladium(II) system with bpy and tyrosylglutamate exhibited the upfield shifts due to the ring current effect, further supporting the existence of such an interaction under comparable steric requirements.[12]

A closer insight into the intramolecular aromatic ring stacking may be obtained from the conformational changes accompanying the interaction. As regards the ternary Pd(II)–dipeptide–aliphatic or –aromatic monoamine systems, Kim and Martin[17] observed

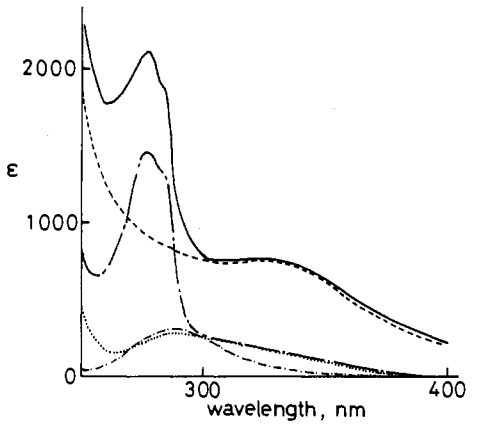


Figure 1. Absorption spectra of binary and ternary Pd(II) complexes: (—) Pd(Tyr-Gly) (pH 7.1); (- -) Pd(Gly-Gly) (pH 7.0); (– – –) Pd(Tyr-Gly)(en) (pH 7.0); (···) Pd(Gly-Gly)(en) (pH 6.9); (·–·) Pd(en)$_2$ (pH 7.0).

the stability enhancement due to the hydrophobic side chains and evaluated the phenyl ring–aromatic ring interaction energy to be larger than the Pd(II)–aromatic ring interaction energy by a ^{1}H NMR spectroscopic method. Sigel et al.[10,18] have detected the upfield shifts of the signals of the ternary complexes involving a metal ion, an aromatic diamine such as bpy, and a second ligand (a nucleotide, an amino acid, or a carboxylate with an aryl or an alkyl group). They concluded that the intramolecular hydrophobic interaction exists between the coordinated aromatic diamine and the side chain aromatic or aliphatic group.

In order to get convincing evidence for intramolecular interactions in copper(II) and other metal complexes among a variety of aromatic rings with and without charged groups, we made a detailed ^{1}H NMR spectral study of ternary Pd(II)–dipeptide–diamine systems with a square-planar N_4 coordination structure formed by the two nitrogens of diamine (DA) and the amino and deprotonated peptide nitrogens of dipeptide (L) (Chart I). Our previous ^{1}H NMR studies[19] have shown that the ternary palladium(II) complexes mimic the structural features of the corresponding copper(II) complexes as concluded from the CD spectra. We here report the stacking interactions in ternary palladium(II) complexes, which have been revealed by the upfield shifts of the proton signals due to the ring current effect and the calculated rotamer populations, and discuss the effects of the aromatic ring size, the phenol OH group, and its phosphorylation on the "unstacked form ⇌ stacked form" equilibrium constants evaluated from the NMR spectra.

Experimental Section

Materials.[20] Tyr-Gly, Tyr-Glu, Trp-Gly, Trp-Glu, Ala-Gly, and Ala-Glu were purchased from Sigma, Phe-Gly was purchased from Peptide Institute, Tyr, Gly-Gly, en, and bpy were purchased from Nakarai, and bphen was purchased from Wako. Pd(II) was used as

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(20) All the dipeptides and amino acids used are the L-enantiomers. The following abbreviations were used for L and DA in the text: Tyr-Glu, tyrosylglutamate; Tyr-Gly, tyrosylglycinate; TyrO⁻-Glu, Tyr-Glu with dissociated phenol OH; TyrO⁻-Gly, Tyr-Gly with dissociated phenol OH; Trp-Glu, tryptophylglutamate; Trp-Gly, tryptophylglycinate; Phe-Glu, phenylalanylglutamate; Phe-Gly, phenylalanylglycinate; Ala-Glu, alanylglutamate; Ala-Gly, alanylglycinate; Htrp, 5-hydroxytryptophanate; Trp, tryptophanate; Tyr, tyrosinate; TyrO⁻, Tyr with dissociated phenol OH; Phe, phenylalaninate; Val, valinate; Ala, alaninate; Pser, *O*-phosphoserinate; Ptyr, *O*-phosphotyrosinate; en, ethylenediamine; bpy, 2,2′-bipyridine; bphen, 4,7-diphenyl-1,10-phenanthroline-4′,4″-disulfonate.

Table I. ^{1}H NMR Chemical Shifts of Dipeptides (L) in Pd(II) Complexes at 25 °C[a]

		chemical shift, ppm					
		Gly		Tyr			
compd	pD	N-terminal CH_2	C-terminal CH_2	α-CH	β-CH_2	H-3	H-2
Gly-Gly[b]	1	3.90	4.08				
	6	3.84	3.80				
	12	3.35	3.78				
Pd(Gly-Gly)	7.0	3.48	3.89				
$Pd(Gly-Gly)_2$	10.6	3.44	3.55				
Pd(Gly-Gly)(en)	5.1	3.48	3.58				
Pd(Gly-Gly)(bpy)	9.2	3.66	3.85				
Pd(Gly-Gly)(bphen)	9.4	3.56	3.98				
Tyr-Gly	3.7		3.93, 3.62 (17.5)[c]	4.20	3.14	6.88	7.18
Pd(Tyr-Gly)	3.5		3.89, 3.69 (16.9)[c]	3.73	2.99	6.94	7.22
Pd(Tyr-Gly)(en)	5.3		3.94, 3.04 (17.1)[c]	3.81	3.08	6.99	7.24
Pd(Tyr-Gly)(bpy)	5.8		4.11, 3.41 (17.2)[c]	4.02	3.12	5.72	6.85
Pd(Tyr-Gly)(bphen)	7.2			4.13	3.14	6.25	7.03

[a] ^{1}H NMR chemical shifts (ppm) of en in various compounds are as follows: en and $Pd(en)_2$, 2.76 (pD 10.0); Pd(Gly-Gly)(en), 2.72; Pd(Tyr-Gly)(en), 2.56 ppm. [b] Nakamura, A.; Jardetzky, O. *Biochemistry* **1968**, *7*, 1226–1230. [c] Geminal coupling constant of Gly in Hz.

