Headline Articles

Calorimetric and ^{195}Pt NMR Studies on Aromatic Ring Stacking between Nucleotides and Platinum DNA Intercalators

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Calorimetric and spectroscopic investigations have been carried out on the stacking interactions between nucleosides or nucleotides (NMP) and platinum DNA intercalators and other planar platinum(II) complexes with heteroaromatic ligands (L). Pt(L)(diamine) (L=1,10-phenathroline (phen), its methyl and nitro derivatives, 2,2′-bipyridine; diamine=ethylenediamine (en), L-2,3-diaminopropionate) reacts with NMP (=adenosine 5′-monophosphate (AMP), guanosine 5′-monophosphate (GMP), inosine 5′-monophosphate (IMP), cytidine 5′-monophosphate (CMP); nicotinamide adenine dinucleotide (NAD), adenosine, cytidine) to give the Pt(L)(diamine)–$(NMP)_n$ adducts (n=1, 2) with $\log K_1$=1.63—3.00 and $\log K_2$=1.47—2.51 at 25 °C at ionic strength=0.1 mol dm^{-3} (NaCl). The 1 : 1 adduct formation mainly depends on the enthalpy change $\Delta H_1°$=−6.5—−26.2 kJ mol^{-1}, and the entropy change ($\Delta S_1°$=−38—20 J mol^{-1} K^{-1}) makes a negative or small contribution. ^{1}H NMR upfield shifts observed for the Pt(phen)(en)–NMP systems have substantiated the presence of stacked adducts in dilute aqueous solution, whereas diquat, a dicationic organic molecule, did not cause appreciable shifts, and hence the adduct formation was negligible. NAD was found to interact with Pt(phen)(en) through the adenine moiety. ^{195}Pt NMR signals of Pt(phen)(en) in water suffered downfield shifts upon addition of NMP, the $\Delta\delta_1$ values being in the order NAD≥AMP≈GMP>IMP. This indicates that stacking causes an electron density decrease of the Pt(II) center. Linear relationships have been detected between the $\Sigma\Delta H_n°$ values and the absorption coefficients ε_n of the difference spectra in the charge transfer region and between $\Sigma\Delta H_n°$ and ^{195}Pt NMR shift differences $\Delta\delta_n$. These observations serve as evidence for the electronic effects of stacking interactions on the stacked rings and the central Pt(II) ion.

The importance of noncovalent interactions is now widely recognized in biology[1] and bio-inspired chemistry.[1,2] Aromatic ring stacking as well as hydrogen bonding and electrostatic bonding plays essential roles in specificity and efficiency of biological reactions.[1,3,4] Whereas interactions between aromatic rings are important for protein stabilization, electron transfer, and various regulatory processes,[5—8] intercalative binding of aromatic chemicals to DNA base pairs has been regarded as the initial step in mutagenesis and drug actions.[9,10] Because of the weakness of stacking and other noncovalent interactions, however, information is rather limited on the modes and energies of such interactions of biomolecules.[5,8,11]

Much attention has been paid to the stacking involving nucleobases such as in the protein–DNA and intercalator–DNA interactions:[12—14] Ribonuclease T1 recognizes a guanine base of RNA by stacking through its phenol moiety of the tyrosine residue,[15] and intercalation of DNA-intercalators most probably depends on stacking with the base pairs and electrostatic interactions with the phosphoric ester moiety. In addition to recent findings on the specific DNA binding by the side chains of arginine and other amino acid residues of zinc finger proteins,[16] information on the structural dependence of the stabilization due to stacking with nucleobases is necessary for understanding the specificity exhibited by protein–DNA and –RNA interactions and for designing base-selective DNA intercalators. Lippard et al. established that platinum(II) complexes with large heteroaromatic rings such as 1,10-phenanthroline (phen) and 2,2′-bipyridine (bpy) effectively

intercalate into DNA base pairs,[14,17] and Barton et al. revealed that chiral ruthenium(II) complexes of phen etc. intercalatively binds with DNA grooves in an enantiospecific manner.[13,18] Sigel et al., on the other hand, have done an extensive series of studies on ternary metal complexes with intramolecular aromatic ring stacking between heteroaromatic ligands and nucleobases, and demonstrated the macroring formation and stability enhancement due to the intramolecular stacking.[19] For ternary Cu(II) and Pd(II) complexes with aromatic amino acids and heteroaromatic ligands such as Cu(bpy)(L-Tyr) (Tyr=tyrosinate), we detected by spectroscopic, potentiometric, and X-ray crystallographic studies that there exists intramolecular stacking between the side-chain aromatic ring and bpy etc. with the separation of ca. 3.5 Å.[20]

We have been interested in the DNA binding by Pt intercalators, and from the studies on the adduct formation in model systems composed of nucleotides or nucleosides (NMP) and [Pt(phen)(en)] (en=ethylenediamine) or similar planar Pt(II) complexes (charges are omitted for clarity), we revealed the intrinsic tendency of NMP to form stacked adducts with Pt intercalators in dilute aqueous solution with the enthalpy change ($\Delta H°$) of as large as -25 kJ mol^{-1}.[21] The aromatic ring stacking and the electrostatic interactions between positively charged Pt intercalators and negatively charged NMP have been found to be cooperative in adduct formation,[21c] although the stacking itself was unfavorable in terms of the entropy change.[21d] However, more information is necessary for understanding the structure dependence of stacking leading to molecular recognition and the electronic effects of stacking on the aromatic rings involved and, in particular, on the metal centers of ternary complexes with intramolecular stacking and of Pt(phen)(en) and other metallointercalators.

In order to acquire further insights into the nature of stacking and structure-stability relationship, we now carried out calorimetric and spectroscopic investigations on the adduct formation between NMP (=AMP, GMP, CMP, NAD, etc.) and Pt(II) complexes, Pt(L)-(diamine) including those reported to be intercalators (L=bpy, phen, Me_2phen, etc.; diamine=en, 2,3-diaminopropionate (dap)) (Fig. 1),[22] in aqueous solution. This paper deals with the bonding modes and stabilities of adducts formed by stacking and electrostatic interactions, the structure-stability relationship, and the electronic effects of Pt intercalator–nucleobase stacking on the central metal ion as viewed from the ^{195}Pt NMR spectra.

Experimental

Reagents. GMP, IMP, CMP, Ado, and Cyt were purchased from Yamasa, AMP from Oriental, NAD from Sigma, and L-dap·2HCl from Tokyo Kasei. Pt(II) complexes [Pt(L)(en)]Cl_2 (L=phen, Me_4phen, Me_2phen, nphen, bpy, en) were prepared from Pt(L)Cl_2 and en according to the literature,[23] and the purity was checked by elemental analysis. Diquat was prepared according to the literature.[24] All other reagents were of analytical grade or of highest grade available and were used without further purification. Distilled and deionized water was used in all the experiments.

