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Ternary Complexes in Solution. 26.[1] Stacking Interactions in the Mixed-Ligand Complexes Formed by Adenosine or Inosine 5′-Triphosphate, 2,2′-Bipyridyl, and Cobalt(II), Nickel(II), Copper(II), or Zinc(II). Evidence for Phosphate-Protonated Complexes

Phalguni Chaudhuri and Helmut Sigel*

Contribution from the Institute of Inorganic Chemistry, University of Basel, CH-4056 Basel, Switzerland. *Received August 18, 1976*

Abstract: The formation of a stacked adduct between 2,2′-bipyridyl and the purine moiety of adenosine or ATP, and inosine or ITP, causes a change in absorption that is best observed by UV-difference spectroscopy. Using Benesi–Hildebrand plots the stability of the adducts (log K_{St} = 0.9–1.4) comprising adenosine or inosine and $M(bpy)^{2+}$, where M^{2+} = Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+}, can be determined. The stabilities of these adducts are comparable to those formed by 2,2′-bipyridyl and adenosine, inosine, ATP, or ITP. However, the stability of the stacked adducts with ATP or ITP in the presence of M^{2+} is increased by several orders of magnitude. This is the result of the metal ion bridge formed between 2,2′-bipyridyl and the phosphate groups of the nucleotides (=NTP). Studies of the bpy–Zn^{2+}–NTP^{4-} systems by ^1H NMR confirm that stacking between the aromatic moieties is enhanced by the coordination of the metal ion. The stability constants for the mixed-ligand complexes $M(bpy)(ATP)^{2-}$ were already known from potentiometric titrations; those for the corresponding ternary systems with ITP have now been determined by the same method. These results agree with the stability constants determined spectrophotometrically. Comparison of the molar absorptivities of all the systems indicates that the stacked isomer dominates in the equilibrium between an opened and stacked form of $M(bpy)(NTP)^{2-}$. This also holds for the protonated ternary complexes $M(bpy)(HITP)^-$, for which the intramolecular stacking interactions could also be confirmed by ^1H NMR. It is concluded that in $M(bpy)(HITP)^-$ the proton is located at the γ-phosphate group. The corresponding complexes with ATP probably also exist but were not observed; this and the sites of proton binding in $M(HATP)^-$ are discussed.

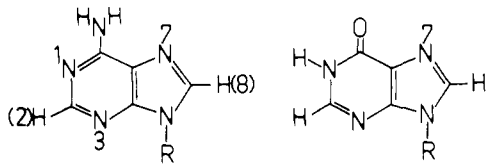
Metal ions have many functions in biological systems; a particularly important one is, together with certain nucleotides, as cofactors for various enzymatic reactions;[2] therefore, metal ion–nucleotide complexes have found much attention.[2,4-6] But due to the ambidentate behavior of the nucleotides the structure of many of their complexes, especially in solution, is not yet clear. For binary complexes it is generally agreed that the stability determining factor is the coordination tendency of the phosphate groups,[2,4,7,8] while the nucleic base moieties may or may not be coordinated, depending on the base and metal ion.[9] This leads in solution to equilibria between macrochelates and complexes having only phosphate coordination, as is known, for example, for the Mn^{2+}–adenosine 5′-triphosphate system.[10] A further problem is the site of protonation in these complexes; for example,[11] in $M(HATP)^-$ the proton may be located either at N(1) of the adenine moiety or at the terminal

phosphate group; which site is preferred depends on the metal ion involved.[12]

In ternary complexes the basicity of a group may change,[9] and some binding sites may be released from the coordination sphere.[13] In addition, if the second ligand contains an aromatic group, a new interaction becomes possible. For example, the mixed-ligand complexes of ATP, tryptophan, and Mn^{2+}, Cu^{2+}, or Zn^{2+} show an intramolecular stacking[14] between the purine and indole residues.[15] Similarly, the complexes formed by 2,2′-bipyridyl, Cu^{2+}, and ATP or ITP can be considered as metal ion bridged stacked bipyridyl-purine adducts;[16,17] i.e., these complexes exist predominantly in a "folded" form.

As such structural features will affect the reactivity of metal ion-nucleotide complexes in vitro[18] and in vivo, we felt it desirable to improve our knowledge of the structures of these complexes. As second ligand we have chosen 2,2′-bipyridyl; this allows a more quantitative evaluation of the mixed-ligand systems[16] than, for example, with the naturally occurring tryptophanate.[15] This quantitative treatment seems at present more important to us than the use of a biologically more meaningful ligand, because stacking depends only on the presence of an aromatic group and not on its origin. Therefore, we have extended our earlier measurements[16] with Cu^{2+} to Co^{2+}, Ni^{2+}, and Zn^{2+}, together with 2,2′-bipyridyl and ATP or ITP (Chart I). For comparison ATP or ITP were partly

Chart I



R = ribosyl 5′-triphosphate

ATP ITP

replaced by adenosine or inosine. The stability of the ternary complexes was determined by potentiometric titrations, while the occurrence of stacking was demonstrated by UV-difference spectra and by ^1H NMR measurements. In addition, evidence was obtained for the formation of the phosphate-protonated complexes, M(bpy)(HITP)$^-$, in which stacking also occurs.