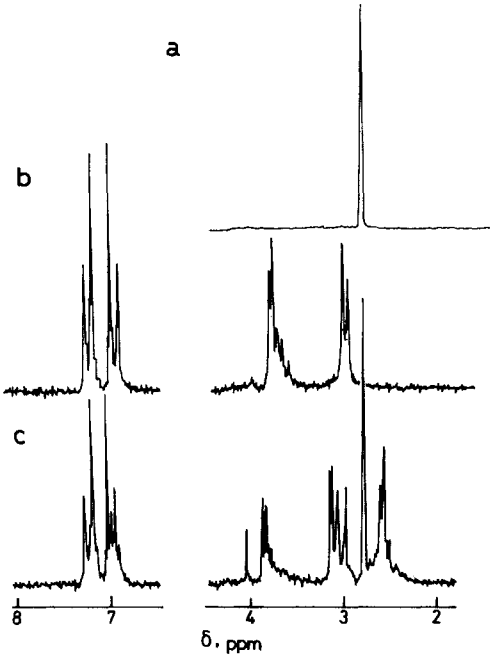


Figure 2. ^{1}H NMR spectra of Pd(Tyr-Gly)(en) and related systems: (a) $Pd(en)_2$ (pD 10.0); (b) Pd(Tyr-Gly) (pD 3.5); (c) Pd(Tyr-Gly)(en) (pD 5.3).

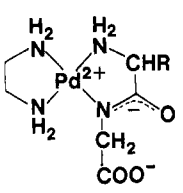
Na_2PdCl_4 obtained from Mitsuwa. Ptyr was prepared according to the literature.[21] Phe-Glu·H_2O was prepared by the usual method and checked by elemental analysis and the specific rotation, $[\alpha]^{18}_D$ 23.2° (*c* 0.2), which agrees with the reported value, $[\alpha]^{22}_D$ 22.8° (*c* 2).[22]

Spectral Measurements. Absorption spectra were recorded in the range 200–450 nm at room temperature with a Union Giken SM-401 instrument and a Hitachi 330 recording spectrophotometer for 1 mM freshly prepared solutions of 1:0:1, 1:1:0, and 1:1:1 Pd–L–DA in water with L = Gly-Gly or Tyr-Gly and DA = en or bpy. The pH values were adjusted with aqueous NaOH.

^{1}H NMR spectra were measured with a JEOL FX-100 NMR spectrometer at 100 MHz in FT mode with homogated decoupling for 20 mM solutions of 1:0:2, 1:2:0, and 1:1:1 Pd–L–DA in deuterium oxide with sodium 2-(trimethylsilyl)propionate-d_4 as internal standard.[23] Measurements were made at 25, 5, 40, and 65 °C in this order with a digital resolution of 0.1 Hz. The samples were prepared by neutralizing the dissociable protons of the ligands including the peptide NH group by adding calculated amounts of NaOH, and the ionic strength was not adjusted (*I* varies).

Results

Electronic and ^{1}H NMR Spectra and Coordination Modes of Ternary Pd(L)(DA) Complexes. Gly-Gly coordinates to Pd(II) through the amino and deprotonated peptide nitrogens and the carboxylate oxygen,[24] exhibiting a broad absorption band mainly due to the d–d transition at ~340 nm as a shoulder (ε ~700, pH 7.0). As shown in Figure 1, the Pd(Tyr-Gly) system at pH 7.1 also exhibits the same d–d band, indicating that the coordination modes of Gly-Gly and Tyr-Gly are the same. The peak at 291 nm (ε 260) exhibited by the Pd(Gly-Gly)(en) system corresponds with that shown by the $Pd(en)_2$ system with an N_4 chromophore. The absorption spectrum of the Pd(Tyr-Gly)(en) system at 250–300 nm is obscured by the absorption due to the phenol ring, but the band at 300–400 nm coincides with that of Pd(Gly-Gly)(en), which shows that Pd(Gly-Gly)(en) and Pd(Tyr-Gly)(en) have the same N_4 chromophore formed by three amino nitrogens and a deprotonated peptide nitrogen, with the carboxylate group uncoordinated (structure **1**). For the Pd(L)(DA) systems with



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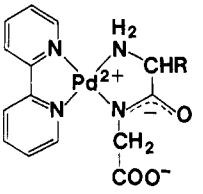

2

DA = bpy or bphen in place of en, we may also reasonably assume a similar donor set in the coordination plane, because bpy and bphen can effectively coordinate to Pd(II) without serious steric hindrance (structure **2**). Participation of the phenol OH group of the Tyr residue was not detected under the present conditions.

The ^{1}H NMR spectra of the Pd(Gly-Gly)(en) and related systems further supported the N_4 coordination mode. While Pd(Gly-Gly) has two singlets at 3.48 and 3.89 ppm, which are assigned to the N-terminal and C-terminal methylene protons, respectively, the ternary system with en exhibits the corresponding signals at 3.48 and 3.58 ppm, respectively, and the en signals at 2.72 ppm, all of which are different from those of the free ligands and the binary complexes. The results show that the carboxylate group is not coordinated in Pd(Gly-Gly)(en). The same spectral changes are observed for Pd(Tyr-Gly)(en) (Figure 2), supporting the formation of the ternary complex with a similar structure. As expected from the Gly-Gly-like mode of coordination, the chemical shifts due to the phenol group of Tyr-Gly suffer no appreciable

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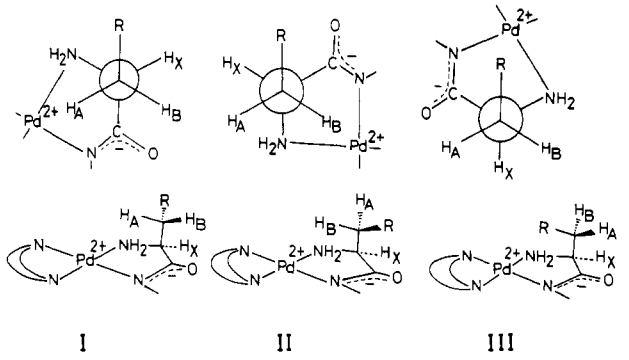
(23) Although the chemical shift of this compound has been reported to be affected by hydrophobic interactions with the other solutes (Mitchell, P. R.; Sigel, H. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 548. Scheller, K. H.; Hofstetter, F.; Mitchell, P. R.; Prijs, B.; Sigel, H. *J. Am. Chem. Soc.* **1981**, *103*, 247–260), it was confirmed to be unchanged under the conditions used in the present study by comparing the shift differences between the signals for the compound and for added ethanol or *tert*-butyl alcohol.
(24) Wilson, E. W.; Martin, R. B. *Inorg. Chem.* **1970**, *9*, 528–532.