Synthesis of [Pt(phen)(L-dap)]ClO_4. To a suspension of Pt(phen)Cl_2 in water containing an equimolar amount of L-dap·2HCl was added aqueous NaOH (2 equiv), and the mixture was heated for 5 h at 90—95 °C, when a clear solution was obtained. After it was concentrated in vacuo, an equimolar amount of aqueous $HClO_4$ was added. The pale yellow precipitate of [Pt(phen)(L-Hdap)]-$(ClO_4)_2$·$2H_2O$ which separated at room temperature was filtered, suspended in water, and neutralized under stirring by an aqueous solution of tetraethylammonium hydroxide. After filtration the solution deposited upon standing a yellowish white powder, which was filtered and air-dried at room temperature. ^{1}H NMR (D_2O) δ=3.267 (AB-q, J=12.5, 5.1, 12.3, 6.7 Hz, 2H), 3.797 (dd, J=6.5, 5.5 Hz, 1H), 7.975 (d, J=8.7, 1H), 8.021 (d, J=8.7 Hz, 1H), 8.038 (dd, J=8.3, 1.5 Hz, 1H), 8.056 (dd, J=8.3, 1.5 Hz, 1H), 8.793 (dd, J=8.3, 1.0 Hz, 1H), 8.858 (dd, J=8.3, 1.0 Hz, 1H), 8.873 (dd, J=5.3, 0.9 Hz, 1H), 9.082 (dd, J=5.3, 0.9 Hz, 1H). Found: C, 29.62; H, 2.98; N, 9.26%. Calcd for [Pt(phen)(L-dap)]ClO_4·$1.5H_2O$ ($C_{15}H_{18}N_4O_{3.5}$Pt): C, 29.79; H, 3.00; N, 9.26%.

Synthesis of Pt(phen)(en)–AMP and –GMP Adducts. To an aqueous solution of [Pt(phen)(en)]-Cl_2 was added an aqueous solution of AMP or GMP, and the yellow precipitate which deposited from the mixture upon standing was collected and air-dried. No NMR spectral measurements were made because of the low solubility of the complexes. Found: C, 32.71; H, 4.12; N, 14.25%. Calcd for [Pt(phen)(en)]·AMP·$5.5H_2O$ ($C_{24}H_{39}N_9O_{12.5}$PPt): C, 32.77; H, 4.47; N, 14.33%. Found: C, 31.91; H, 3.93; N, 13.61%. Calcd for [Pt(phen)-(en)]·GMP·$6H_2O$ ($C_{24}H_{40}N_9O_{14}$PPt): C, 31.86; H, 4.46; N, 13.93%.

Calorimetry. Calorimetric titrations were carried out with the use of a computer-controlled titration assembly[25] at 25 °C for aqueous solutions of Pt(L)(en) (0.6—20 mM) by adding 50 and 100 mM NMP at ionic strength (I)=0.1 and 0.2 M NaCl (1 M=1 mol dm^{-3}), respectively. The concentration of Ado as titrant was 15 mM due to low solubility. Correction for the heat evolved with dilution and protonation or deprotonation of the titrant due to small pH changes was made by titrating 0.1 or 0.2 M NaCl with NMP. The NMP/Pt(L)(en) concentration ratio was 3—10 at end of each titration, and the total data points collected in 5—10 titrations were 34—68. The experimental data were analyzed according to the following equilibrium (Eq. 1) by a nonlinear least-squares treatment:[25]

$$\mathrm{Pt(L)(en)} + n\ \mathrm{NMP} \overset{\beta_n}{\rightleftarrows} \mathrm{Pt(L)(en)–(NMP)}_n$$

$$\beta_n = K_1 \cdots K_n = \frac{[\mathrm{Pt(L)(en)–(NMP)}_n]}{[\mathrm{Pt(L)(en)}][\mathrm{NMP}]^n}$$

$$-2.303\ RT \log K_n = -\Delta H_n° + T\Delta S_n° \qquad (1)$$

where β_n and K_n are the overall and successive stability con-

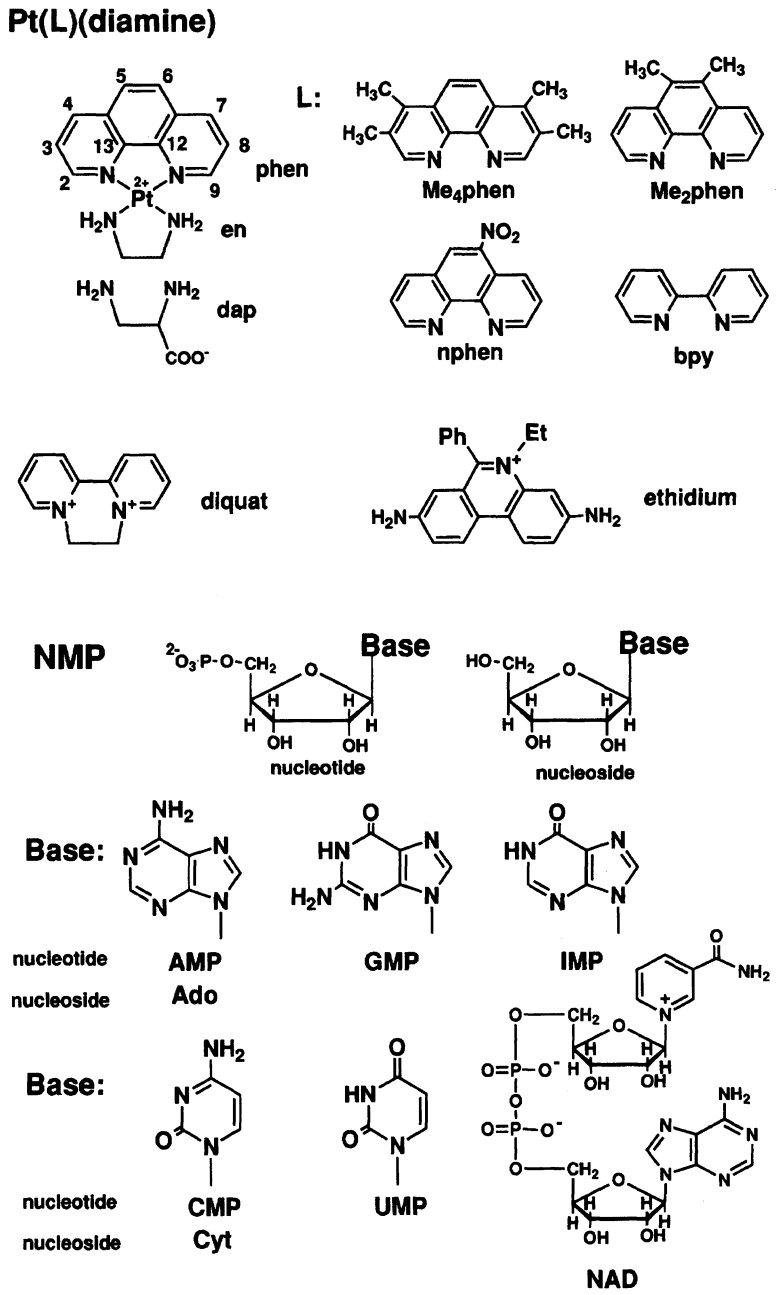

Fig. 1. Structures of Pt(L)(en or dap) and NMP used.

stants, respectively, and R and T are the gas constant and the absolute temperature, respectively. Minimization was applied for the sum of the squares of differences between observed and calculated heat values. ΔH_n° and $\log \beta_n$ values were calculated successfully by considering both $n=1$ and 2. For the systems with L=Me2phen it was necessary to consider adducts with n=1,2, and 4 for the measurements up to the Pt concentration of 40 mM.