Experimental Section

Materials. The metal(II) perchlorates (all purum) were from Fluka AG, Buchs, Switzerland; the concentrations of their stock solutions were determined with ethylenediamine *N,N,N′,N′*-tetraacetate. Sodium perchlorate, 2,2′-bipyridyl (both purissimum), and D_2O (99.75%) were also from Fluka AG, and adenosine (for biochemical purposes) was from Merck AG, Darmstadt, Germany. Inosine (p.A., >99.5%) and the disodium salts of ATP and ITP were purchased from Serva Feinbiochemica GMBH, Heidelberg, Germany (for specifications, see ref 16). The sodium tripolyphosphate was the same as used recently.[15] All other materials were reagent grade and used without further purification. Double distilled water was used for preparing all solutions.

Apparatus. The absorbance spectra were measured with a Varian Techtron spectrophotometer (Model 635) using a Honeywell electronic recorder 196. Some of the experiments were repeated on a Cary 118 spectrophotometer with the same result. The pH was measured with a Metrohm potentiometer E 353 B and potentiometric titrations were carried out with a potentiograph E 336 using type UX glass electrodes. The ^1H NMR spectra were recorded with a Bruker WH-90FT spectrometer at 90 MHz.

Determination of Equilibrium Constants by Potentiometric Titrations. The acidity constant $K^H_{H(ITP)}$ due to the deprotonation of the γ-phosphate group of ITP was determined from automatic titrations with 5×10^{-2} M NaOH of aqueous solutions (50 mL, under N_2) containing 9×10^{-4} M $HClO_4$ and $NaClO_4$ ($I = 0.1$; 25 °C) in the presence and absence of 6×10^{-4} M ITP^{4-} (for its preparation and handling, see ref 9). The differences between such a pair of titrations were measured, and the constant was calculated from the pH range which corresponded to a degree of neutralization between 0.1 and 0.9.

The stability constants $K^M_{M(ITP)}$ and $\beta^M_{M(bpy)(ITP)}$ for the binary and ternary complexes, respectively, were measured under the same conditions as for the acidity constant, but $M(ClO_4)_2$ and 2,2′-bipyridyl were added to give the ratios M^{2+}-ITP = 1:1 for the binary and M^{2+}-bpy-ITP = 1:1:1 for the ternary systems. Titrations of solutions without ligand were used as a basis for the evaluation. At least three (usually four) independent titration curves were measured.

The calculation of $K^M_{M(ITP)}$ for the binary complexes was done by considering the species H^+, $H_2(ITP)^{2-}$ ($K^H_{H_2(ITP)} = 2.1$ at $I \sim 0.2$ and 37 °C),[17] $H(ITP)^{3-}$, ITP^{4-}, M^{2+}, and $M(ITP)^{2-}$.[19] The overall stability constant $\beta^M_{M(bpy)(ITP)}$ for the ternary complexes was computed[19] by taking into account H^+, $H_2(ITP)^{2-}$, $H(ITP)^{3-}$, ITP^{4-}, $M(ITP)^{2-}$, $H_2(bpy)^{2+}$ (cf. ref 20), $H(bpy)^+$, bpy, $M(bpy)^{2+}$, $M(bpy)_2{}^{2+}$ (cf. ref 21), M^{2+}, and $M(bpy)(ITP)^{2-}$. Only this pH region was evaluated where the formation of protonated complexes was insignificant (cf. Results and Discussion sections). It may be noted that with certain metal ions, e.g., Zn^{2+}, the species $Zn(bpy)_2(ITP)^{2-}$ could also occur in low concentrations; the values of $\beta^M_{M(bpy)(ITP)}$ did not show a trend in dependence on pH, and therefore the concentration of $Zn(bpy)_2(ITP)^{2-}$ is too low to have a significant effect on the results.

Determination of the Stability Constants of 2,2′-Bipyridyl-Purine Adducts by Spectrophotometric Measurements. The UV-difference spectra were taken in 1-cm quartz cells by placing in the reference beam one cell with $[M(ClO_4)_2]$ = [bpy] and a second one with the nucleoside or nucleotide; the sample beam contained one cell with the mixed system and one with water. $NaClO_4$ was added to all four solutions to maintain $I = 0.1$ (25 °C); the desired pH was adjusted to within ±0.02 log unit[16] (cf. figure legend).