I II III

Figure 3. Three staggered rotamers of N-terminal amino acid in Pd(L)(DA).

change upon complex formation. The Pd(Tyr-Gly)(bpy) system shows the geminal coupling constant ($|J_{AB}|$ = 17.1 Hz) of the Gly protons, which is intermediate between that of the free ligand (17.5 Hz) and that of the coordinated ligand (16.9 Hz), indicating again that the carboxylate group is free from coordination. Table I summarizes the chemical shifts for various binary and ternary systems in deuterium oxide.

Estimation of Rotamer Populations in Ternary Complexes. The conformations of amino acid residues in coordinated dipeptides can be described by three staggered rotamers, I, II, and III, viewed through the C_α–C_β bond (Figure 3).[25] Intramolecular interactions between the aromatic rings of DA and the aromatic side chain of L in Pd(L)(DA) with an N_4 coordination require rotamer III, which enables the side chain of L to be positioned above the Pd(II) coordination plane. The fractional populations P_I, P_{II}, and P_{III} for rotamers I, II, and III, respectively, are considered to indicate the rotamers' relative contribution to the side chain conformation in Pd(L)(DA), so that P_{III} should be large for complexes with significant intramolecular aromatic ring stacking.

Calculation of the fractional populations for Pd(II)–peptide systems has been successfully carried out for various di- and tripeptides by Kozlowski et al.[26] and Martin et al.[17,27] according to

$$P_I = (J_{AX} - J_g)/(J_t - J_g) \qquad P_{II} = (J_{BX} - J_g)/(J_t - J_g)$$
$$P_{III} = [J_t + J_g - (J_{AX} + J_{BX})]/(J_t - J_g) \qquad (1)$$
$$P_I + P_{II} + P_{III} = 1$$

where J_{AX}, J_{BX}, J_g, and J_t are ^{1}H NMR coupling constants. The $J_{AX} + J_{BX}$ values necessary for calculating P_{III} are positive for α-amino acids[28] and were obtained from the spectra for the Pd(L)(DA) systems with L = Tyr-Glu, TyrO$^-$-Glu, Tyr-Gly, TyrO$^-$-Gly, Phe-Glu, Phe-Gly, or Trp-Gly and DA = en, bpy, or bphen. The α-CH and β-CH_2 proton signals of Tyr and Phe in the complexes are observed at 3.8–4.1 and 3.0–3.3 ppm, respectively, and those for Trp at 3.9–4.5 and 3.1–3.4 ppm, respectively. Since the chemical shift differences between the two β-CH_2 protons were very small, the spectra exhibited the AA'X pattern and only the sums, $J_{AX} + J_{BX}$, were obtained for most of the ternary systems. The P_{III} values were then calculated from the coupling constants according to eq 1 by using the values J_t = 13.3 and J_g = 2.4 Hz recommended for three-spin α-amino acids by Martin[29] (Table II). With Pd(L)(en) as standards, we see P_{III} increases of 0.3–0.4 for the systems with L = Tyr-Glu and DA = bpy or bphen and 0.3 for Pd(Trp-Glu)(bpy), which clearly indicates that rotamer III is favored in the complexes where aromatic ring stacking is expected to take place between the coordinated ligands. Interestingly, deprotonation of the Tyr phenol OH group decreases the P_{III} values at 25 °C from 0.65–0.8 to 0.4–0.5 for Pd(TyrO$^-$-Glu)(DA) (DA = bpy or bphen), whereas

Table II. Fractional Populations of Rotamer III (P_{III}) of Aromatic Amino Acid Residues in Pd(L)(DA) Complexes Estimated from the Sums of Vicinal Coupling Constants, $J_{AX} + J_{BX}$, and Their Temperature Dependences[a]

		DA					
		en		bpy		bphen	
L	temp, °C	$J_{AX} + J_{BX}$, Hz	P_{III}	$J_{AX} + J_{BX}$, Hz	P_{III}	$J_{AX} + J_{BX}$, Hz	P_{III}
Trp-Glu	5	10.1	0.51				
	25	10.5	0.48	7.6	0.74		
	40	11.0	0.43				
	65	11.5	0.39				
Tyr-Glu	5	11.0	0.43	7.1	0.79		
	25	11.5	0.39	8.6	0.65	7.0	0.80
	40	11.2	0.41	9.3	0.59	7.9	0.72
TyrO$^-$-Glu	5	9.5	0.57	10.1	0.51		
	25	11.7	0.37	11.0	0.43	10.2	0.51
	40	12.0	0.34	11.5	0.39	11.0	0.43
	65			10.5	0.48		
Phe-Glu	5	11.5	0.39	9.6	0.56		
	25	11.7	0.37	9.8	0.54		
	40	11.7	0.37				
	65	12.2	0.32				
Trp-Gly	5	8.8	0.63				
	25	9.5	0.57				
	40	10.1	0.51				
	65	9.7	0.55				
Tyr-Gly	5	8.5	0.66	7.1	0.79		
	25	8.7	0.64	8.4	0.67		
	40	9.5	0.57	8.7	0.64		
	65	9.6	0.56	9.3	0.59		
TyrO$^-$-Gly	5			8.4	0.67		
	25	10.0	0.52	9.0	0.61		
	40	10.0	0.52	9.8	0.54		
	65			9.3	0.59		
Phe-Gly	5			8.4	0.67		
	25	10.3	0.50	9.5	0.57		
	40	10.9	0.44	9.0	0.61		
	65	11.7	0.37				
Ptyr	5	10.3	0.50				
	25	10.8	0.45				
	40	10.5	0.48				

[a] $P_{III} = [15.7 - (J_{AX} + J_{BX})]/10.9$.