Spectral Measurements. Absorption and circular dichroism (CD) spectra were recorded at 25 °C on a Shimadzu UV 3101PC spectrophotometer and a JASCO J-720 spectropolarimeter, respectively, in 0.1-cm path length quartz cells. ^{1}H and ^{195}Pt NMR spectra were recorded at 24 °C on a Varian VXR-300S spectrometer with D lock. A short pulse interval (0.1 s) on ^{195}Pt NMR made it possible to measure the ^{195}Pt NMR spectra at low Pt concentrations (ca. 0.3 mM). An Iwaki M-225 pH meter with an Iwaki IW-050 combined electrode was used for pH measurement. The ionic strength of the solutions was adjusted at 0.1 or 0.2 M (NaCl). Chemical shifts were defined as downfield shifts relative to the standard and measured by using t-butyl alcohol as ^{1}H internal standard and $[Pt(en)_2]Cl_2$ as ^{195}Pt external standard. Values of ^{1}H chemical shifts were converted to the TMS scale by adding 1.253 ppm.

The NMR chemical shift δ_n of the Pt(L)(en)–$(NMP)_n$ adduct and its absorption coefficient ε_n were estimated by Eqs. 2 and 3:

$$\delta_{obsd} - \delta_0 = f_1(\delta_1 - \delta_0) + f_2(\delta_2 - \delta_0) + f_d(\delta_d - \delta_0)$$
$$= f_1\Delta\delta_1 + f_2\Delta\delta_2 + f_d\Delta\delta_d \quad (2)$$

$$\varepsilon_{obsd} = f_0\varepsilon_0 + f_1\varepsilon_1 + f_2\varepsilon_2 + f_d\varepsilon_d \quad (3)$$

where δ_{obsd} and ε_{obsd} are the observed chemical shift and the observed absorption coefficient based on the Pt concentration, respectively, and f_n, δ_n, and ε_n are the fractional population, chemical shift, and absorption coefficient of Pt(L)(en)–$(NMP)_n$, respectively. The suffix 0 refers to the monomer and d to the dimer of Pt(L)(en), and $\Delta\delta_n$ is the shift due to the $1:n$ adduct formation. δ_0 was obtained by extrapolation of δ values to infinite dilution, and positive $\Delta\delta_n$ values are defined as referring to upfield shifts for ^{1}H and downfield shifts for ^{195}Pt NMR. The equilibrium constants K_D for the dimer formation is defined by Eq. 4:

$$2\text{Pt(L)(en)} \overset{K_D}{\rightleftharpoons} \{\text{Pt(L)(en)}\}_2$$

$$K_D = \frac{[\{\text{Pt(L)(en)}\}_2]}{[\text{Pt(L)(en)}]^2} \quad (4)$$

From the concentration dependence of ^{1}H NMR chemical shifts for Pt(phen)(en), the K_D and $\Delta\delta_d$ values were evaluated from δ_{obsd} for each proton of L by the usual method. After the f_n values were calculated from $\log K_n$, $\Delta\delta_d$, and ε_d determined, $\Delta\delta_n$ and ε_n values were obtained by using the nonlinear minimization computer package SAS.[26] The procedure for the calculations is schematically shown in Fig. 2.

Results

Self-Association of Pt(phen)(en). There are two models for treating self-association in solution; (1) the equilibrium between monomer and dimer (Eq. 4) and (2) the n-mer formation with equal successive stability constants.[27] Our assumption that self-association is expressed by the dimer formation (Eq. 4) in this study is based on the ^{1}H NMR chemical shifts for monomeric Pt(phen)(en) in aqueous solution, which were constant to within 0.001 ppm in the concentration range 10^{-5}—10^{-3} M.

With the increase of the Pt(phen)(en) concentration the H(phen) signals shifted upfield, which is ascribed to self-association. The upfield shifts due to the ring current effect indicated that face-to-face stacking occurs between the two phen rings in the dimer.[28] The order of ^{1}H NMR upfield shifts of phen, H-5>H-3>H-1>H-2, and the downfield shift of H(en) suggest that the dimer is best described by the structure shown in Fig. 3, where the Pt(II) centers are positioned far from each other due to repulsion between the positive charges. The $\log K_D$ and $\Delta\delta_d$ values at 24 °C were evaluated from the largest shift of H-5 to be 0.254 and 1.755 ppm, respectively. The $\log K_D$ value of Pt(phen)(en) is smaller than that of phen itself ($\log K_D$=1.49) determined in the same way, showing that the self-association is inhibited probably by the repulsion between the Pt(II) charges. The $\Delta\delta_d$ and δ_0 values for ^{195}Pt NMR were calculated from the K_D value of Pt(phen)(en) as follows (values in parentheses denote estimated standard deviations): $\Delta\delta_d(^{195}\text{Pt})$=95(13); $\delta_0(^{195}\text{Pt})$=171.6(0.1) ppm. The ε_0 and ε_d values at 370 nm were also evaluated to be 410(10) and 2500(300) from the K_D value.

Calorimetric Studies. As already revealed by the X-ray crystal structure analysis of [Pt(bpy)(en)]·AMP etc.,[29] the Pt(II) complexes and NMP afford stacked adducts, and this is further substantiated by isolation of the adducts of [Pt(phen)(en)] with AMP and GMP. Analysis of the calorimetric titration curves of the [Pt(L)(diamine)]–NMP systems by the method of nonlinear least-squares afforded the $\log\beta_n$ values and hence the successive stability constants $\log K_n$ for the adducts illustrated in Chart 1.