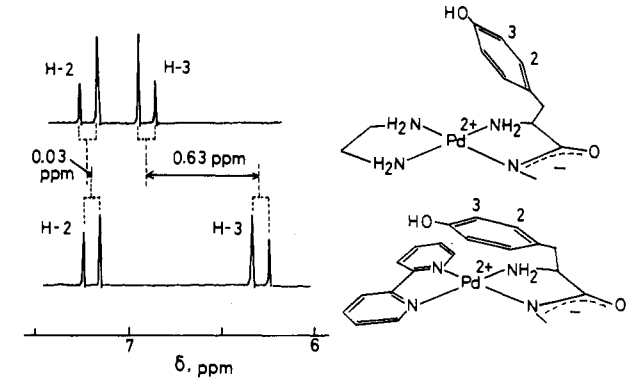


Figure 4. Upfield shifts due to the Pd^{II}/bpy moiety observed for Pd(Tyr-Glu)(bpy).

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Table III. Upfield Shifts Observed for Aromatic Amino Acid Residues in Pd(L)(DA) Complexes at 25 °C[a]

	upfield shift, ppm							
	bpy				bphen			
L	H-5,6	H-4	H-3	H-2	H-5,6	H-4	H-3	H-2
Trp-Glu	0.72				1.44			
Tyr-Glu			0.66	0.05			1.04	0.14
$TyrO^-$-Glu			0.38	0.00			0.30	−0.09
Phe-Glu		0.67	0.47	0.06		1.00	0.70	0.06
Trp-Gly					1.59			
Tyr-Gly			0.74	0.21			1.27	0.39
$TyrO^-$-Gly			0.53	0.11			0.57	0.08
Phe-Gly		0.70	0.49	0.17		1.12	0.83	0.26
Ptyr							0.24	−0.02

[a] The upfield shifts were obtained by taking the differences from the following chemical shifts (ppm) of Pd(L)(en) complexes as standards, and negative values denote downfield shifts: Trp-Glu 7.28 (H-5,6); Tyr-Glu 6.91 (H-3), 7.23 (H-2); $TyrO^-$-Glu 6.68 (H-3), 7.09 (H-2); Phe-Glu 7.44 (H-2,3,4); Trp-Gly 7.26 (H-5,6); Tyr-Gly 6.99 (H-3), 7.24 (H-2); $TyrO^-$-Gly 6.71 (H-3), 7.07 (H-2); Phe-Gly 7.41 (H-2,3,4); Ptyr 7.34 (H-2,3). Reproducibility of the shifts was usually ±0.01 ppm.

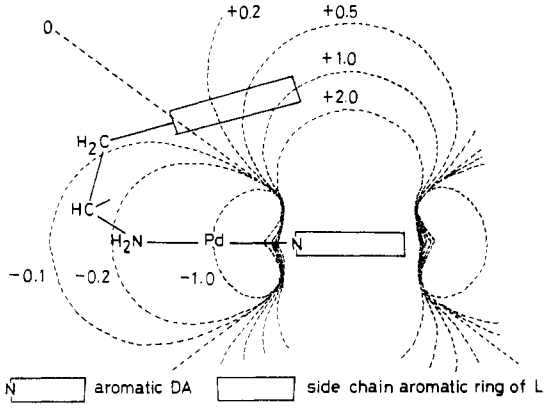

Figure 5. Upfield and downfield shifts due to the ring current of benzene according to Johnson and Bovey.[30,31] The positive and negative values denote the upfield and downfield shifts in ppm, respectively.

the value for $Pd(TyrO^-\text{-}Glu)(en)$ is affected much less. This is interpreted as due to the release of the side chain from a fixed conformation and accordingly to the inhibition of the stacking interaction by the negative charge of the phenolate oxygen moiety. The P_{III} values for most of the Pd(L)(bphen) systems could not be calculated because of the broad NMR spectra.

Upfield Shifts due to Ring Current Effect. The stacking of aromatic rings gives rise to upfield shifts of the ring protons and other protons in the vicinity of the rings due to the ring current effect.[30] They are governed by the position of the protons relative to the ring, so that the magnitude of the shift $\Delta\delta$ may serve as a measure of the extent of stacking. Figure 4 shows that relative to the corresponding signals of Pd(Tyr-Glu)(en) the Tyr H-2 and H-3 signals of Pd(Tyr-Glu)(bpy) shift upfield by 0.03 and 0.63 ppm, respectively, indicating that Tyr H-3 is close above the pyridine ring of bpy as a result of the stacking. This is evident from the still larger $\Delta\delta$ values for the Phe H-4 signal of Pd(Phe-Gly)(bphen) and the H-5 and H-6 signals of Trp in Pd(Trp-Gly)(bphen) (Table III). Although the latter signals are not clearly resolved, the upfield shifts expressed as their averages are larger than those detected for Tyr and Phe. The protons suffering small downfield shifts of the signals are inferred to lie in the region beside the relevant aromatic ring according to the diagram showing the ring current effect of the benzene ring (Figure 5).[30,31] The upfield shifts due to the Tyr phenol ring were observed for the α-protons of bpy ($\Delta\delta \simeq 0.15$ ppm) and the

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(31) Johnson, C. E., Jr.; Bovey, F. A. *J. Chem. Phys.* **1958**, *29*, 1012–1014.

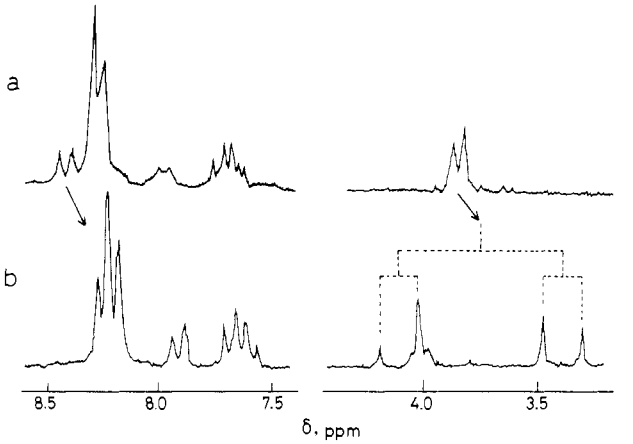


Figure 6. Upfield shifts of bpy α-proton and Gly methylene proton signals due to the phenol ring of Tyr in Pd(Tyr-Gly)(bpy): (a) Pd(Ala-Gly)(bpy) (pD 8.5); (b) Pd(Tyr-Gly)(bpy) (pD 5.8).

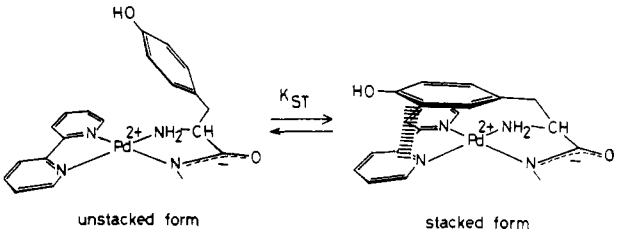


Figure 7. Proposed stacking equilibrium in Pd(Tyr-X)(bpy).

protons of the C-terminal amino acid ($\Delta\delta \simeq 0.1$ ppm) in Pd(Tyr-Gly)(bpy) (Figure 6).[32] The side chain conformation favoring the Pd(II)–aromatic ring interaction can be deduced from the ring current effect of the Tyr phenol ring on the methylene proton signal of en in Pd(Tyr-Gly)(en), which shifts upfield by 0.18 ppm relative to the signal in Pd(Ala-Gly)(en).

The observed upfield shifts could have been caused by the Pd(II)–aromatic ring interaction and/or intermolecular aromatic ring stacking. However, possible effects of the former are canceled by taking the shift differences between Pd(L)(DA) (DA = bpy or bphen) and Pd(L)(en), and those of the latter can be neglected because no chemical shift differences were observed upon variation of the sample concentrations between 10 mM and 100 mM.

Evaluation of the Equilibrium between Stacked and Unstacked Forms. The strength of intramolecular aromatic ring stacking in Pd(II) complexes in solution may be evaluated by K_{ST} or K (eq 2) according to Sigel et al.[10] (Figure 7). The constant K is

$$\underset{\text{unstacked form}}{L\text{—}Pd\text{—}DA} \overset{K_{ST}}{\rightleftharpoons} \underset{\text{stacked form}}{L\text{—}Pd\text{—}DA}$$

$$K_{ST} = \frac{[\text{stacked form}]}{[\text{unstacked form}]} \tag{2}$$

$$K = K_{ST} + 1 = \frac{[\text{unstacked form}] + [\text{stacked form}]}{[\text{unstacked form}]}$$

related to P_{ST}, the fractional population of the stacked species, by eq 3.

$$P_{ST} = \frac{[\text{stacked form}]}{[\text{unstacked form}] + [\text{stacked form}]} \tag{3}$$

$$K = \frac{1}{1 - P_{ST}}$$

On the assumption that only intramolecular stacking causes the

(32) The α-proton signal of Glu in Pd(Tyr-Glu) shifts upfield by ~0.4 ppm relative to that in Pd(Ala-Glu).

Table IV. Observed Upfield Shifts ($\Delta\delta$), Populations of Stacked Forms (P_{ST}), and Stability Constants (log K) for Pd(L)(DA) at Various Temperatures and Thermodynamic Parameters (I Varies)[a]

		DA											
		bpy						bphen					
L	temp, °C	$\Delta\delta$, ppm	P_{ST}	log K	$\Delta G^{\circ\prime}$, kJ mol^{-1}	$\Delta H^{\circ\prime}$, kJ mol^{-1}	$\Delta S^{\circ\prime}$, J mol^{-1} K^{-1}	$\Delta\delta$, ppm	P_{ST}	log K	$\Delta G^{\circ\prime}$, kJ mol^{-1}	$\Delta H^{\circ\prime}$, kJ mol^{-1}	$\Delta S^{\circ\prime}$, J mol^{-1} K^{-1}
Trp-Glu	5	0.77	0.77	0.64	−3.4								
	25	0.72	0.72	0.55	−3.1	−5.4	−7	1.44	0.89	0.95	−5.3	−17.1	−39
	40	0.70	0.70	0.52	−3.1			1.36	0.84	0.79	−4.7		
	65	0.65	0.65	0.46	−3.0			1.21	0.75	0.60	−3.9		
Tyr-Glu	5	0.71	0.70	0.53	−2.8			1.13	0.79	0.68	−3.6		
	25	0.66	0.65	0.46	−2.6	−4.3	−5	1.04	0.73	0.56	−3.1	−6.4	−11
	40	0.64	0.63	0.44	−2.6			1.00	0.70	0.52	−3.1		
	65	0.59	0.58	0.38	−2.5			0.92	0.64	0.45	−2.9		
TyrO$^-$-Glu	5	0.39	0.39	0.21	−1.1			0.20	0.14	0.07	−0.4		
	25	0.38	0.38	0.20	−1.1	−0.8	1	0.30	0.21	0.10	−0.6	−1.5	7
	40	0.37	0.37	0.20	−1.2			0.31	0.22	0.11	−0.7		
	65	0.35	0.35	0.18	−1.2			0.34	0.24	0.12	−0.8		
Phe-Glu	5	0.44	0.43	0.24	−1.3			0.51	0.35	0.19	−1.0		
	25	0.47	0.47	0.27	−1.5	0.3	6	0.70	0.49	0.29	−1.6	2.6	13
	40	0.47	0.47	0.27	−1.6			0.70	0.49	0.29	−1.7		
	65	0.45	0.45	0.26	−1.7			0.68	0.48	0.28	−1.8		
Trp-Gly	25							1.59	0.98	1.73	−9.7		
	40							1.53	0.94	1.26	−7.6	−50.6	−137
	65							1.28	0.79	0.68	−4.4		
Tyr-Gly	5	0.79	0.78	0.66	−3.5			1.36	0.95	1.31	−7.0		
	25	0.74	0.73	0.57	−3.2	−5.9	−9	1.27	0.89	0.95	−5.3	−20.4	−49
	40	0.72	0.71	0.54	−3.2			1.23	0.86	0.85	−5.1		
	65	0.66	0.65	0.46	−3.0			1.08	0.76	0.61	−3.9		
TyrO$^-$-Gly	5	0.58	0.57	0.37	−2.0			0.60	0.42	0.24	−1.3		
	25	0.53	0.52	0.32	−1.8	−3.4	−5	0.57	0.40	0.22	−1.2	−1.3	−0
	40	0.50	0.50	0.30	−1.8			0.56	0.39	0.22	−1.3		
	65	0.45	0.45	0.26	−1.7			0.51	0.36	0.19	−1.2		
Phe-Gly	5	0.52	0.51	0.31	−1.7								
	25	0.49	0.49	0.29	−1.6	−1.8	−0	0.83	0.58	0.38	−2.1	−2.0	1
	40	0.47	0.47	0.27	−1.6			0.81	0.57	0.36	−2.2		
	65	0.45	0.45	0.26	−1.7			0.77	0.53	0.34	−2.2		
Ptyr	5							0.23	0.16	0.08	−0.4		
	25							0.24	0.17	0.08	−0.4	0.1	2
	40							0.23	0.16	0.08	−0.5		
	65							0.24	0.17	0.08	−0.5		