Table 1 summarizes the $\log K_n$ values and the relevant thermodynamic parameters $\Delta H_n°$ and $\Delta S_n°$ obtained. The $\log K_1$ and $\log K_2$ values are in the ranges 1.63—3.00 and 1.47—2.51, respectively, the former being equal to or greater than the latter in most cases. The $\log K_1$ values are in excellent agreement with the values determined previously by using the absorption and CD spectral data. The $\Delta H_1°$ and $\Delta H_2°$ values were −6.5—−26.2, 2.6—−20.0 kJ mol^{-1}, respec-

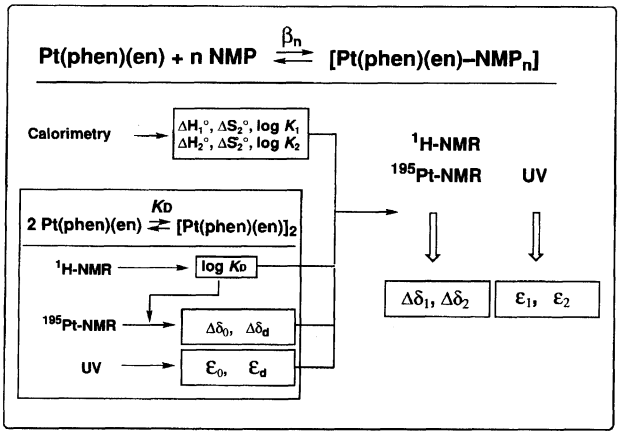

Fig. 2. Schematic representation of the procedure for calculating ^{1}H and ^{195}Pt NMR shift values $\Delta\delta_n$ and absorption coefficients ε_n (n=1, 2).

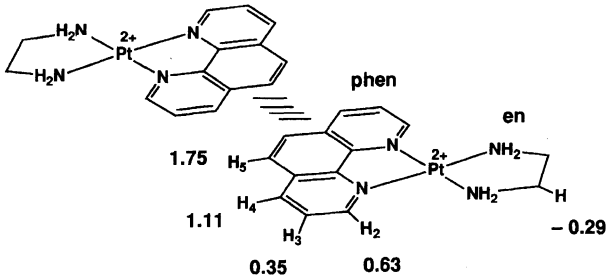

Fig. 3. Proposed structure of the dimer of Pt(phen)(en) and calculated ^{1}H NMR upfield shifts.

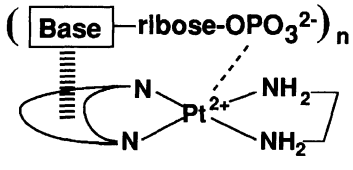

Chart 1.

Table 1. Stability Constants, $\log K_n$, and Thermodynamic Parameters, $\Delta H_n°$, and $\Delta S_n°$, for Formation of Pt(L)(diamine)–NMP Adducts in Water at 25 °C[a)]

L	Diamine	NMP	I	$\log K_1$	$\Delta H_1°$	$\Delta S_1°$	$\log K_2$	$\Delta H_2°$	$\Delta S_2°$	Others
phen	en	AMP	0.1	2.51[b)]	−25.6[b)]	−38	1.89 (0.06)	−6.2 (1.0)	15	
phen	en	AMP	0.2	2.48 (0.04)	−21.1 (0.6)	−23	1.56 (0.07)	−17.7 (2.1)	−29	
phen	en	Ado	0.1	2.21 (0.07)	−15.8 (1.3)	−11	2.18 (0.05)	2.6 (0.6)	50	
phen	en	Ado	0.2	2.24 (0.08)	−14.4 (1.3)	−6	2.39 (0.12)	2.4 (0.6)	54	
phen	en	GMP	0.1	2.49[c)]	−26.2[c)]	−40[c)]	1.91 (0.06)	−6.2 (0.9)	16	
phen	en	GMP	0.2	2.47 (0.08)	−20.0 (1.2)	−20	1.84 (0.09)	−14.2 (0.9)	−12	
phen	en	IMP	0.1	2.34[c)]	−11.9[c)]	5[c)]	1.62 (0.16)	−8.1 (3.0)	4	
phen	en	CMP	0.1	2.17 (0.08)	−6.5 (0.6)	20	1.51 (0.18)	−3.9 (1.8)	16	
phen	en	Cyt	0.1	1.63 (0.13)	−5.0 (0.9)	15	1.48 (0.09)	−3.7 (0.8)	16	
phen	en	NAD	0.1	2.32 (0.04)	−18.2 (0.5)	−17	1.53 (0.04)	−15.5 (0.8)	−23	
phen	L-dap	NAD	0.1	2.11 (0.07)	−19.7 (1.5)	−26	1.47 (0.09)	−11.9 (0.9)	−12	
Me$_4$phen	en	AMP	0.1	3.07[b)]	−22.9[b)]	−18	2.30 (0.10)	−20.0 (0.8)	−23	
Me$_4$phen	en	Ado	0.1	2.74 (0.14)	−16.1 (2.1)	−2	2.51 (0.09)	−12.1 (1.5)	7	
Me$_2$phen	en	AMP	0.1	3.00 (0.03)	−23.3 (0.3)	−21	2.49 (0.03)	−9.8 (0.4)	15	d)
nphen	en	AMP	0.1	2.44 (0.06)	−22.7 (1.1)	−30	1.84 (0.03)	−6.1 (1.0)	15	
bpy	en	AMP	0.1	2.30[b)]	−18.0[b)]	−16	1.83 (0.12)	−2.9 (1.5)	25	
bpy	en	Ado	0.1	1.91 (0.15)	−14.2 (2.8)	−11				

a) Values in parentheses denote estimated standard deviations. $\Delta H_n°$ and $\Delta S_n°$ are expressed in kJ mol^{-1} and J mol^{-1} K^{-1}, respectively. b) Data taken from Ref. 21b. c) Data taken from Ref. 21d. d) $\log \beta_4/\beta_2 = 4.68$ (0.11), $\Delta H°_{\beta_4/\beta_2} = 1.7$ (0.2).

tively, whereas $\Delta S_1°$ and $\Delta S_2°$ values were −38—20 and −29—54 J mol^{-1}K^{-1}, respectively. The results show that 1:1 adduct formation is mainly dependent on $\Delta H_1°$ and that $\Delta S_1°$ usually makes a negative contribution.

On the other hand, the 1:2 adduct formation is dependent on both $\Delta H_2°$ and $\Delta S_2°$ and in some cases either $\Delta H_2°$ or $\Delta S_2°$. In contrast to the major contribution of $\Delta H_1°$ to the 1:1 adduct formation, the effect of $\Delta H_2°$ on the 1:2 adduct formation is rather small. We found systematic correlations at different ionic strengths between $\Delta H_n°$ and $\Delta S_n°$ for both 1:1 and 1:2 adduct formations as depicted in Fig. 4, where the $\Delta H_1°$ and $\Delta H_2°$ values for Pt(phen)(en)–NMP are plotted against $\Delta S_1°$ and $\Delta S_2°$, respectively, to give a linear relationship for various NMP. Similar plots for Pt(L)(en)–AMP systems did not show such a correlation, and this suggests that the adduct formation may depend on the structure of the Pt(II) complexes, so that its thermodynamics is different for different L's.