[a] The differences between the observed log K values and those obtained from the thermodynamic parameters were usually within 0.02.

upfield shift, which is directly proportional to the extent of stacking, P_{ST} may be calculated from the observed $\Delta\delta$ values and the shifts expected for complete stacking, $\Delta\delta_{calcd}$

$$P_{ST} = \frac{\Delta\delta}{\Delta\delta_{calcd}} \quad (4)$$

The $\Delta\delta_{calcd}$ values may be estimated by the upfield shifts for complete stacking in binary systems[33] or according to Johnson and Bovey[30,31] by using the CPK models (Figure 5);[34,35] because the aromatic rings of DA and the α-carbon of L are reasonably fixed in a plane by coordination to Pd(II), wagging of the side chain on the surface of DA does not seem to affect significantly the upfield shifts as far as the rings of L and DA overlap each other. For Tyr, Ptyr, and Tyr- and Phe-containing dipeptides, C-3 lies close to the aromatic nitrogen of DA (Figure 8a),[12] from which the $\Delta\delta_{calcd}$ values of H-3 were estimated to be 1.01 and 1.43 ppm for DA = bpy and bphen, respectively, by assuming that the pyridine ring in DA approximates the benzene ring and that the distance between the stacked aromatic rings is 3.35 Å.[36] The average value for H-5 and H-6 of Trp in Pd(Trp-Glu)(bpy) was calculated to be 1.00 ppm for the stacking as shown in Figure 8b. Since the overlapping between the indole ring of Trp and bphen in Pd(Trp-X)(bphen) (X = Gly or Glu) is possible in several ways, we used the average chemical shift (1.62 ppm) of the H-5 (1.46

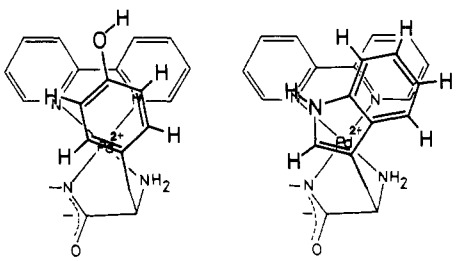


Figure 8. Examples of stacked structures of Pd(Tyr-X)(bpy) (a) and Pd(Trp-X)(bpy) (b) employed for calculation of complete upfield shifts.

(33) (a) Mitchell, P. R.; Prijs, B.; Sigel, H. *Helv. Chim. Acta* **1979**, *62*, 1723–1735. (b) Sigel, H.; Fischer, B. E.; Farkas, E. *Inorg. Chem.* **1983**, *22*, 925–934. (c) Scheller, K. H.; Sigel, H. *J. Am. Chem. Soc.* **1983**, *105*, 3005–3014. (d) Scheller, K. H.; Sigel, H. *J. Am. Chem. Soc.* **1983**, *105*, 5891–5900. (e) Farkas, E.; Fischer, B. E.; Griesser, R.; Rheinberger, V. M.; Sigel, H. *Z. Naturforsch., B: Anorg. Chem., Org. Chem.* **1979**, *34b*, 208–216.

(34) Davis, J. S.; Hakeem, E. *J. Chem. Soc., Perkin Trans. 2* **1984**, 1387–1392.

(35) The ring current effect for metal complexes may be treated in the same way as that for free ligands: Abraham, R. J.; Leighton, P.; Sanders, K. M. *J. Am. Chem. Soc.* **1985**, *107*, 3472–3478.

(36) Average spacings of 3.3–3.4 Å have been revealed for intramolecularly stacked bpy and the adenine ring of AMP (adenosine 5′-monophosphate) in $[Cu(bpy)(AMP)(H_2O)](NO_3)_2 \cdot 6H_2O$ by X-ray crystal structure analysis: Aoki, K. *J. Am. Chem. Soc.* **1978**, *100*, 7106–7108.

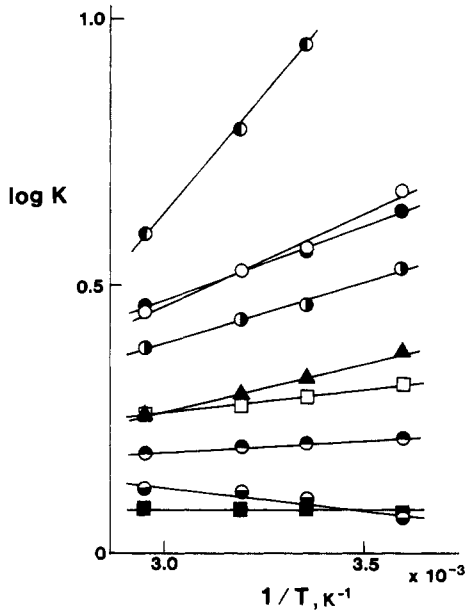


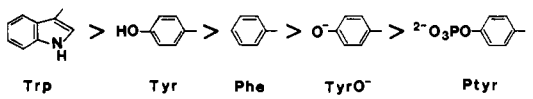
Figure 9. Temperature dependences of log K values for ternary Pd(L)(DA) complexes: (◐) Pd(Trp-Glu)(bphen); (○) Pd(Tyr-Glu)(bphen); (●) Pd(Trp-Glu)(bpy); (◑) Pd(Tyr-Glu)(bpy); (▲) (TyrO⁻-Gly)(bpy); (□) Pd(Phe-Gly)(bpy); (⊖) Pd(TyrO⁻-Glu)(bpy); (⊖) Pd(TyrO⁻-Gly)(bphen); (■) Pd(Ptyr)(bphen).

ppm) and H-6 (1.78 ppm) signals obtained by considering two more structures in addition to those in Figure 8b. The P_{ST} and log K values calculated from the $\Delta\delta$ values are shown in Table IV. The systems with Ptyr in place of L exhibited the lowest P_{ST} and log K values.