Absorption and CD Spectra. The absorption spectra of Pt(phen)(en) at pH 7—8 in the region 350—380 nm changed with addition of NMP as typically shown for NAD in Fig. 5; the 358-nm peak of Pt(phen)(en) shifted to a longer wavelength with an increase in the intensity at 370—380 nm, indicating the

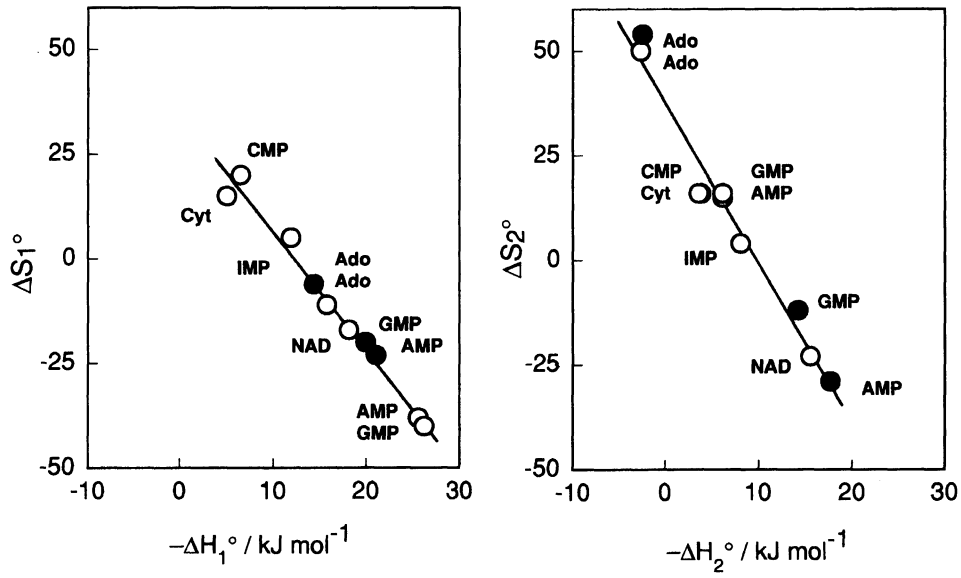

Fig. 4. Linear correlations between $-\Delta H_n°$ and $\Delta S_n°$ for Pt(phen)(en)–NMP systems (n=1, 2). I: ○, 0.1 M; ●, 0.2 M.

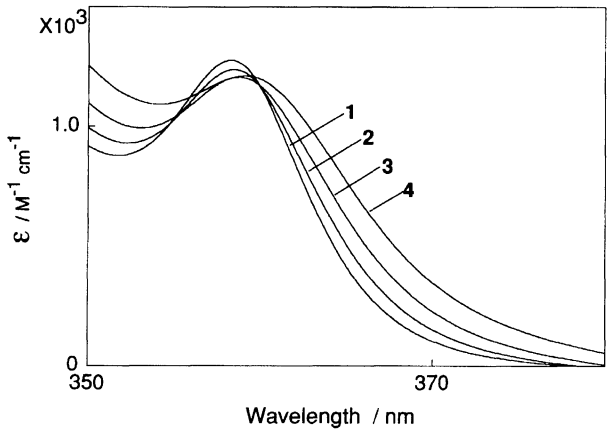

Fig. 5. Absorption spectra for Pt(phen)(en)–NAD systems at pH 7—8 and I=0.1 M. [Pt]=2.3 mM. Concentrations of NAD (mM): curve 1, 0; curve 2, 1.7; curve 3, 2.7; curve 4, 10.9.

formation of the adduct Pt(L)(en)–$(NAD)_n$. Deviation of the spectral curves from the isosbestic points is ascribed to the formation of the additional adduct with n=2. The difference spectrum, obtained by subtracting from the spectrum of the Pd(L)(en)–NAD system the spectrum of each component, revealed a maximum at 370 nm, which can be assigned to the charge transfer between coordinated phen and the adenine ring of NAD. AMP used in place of NAD gave a similar difference spectrum with a maxima centered at 370 nm. The intensity of the absorption spectrum of Pt(phen)-(en) at 366 nm decreased from 580 to 404 upon dilution (Pt concentration=0.25—25 mM) because of the dissociation of the dimer to the monomer. These spectral changes were used for calculation of the absorption coefficients ε_0, ε_1, ε_2, and ε_d for Pt(L)(en)–NMP systems (NMP=AMP, GMP, NAD).

Positive CD peaks of the Pt(L)(en)–AMP systems observed at 280—350 nm show the proximal effect of the optically active ribose moiety of NMP on the Pt-(L)(en) chromophore, which supports that both groups are in close proximity probably due to adduct formation (Fig. 6). Large CD peaks were observed for L= Me_4phen. Pt(phen)(L-dap), where L-dap is coordinated

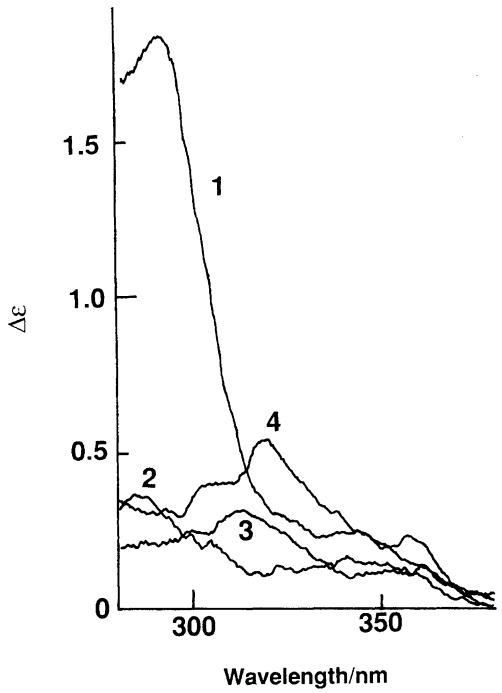

Fig. 6. CD spectra for 1:1 Pt(L)(en)–AMP systems at pH 7—8 and I=var. [Pt]=0.5 mM. L: curve 1, Me_4phen; curve 2, phen; curve 3, nphen; curve 4, bpy.

through the two amino groups similar to en with the deprotonated side-chain carboxylato group free from coordination, also showed positive CD peaks at 350—380 nm.

^{1}H and ^{195}Pt NMR Spectra. Addition of NMP to 2.3 mM solutions of Pt(phen)(en) in water caused ^{1}H NMR upfield shifts of phen, which is due to the ring current effect[28] of the base moiety of NMP stacked with L. The $\Delta\delta_1$, $\Delta\delta_2$, and $(\Delta\delta_2-\Delta\delta_1)$ values of H-4(phen) were 0.119—0.190, 0.149—0.246, and −0.010—0.100 ppm, respectively (Table 2). Upfield shifts observed for phen serve as strong evidence for face-to-face stacking in the 1:1 and 1:2 adducts. The $\Delta\delta_n$ values of H-5(phen) were larger than those for H-4(phen).

^{195}Pt NMR signals of Pt(phen)(en) in water shifted downfield upon addition of NMP and finally reached

Table 2. Calculated ^{195}Pt NMR Downfield Shifts (ppm) and ^{1}H Upfield Shifts (ppm) for 1:1 and 1:2 Pt(phen)(en)–NMP Adducts[a]

	I	Pt[b]		H-4(phen)[c]		H-5(phen)[d]	
NMP	M	$\Delta\delta_1$	$\Delta\delta_2$	$\Delta\delta_1$	$\Delta\delta_2$	$\Delta\delta_1$	$\Delta\delta_2$
AMP	0.1	21.5 (1.6)	28.5 (0.8)	0.173 (0.018)	0.163 (0.025)	0.206 (0.023)	0.334 (0.014)
	0.2	24.5 (2.7)	31.2 (1.8)	0.146 (0.015)	0.246 (0.010)	0.243 (0.025)	0.354 (0.020)
GMP	0.1	23.2 (1.6)	24.5 (0.9)	0.132 (0.018)	0.182 (0.010)	0.158 (0.067)	0.190 (0.038)
	0.2	21.9 (1.7)	31.6 (1.0)				
IMP	0.1	9.8 (1.3)	16.0 (0.9)	0.119 (0.008)	0.149 (0.006)	0.174 (0.015)	0.229 (0.010)
NAD	0.1	23.6 (3.2)	32.8 (2.6)	0.190 (0.018)	0.240 (0.014)	0.264 (0.017)	0.388 (0.013)

a) Values in parentheses denote estimated standard deviations. b) $\Delta\delta_0$(Pt)=179.7 ppm relative to $Pt(en)_2$. c) $\Delta\delta_0$(H−4)=7.710 ppm. d) $\Delta\delta_0$(H−5)=6.966 ppm.

constant values (Fig. 7). The order of the ^{195}Pt shifts with respect to NMP was NAD>AMP>GMP>IMP. The $\Delta\delta_n$ values of the Pt(phen)(en)–$(NMP)_n$ adducts calculated from the ^{195}Pt shifts are listed in Table 2, which shows that the $\Delta\delta_1$ and $\Delta\delta_2$ values are 9.8—24.5 and 16.0—32.8 ppm, respectively. The $\Delta\delta_n$ values for CMP were excluded because of large standard deviations. The $(\Delta\delta_2-\Delta\delta_1)$ values were 1.3—9.7 ppm, indicating that downfield shifts for the 1:2 adduct formation are smaller than those for the 1:1 adduct formation.

Discussion

Adduct Formation in Pt(L)(en)–NMP Systems. Previous absorption and CD spectral studies showed that Pt(phen or bpy)(en) forms 1:1 adducts with NMP (=AMP, GMP, IMP, CMP) at the Pt concentration≤1 mM.[21] Simulation of the present calorimetric titration data for various Pt(L)(en)–NMP systems further indicated the presence of 1:2 adducts at higher Pt concentrations (0.6—20 mM Pt(L)(en); NMP/Pt(L)(en)=0—10). An additional adduct (n=4) was detected for Pt(Me_2phen)(en)–AMP, and formation of higher adducts ($n\geq2$) was supported by the ^{13}C NMR spectroscopy. Figure 8 shows that with the addition of GMP to Pt(Me_2phen)(en) the C-12 signal of Me_2phen moves upfield due to 1:1 adduct formation but that the shift values become smaller upon further addition of GMP, indicating that higher adduct species are formed. This is evidenced by the X-ray crystal structure analysis of the Pt(phen)(en)–3′-CMP adduct with the –BP–BP–I–BP–BP–I– stacking mode (BP= base pair; I=intercalator) which involves NMP–NMP stacking.[29d]

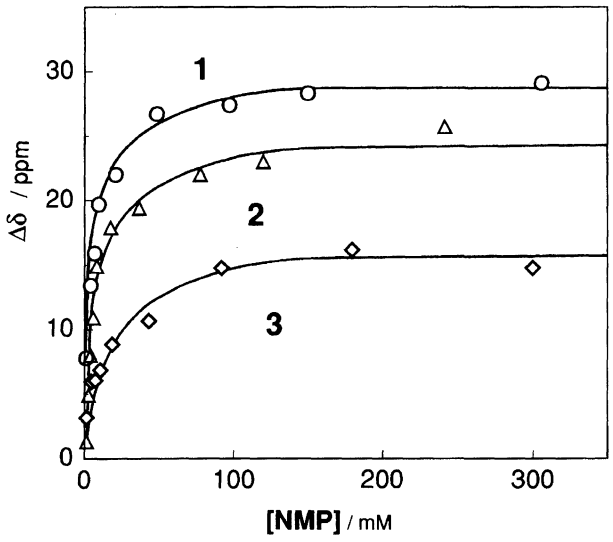

Fig. 7. Concentration dependences of the ^{195}Pt NMR downfield shifts $\Delta\delta$ for Pt(phen)(en)–NMP systems at pH 7—8 and I=0.1 M. NMP: curve 1, AMP; curve 2, GMP; curve 3, IMP.

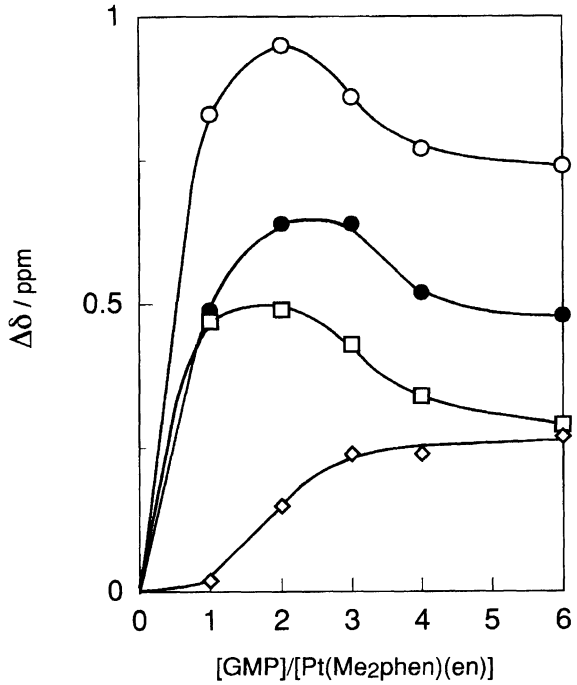

Fig. 8. Concentration dependences of the ^{13}C NMR upfield shifts of Me_2phen in the Pt(Me_2phen)(en)–GMP system at pH 7—8 at 70 °C. [Pt]=50 mM. ○, C-12; ●, C-2; □, C-3; ◇, C-4.