Temperature Dependence of Stacking Interactions. Because the stacking interaction is intrinsically weak ($\Delta G° \simeq -6$ kJ mol^{-1} for stacking of the benzene rings of Phe[2a,37]) and requires a certain conformation, it will be weakened with the increase of temperature, which favors rapid interconversion between the rotamers. As shown in Table II, the P_{III} values for Pd(L)(DA) (DA = bpy or bphen) gradually decrease with temperature increase. In accordance with this, the upfield shifts noticed for them decrease in the same way, while those of Pd(L)(en) remain unchanged (Table IV). The log K values, which were calculated from the P_{ST} values obtained from the NMR spectra at 5, 25, 40, and 65 °C, also decrease at higher temperatures. Plots of log K against $1/T$ gave satisfactorily straight lines (Figure 9), from which the enthalpy and entropy changes shown in Table IV were calculated.

Discussion

Structure–Stability Relationship of Aromatic Ring Stacking. The upfield shifts due to the ring current effect observed for the systems with L and DA involving aromatic rings serve as evidence for the aromatic ring stacking in the complexes. The equilibrium constants for the stacking interaction can be evaluated from P_{III} on the basis of the coupling constants and from P_{ST} on the basis of the upfield shifts. We see from Tables II and IV that the P_{III} values are always higher than the P_{ST} values, though the stability sequence of the stacking expressed in terms of these values is the same. Because the side chain of rotamer III is not necessarily stacked with bpy or bphen, it is more appropriate to evaluate log K from P_{ST}. The stability as expressed by log K decreases in the following order of the side chain aromatic rings in Pd(X-Glu)(DA), Pd(X-Gly)(bphen), and Pd(Ptyr)(bphen):



The phenolate ring is placed before the phenyl ring in the stability series for Pd(X-Gly)(bpy). The order for DA, bphen > bpy, holds for L = X-Gly and X-Glu all involving an uncharged aromatic ring. Apart from the dipeptide complexes, Pd(Ptyr)(bphen) with a dinegative phospho ester group has the lowest log K value (0.08), indicating negligible stacking interactions. These findings are in agreement with the conclusion drawn by potentiometry for Cu(AA)(DA) (AA = aromatic amino acid and DA = bpy, 1,10-phenanthroline, 2-(aminomethyl)pyridine, or histamine),[12] where the stability of stacking has been found to be in the order (of AA) Htrp > Trp > Tyr > Phe > TyrO⁻ > Val ≃ Ala ≃ Pser ≃ Ptyr for various aromatic DA's; for example, Cu(Trp)(bpy) and Cu(Tyr)(bpy) have log K values of 1.19 and 0.90, respectively. The differences between the absolute values for Pd(II) and Cu(II) complexes probably arise from the differences in the coordination mode due to the deprotonated peptide nitrogen and the steric hindrance arising from the C-terminal amino acid residue. In fact the log K value for Pd(TyrO⁻)(bpy) calculated from the upfield shift of the H-3 signal (0.49 ppm) relative to that for Pd(TyrO⁻)(en) is 0.29 at 25 °C, which agrees well with the value (0.25) for the corresponding Cu(II) complex.

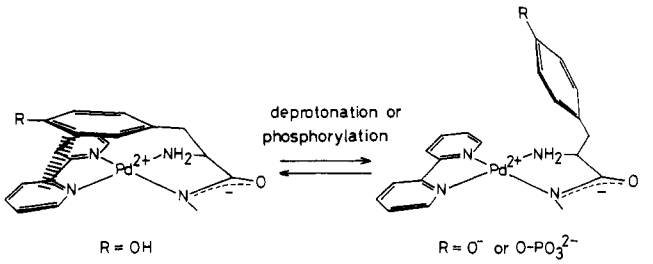


Figure 10. Proposed mode of regulation of stacking interaction in Pd(Tyr-X)(bpy) by modification of the phenol OH group.

The thermodynamic parameters shown in Table IV indicate that the stability of stacking ($\Delta G°'$ = −1 to −10 kJ mol^{-1} at 25 °C) largely depends on $\Delta H°'$, which is <0 for most systems, and the fact that $\Delta S°'$ often makes a negative contribution. Such a tendency has been known for the self-stacking of nucleic bases[38] and is more pronounced in the systems containing the Tyr and Trp residues with larger log K values, which implies that the stacking is assisted by $\Delta H°'$ due to charge transfer from the aromatic ring of L to the coordinated aromatic DA. This is further substantiated by the fact that the phenol OH group and the pyrrole moiety of the indole ring enhance the stability by 0.12–0.57 and 0.20–1.35 log units, respectively, as compared with the benzene ring in Phe. For the ternary Cu(II) complexes, a log K increase of ~0.3 has been assigned to these groups.[12]

Higher log K values for dipeptides X-Gly than those for X-Glu may be interpreted as due to the steric hindrance or competition between the N-terminal aromatic ring and the β-carboxylate group of Glu, both of which prefer to be positioned above the Pd(II) coordination plane.

Regulation of Stacking by an Ionizable Group Attached to the Aromatic Ring and Its Biological Relevance. Dissociation of the phenol OH group in the Cu(Tyr)(bpy) system has been found to decrease the log K value by 0.65, and more importantly phosphorylation of the OH group has nearly completely blocked the stacking interaction by a stability decrease of 1.04.[12] The present NMR study on the related Pd(Tyr-X)(DA) systems (X = Gly or Glu and DA = bpy or bphen) reveals that stacking between the phenolate ring and DA is destabilized by ~0.25 for DA = bpy and 0.46 (X = Glu) and 0.73 (X = Gly) for DA = bphen, which demonstrates the dependence of the interaction on the charged group attached to the ring and the regulation of stacking as a consequence (Figure 10). Table IV indicates that the stability decrease is attributed to the increase of $\Delta H°'$ probably resulting from desolvation of the charged group upon stacking.