Structure Dependences of Thermodynamic Parameters for Adduct Formation in Pt(L)(en)–NMP Systems. Adduct formation of Pt(L)(en) with NMR is based on the cooperative interactions involving stacking and electrostatic interactions, which should give rise to the structure dependences of $\Delta H°$. The $-\Delta H_1°$ values for AMP decrease in the order of L, phen≥Me_2phen≈Me_4phen≈nphen>bpy, whereas the log K_1 values are in the order Me_2phen≈Me_4phen>phen≈nphen≥bpy (Table 1). We see from these results that large aromatic rings favor the π–π interaction with CT and that the aliphatic substituents stabilize the adduct by the hydrophobic effect. The importance of the charge transfer in Pt(phen)(en)–NMP is apparent from the $-\Delta H_1°$ values for NMP, AMP≈GMP>IMP, indicating that the amino group as an electron donor to the purine base of AMP and GMP enhances the charge transfer from the nucleobase to coordinated phen and increases the adduct stability.[21d] In this connection no liberation of heat was observed for the reaction of Pt(phen)(en) with uridine 5′-monophosphate involving a pyrimidine base devoid of the amino group.

Contribution of electrostatic interactions between Pt(II) and the phosphate moiety of AMP to the stability of the adducts is apprent from comparison of the $-\Delta H_1°$ and log K_1 values of nucleotides and nucleosides, which are in the order AMP>Ado for Pt(L)(en) (L=Me_4phen, phen, bpy) and CMP>Cyt for Pt(phen)(en). In addition, increase in the ionic strength from 0.1 to 0.2 M caused a decrease in $-\Delta H_1°$ from 25.6 and 26.2 to 21.1

and 20.0 kJ mol^{-1} for Pt(phen)(en)–AMP and –GMP systems, respectively, whereas almost no such effect was observed for $-\Delta H_1°$(15.8 (I=0.1) and 14.4 kJ mol^{-1} (I=0.2)) of the Pt(phen)(en)–Ado adduct.

Nucleosides Ado and Cyt form 1:2 adducts with Pt(phen)(en) more easily than the corresponding nucleotides, AMP and CMP, respectively. No other obvious structure dependences were observed for 1:2 adduct formation in contrast to 1:1 adduct formation, and this may be due to an equilibrium between the structures of the 1:2 adduct, a sandwich structure (NMP–Pt(L)(en)–NMP) and a pile structure (Pt(L)-(en)–NMP–NMP) (Fig. 9).

Adduct Formation in Pt(phen)(en)–NAD Systems. The NAD molecule is composed of two parts, the AMP and the nicotinamide monophosphate moiety, the latter of which has a pyridinium ring. On the basis of the ring size and the electron density the adenine ring is expected to stack with phen preferentially, and in fact the log K_1 and $-\Delta H_1°$ values have been found to be in the following order (log K_1, $-\Delta H_1°$): AMP (2.51, 25.6)≥NAD (2.32, 18.2)>CMP (2.17, 6.5)≫Cyt (1.63, 5.0), which indicates that the interaction occurs between the adenine ring of NAD and the coordinated phen ring. The log K_1 and $-\Delta H_1°$ values decreased with the charge on NMP, $AMP^{2-}>NAD^{1-}>$ Ado. The difference absorption spectrum for the Pt-(phen)(en)–NAD system was similar to that of Pt-(phen)(en)–AMP, and ^{1}H NMR upfield shifts due to the ring current effect arising from the face-to-face stacking were observed for H-2 (0.24 ppm) and H-8 (0.12 ppm) of the adenine ring while the shifts for the nicotinamide ring were less than 0.03 ppm and downfield. We conclude from these observations that NAD forms the adduct with Pt(phen)(en) mainly by the stacking of the adenine moiety. In this connection, X-ray crystal structure analysis of the lithium salt of NAD revealed the intermolecular stacking between the adenine and pyridinium rings.[30] No ^{1}H NMR shifts were observed for NAD in the presence of diquat, a dicationic bipyridinium compound, which shows that no stacking takes place between NAD and diquat and hence that the formal charges on Pt(II) in Pt(phen)(en) and diquat may have different effects.

^{195}Pt NMR Downfield Shifts and Absorption Coefficients of Adducts. Charge transfer due to stacking interactions in metal–nucleotide systems has been detected in the region 300—400 nm,[19,21a,21c,31] and the importance of stacking for specific interactions has been demonstrated for ternary Cu(II) complexes involving aromatic amino acids[20,32] and 1:1 Pt-(phen)(en)–NMP systems,[21d] though the contribution of the charge transfer to the stacking has been unknown. As a measure of evaluating contribution of the charge transfer to the stacking in 1:1 and 1:2 adducts, the ε_1 and ε_2 values were plotted against $-\Delta H_1°$ and $-(\Delta H_1°+\Delta H_2°)$, respectively (Fig. 10), which suggests that the charge transfer plays a major role in 1:1 and 1:2 adduct formations and that the 1:2 adduct takes the form of the sandwich type for the charge transfer to occur. We concluded previously from the extended Hückel molecular orbital calculation that the adduct formation between Pt(bpy)(en) and AMP is governed by the charge transfer interactions between the π orbital of AMP (nextHOMO) and the π^* orbital of Pt(bpy)-(en) (LUMO).[21c]

The electronic effect of stacking on the aromatic rings involved and especially on the central Pt(II) ion may be best seen from the ^{195}Pt NMR, whose chemical shifts can spread over 2000 ppm and are sensitive to the electron density on Pt(II).[33] Upon adduct formation the

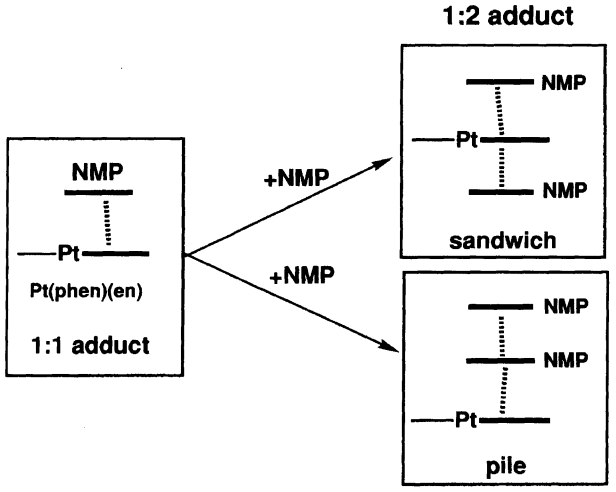

Fig. 9. Possible structures of 1:2 Pt(phen)(en)–NMP adducts.

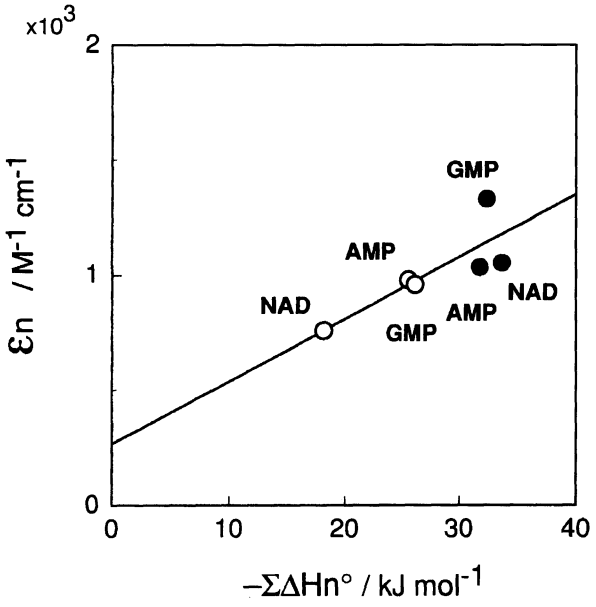

Fig. 10. Correlation between $-\Sigma\Delta H_n°$ and ε_n calculated for Pt(phen)(en)–NMP systems at I=0.1 M. NMP=AMP, GMP, NAD. n: ○, 1; ●, 2.

^{195}Pt NMR signals were observed to shift downfield by 8—33 ppm. Since the signals of Pt(IV) complexes were observed downfield relative to those of Pt(II) complexes, the downfield shifts are compatible with a lower electron density on Pt(II). That the downfield shifts are due to stacking is concluded from the correlations between $-\Sigma\Delta H_n^\circ$ values and the ^{195}Pt chemical shift differences $\Delta\delta_n$ (Fig. 11) and between the $-\Sigma\Delta H_n^\circ$ values and the absorption coefficients ε_n (Fig. 10). To our knowledge these are the first evidence for the direct relationship between the stacking with charge transfer and the electron density on the Pt atom. The mechanism of the charge transfer through the Pt(II) ion remains to be explained.

Comparison between the Adduct Formations by Pt(L)(en) and Organic Intercalators and Concluding Remarks. Ethidium, a well established DNA intercalator,[34] and Pt(phen)(en) are a monocationic and a dicationic ion, respectively, with the aromatic ring of comparable size. We reported in a previous paper that ethidium bromide forms adducts with AMP, GMP, and IMP with smaller $-\Delta H_1^\circ$ values (4.0—9.3 kJ mol^{-1} at I=0.2 M(NaCl)[21d] as compared with those found for Pt(phen)(en) (11.9—26.2 kJ mol^{-1}). This indicates that the ethidium–NMP binding energy is much smaller than that for Pt(phen)(en)–NMP. Because of the difference in the contribution by the entropy change which is positive for ethidium, the log K_1 values are 1.9—2.0 for AMP and GMP, which are not much smaller than those for Pt(phen)(en). In contrast to ethidium, diquat which is structurally comparable with phen caused only very small ^{1}H NMR downfield shifts ($\leq$0.1 ppm) of AMP and liberated no heat ascribable to adduct formation, and therefore the fractional population of the stacked species must be very small. These results suggest that the central Pt(II) ion of Pt(phen)(en) may take part in the interaction with nucleobases via coordinated phen and thus play a unique role in the adduct formation.

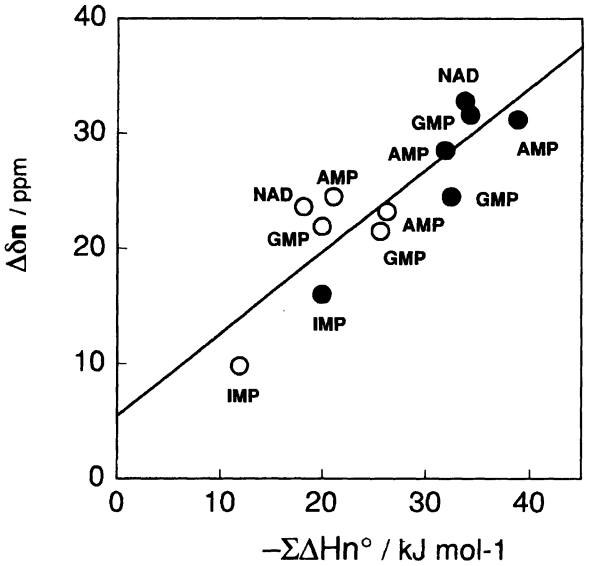

Fig. 11. Correlation between $-\Sigma\Delta H_n^\circ$ and $\Delta\delta_n$ of ^{195}Pt NMR downfield shifts calculated for Pt(phen)(en)–NMP systems at I=0.1 and 0.2 M. n: ○, 1; ●, 2.

We found a linear relationship between ΔH_n° and ΔS_n° as shown in Fig. 4, where a linear correlation holds for NMP but not for Pt(II) complexes. Charge transfer due to intramolecular ligand–ligand stacking interactions has been reported for ternary metal complexes involving nucleotides and heteroaromatic ligands such as phen[19,31] and ternary Cu(II) complexes involving aromatic amino acids in place of nucleotides.[20] The present and previously reported 1:1 and 1:2 Pt(phen)(en)–NMP systems also exhibit the charge-transfer band in the region 300—380 nm.[21a,21c] The intensities ε_n of the difference spectra for 1:1 and 1:2 adducts plotted against $-\Sigma\Delta H_n^\circ$ (n=1, 2) gave a linear relationship (Fig. 10), which suggests that the observed enthalpy change is mainly due to the charge-transfer interaction between the stacked rings and that the adduct with n=2 must take the sandwich form. This relationship and the ΔH_n°–ΔS_n° relationship (Fig. 4) may be characteristic of the adduct formation between Pt(phen)(en) and nucleobases.

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22) The following abbreviations were used throughout the text: Ado, adenosine; Cyt, cytidine; AMP, adenosine 5′-monophosphate; GMP, guanosine 5′-monophosphate; IMP, inosine 5′-monophosphate; CMP, cytidine 5′-monophosphate; 3′-CMP, cytidine 3′-monophosphate; NAD, nicotinamide adenine dinucleotide; dap, 2,3-diaminopropionate; Hdap, 2,3-diaminopropionic acid; en, ethylenediamine; bpy, 2,2′-bipyridine; phen, 1,10-phenanthroline; Me_2phen, 5,6-dimethyl-1,10-phenanthroline; Me_4phen, 3,4,7,8-tetramethyl-1,10-phenanthroline; nphen, 5-nitro-1,10-phenanthroline; NMP=AMP, GMP, IMP, CMP, NAD, Ado, or Cyt; L=bpy, phen, Me_2phen, Me_4phen, or nphen; diamine=en, dap, or Hdap.
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