The hydrophobic interactions in which the aromatic side chains of peptides or proteins are involved can contribute to specificity of reactions or molecular recognition in biological systems. For

(37) Scheraga, H. A. *Acc. Chem. Res.* **1979**, *12*, 7–14.

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the opioid peptides with the N-terminal tyrosyl residue, the bonding with the receptor could involve the stacking interaction between the phenol group of Tyr and the aromatic side chain of the receptor, possibly the imidazole ring which has been reported to be located at the binding site.[15] In line with this, the receptor has been found to interact with a dopamine analogue containing a diphenol moiety.[39] In low molecular weight complexes, stacking has been concluded to occur between the Cu(II)-coordinated histidine imidazole ring and the aromatic side chain of Tyr, Trp, or Phe.[10,11] The present NMR study offers convincing evidence for the stacking interaction between an aromatic amine and the side chain of a peptide both firmly coordinated. Metal ions may assist the interaction in two ways: they put the interacting groups close to each other in the coordination sphere and/or enhance the charge transfer between the stacked rings by reducing the electron density of coordinated aromatic amines. Model studies aimed at demonstrating the latter point are in progress in our laboratory.

Regulation of stacking by introducing a charged or a hydrophilic group into the stacked ring is clearly seen from the log K values obtained for Pd(TyrO$^-$-X)(DA) (X = Gly or Glu and DA = bpy or bphen) and Pd(Ptyr)(bphen). This confirms the earlier observation on the Cu(II) complexes and may suggest regulation of biological reactions by tyrosine phosphorylation or sulfation;[40,41] while dissociation of the phenol OH group requires a strong base, its phosphorylation takes place in vivo by kinases and may break up possible stacked structures. In this connection, it is worth mentioning that phosphorylation of Tyr residues is regarded as the key step in the growth of cancer cells[40] and that sulfation of the Tyr residue of enkephalin[42] and phosphorylation of the opiate receptor site[43] inhibit the physiological reactions.

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Registry No. Pd(Trp-Glu)(en), 99546-63-1; Pd(Trp-Blu)(bpy), 99546-64-2; Pd(Tyr-Glu)(en), 99546-65-3; Pd(Tyr-Glu)(bpy), 99546-66-4; Pd(Tyr-Glu)(bphen), 99546-67-5; Pd(TyrO$^-$Glu)(en), 99546-68-6; Pd(TyrO$^-$-Glu)(bpy), 99546-69-7; Pd(TyrO$^-$-Glu)(bphen), 99546-70-0; Pd(Phe-Glu)(en), 99546-71-1; Pd(Phe-Glu)(bpy), 99546-72-2; Pd(Trp-Gly)(en), 99546-73-3; Pd(Tyr-Gly)(en), 99546-74-4; Pd(Tyr-Gly)(bpy), 99546-75-5; Pd(TyrO$^-$-Gly)(en), 99546-76-6; Pd(TyrO$^-$-Gly)(bpy), 99546-77-7; Pd(Phe-Gly)(en), 99546-78-8; Pd(Phe-Gly)(bpy), 99546-79-9; Pd(Ptyr)(en), 99546-80-2; Pd(Trp-Glu)(bphen), 99546-81-3; Pd(Phe-Glu)(bphen), 99546-82-4; Pd(Trp-Gly)(bphen), 99546-83-5; Pd(Tyr-Gly)(bphen), 99546-84-6; Pd(TyrO$^-$-Gly)(bphen), 99546-85-7; Pd(Phe-Gly)(bphen), 99559-67-8; Pd(Ptyr)(bphen), 99546-86-8; Pd(Gly-Gly), 99546-87-9; Pd(Gly-Gly)$_2$, 99546-88-0; Pd(Gly-Gly)(en), 62424-35-5; Pd(Gly-Gly)(bpy), 99546-89-1; Pd(Gly-Gly)(bphen), 99546-90-5; Pd(Tyr-Gly), 99546-91-5; Pd(en)$_2^{2+}$, 22573-08-6; Pd(Ala-Gly)(bpy), 99546-92-6.

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NMR Investigation of the Lanthanide Complexes with a 14-Membered Polyaza Polyacetic Macrocycle, TETA. Another Rare Example of Nonlabile Lanthanide Compounds

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The 14-membered macrocycle TETA (1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) forms unusually rigid lanthanide complexes whose solution properties have been investigated by ^{1}H and ^{13}C NMR spectroscopy after deuterium labeling of the ligand. The conformational analysis was based on the previously published crystallographic structure of TbTETA. A quantitative agreement is obtained between the dodecahedral geometry found in the solid and the spectra of paramagnetic YbTETA, provided the complete form of the dipolar equation is used in the calculations. The temperature dependence of the ^{1}H spectra of YbTETA and of the ^{13}C spectra of diamagnetic LuTETA is interpreted as arising from an exchange between two equivalent dodecahedral geometries. The kinetic parameters of this process were obtained by band-shape analysis in the case of LuTETA. The free energy of activation ($\Delta G^*_{25°C}$ = 63.7 kJ mol^{-1}) is remarkably high for a lanthanide complex, a special feature ascribed to the steric requirements of the macrocyclic ring. The dynamic behavior of the TETA complexes depends on the ionic radius of the metals: EuTETA behaves like the heavier lanthanide complexes, while PrTETA assumes a highly asymmetric geometry that is rigid only at −55 °C.

Introduction

Structural inferences made with lanthanide shift reagents[1] must be accepted with reservation for a number of reasons. Foremost among them are the unknown origin of the induced NMR shifts, the low symmetry of the lanthanide complexes, and the high lability of these compounds.[2] Separating the various contributions to the NMR shifts can be achieved by published procedures[1,3] provided a number of conditions are fulfilled or, better, can be avoided by selecting the ion Yb(III) as paramagnetic center since this ion induces shifts that are essentially dipolar in origin. However, the lack of symmetry of the lanthanide complexes remains one of the key questions in the analysis of the NMR spectra of these compounds. Indeed, the vast majority of the lanthanide complexes exhibit at best a C_2 axis and the complete form of the

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