As the absorption of the reference solution containing M^{2+}-bpy is rather high, the effect of this solution on the stability constant, λ_{max}, and the molar absorptivity ϵ was studied. In the concentration range from 10^{-4} to 8×10^{-4} M, the stability constant and λ_{max} remain constant while ϵ decreases by about 30% with increasing $[M^{2+}/bpy]$. Changing the slit width of the spectrophotometer from 0.5 to 1.0 and 2.0 nm decreases ϵ by about 30%, while λ_{max} and the stability constant again remain practically constant. In general, the experiments were carried out with $[M^{2+}]$ = [bpy] = 4×10^{-4} M and the slit width was adjusted to 2.0 nm. As a consequence, there is some stray light present and the measured molar absorptivities (ϵ) are somewhat low, but in the present cases the stability constants determined are independent of the latter as indicated above. In addition, the relative values of ϵ can be compared, provided they were obtained under the same conditions. These difficulties must also be considered if different spectrophotometers are used. The UV-difference spectra recorded now were more symmetrical, i.e., more bell shaped than those obtained recently,[16] but the overall shape was similar (for details, see the tables).

There was a further problem in the Co^{2+}- and Ni^{2+}-NTP systems; a M^{2+}-phosphate interaction apparently contributes somewhat to the absorbance in the 315-nm region. To compensate for this we added triphosphate to the reference cell which already contained M^{2+} and bpy. This is possible because $M(HTP)^{2-}$ and $M(NTP)^{2-}$ are of comparable stability.[8] With Co^{2+}, $NaClO_4$ was not added into this reference cell; Na^+ may compete too much for coordination at HTP^{4-} in this case. In all the experiments with Co^{2+} the solutions were flushed with N_2 during preparation to minimize oxidation to Co^{3+}. With these precautions also for the Co^{2+} and Ni^{2+} systems normal difference spectra were observed, and reasonable stability constants were obtained. Finally, it should be noted that with the Cu^{2+}- and Zn^{2+}-NTP systems, addition of TP has no effect on λ_{max}, on ϵ, or on the stability constant.

The evaluation of the data was carried out by Benesi-Hildebrand plots, i.e., the stability constant was obtained from the intercept of the straight line with the *y* axis, while ϵ was calculated from the intercept with the *x* axis.[16] High complex stability leads to a line parallel to the *y* axis; hence, ϵ can still be calculated, although only a lower limit for the stability constant can be estimated. All straight lines were drawn according to least-squares methods. The weak self-association[22] of the nucleosides and nucleotides is negligible.

The ranges of error given are *three* times the standard deviation throughout; this was done to make sure that only differences, which are certainly significant, are interpreted and that these do not arise

Table I. Logarithms of the Stability Constants of the Binary Complexes, $M(NTP)^{2-}$, and the Ternary Complexes, $M(bpy)(NTP)^{2-}$, as Determined by Potentiometric Titrations (25 °C; I = 0.1, $NaClO_4$)[a]

NTP^{4-}	M^{2+}	Log $K^{M}_{M(NTP)}$	Log $\beta^{M}_{M(bpy)(NTP)}$	Log $K^{M(bpy)}_{M(bpy)(NTP)}$	Δ log K_M
ATP^{4-}	Co^{2+}	4.86 ± 0.07		4.79 ± 0.05	−0.07
	Ni^{2+}	4.85 ± 0.04		4.45 ± 0.05	−0.40
	Cu^{2+}	6.38 ± 0.09		6.91 ± 0.15	+0.53
	Zn^{2+}	5.21 ± 0.06		5.26 ± 0.03	+0.05
ITP^{4-}	Co^{2+}	4.81 ± 0.03	10.79 ± 0.03	4.73	−0.08
	Ni^{2+}	4.73 ± 0.03	11.57 ± 0.02	4.44	−0.29
	Cu^{2+}	5.99 ± 0.04	14.03 ± 0.08	6.03	+0.04
	Zn^{2+}	5.02 ± 0.03	10.34 ± 0.04	5.04	+0.02

[a] Acidity constants of the nucleotides: $pK^{H}_{H(ATP)}$ = 6.42 ± 0.05 and $pK^{H}_{H(ITP)}$ = 6.45 ± 0.01. The range of error given corresponds to three times the standard deviation. The data for the ATP systems are taken from our earlier work.[25]

from any systematic errors. In those cases where the error could only be estimated, this was done with the same aim in mind.

^{1}H NMR Measurements. D_2O was used as solvent and sodium 3-(trimethylsilyl)-1-propanesulfonate as an internal standard (25 °C). The use of this standard in the presence of metal ions and ligands with aromatic moieties is only applicable in rather dilute solutions due to hydrophobic interactions between the trimethylsilyl group and the aromatic moiety.[23] The pD of the solutions was adjusted to the desired value ±0.05 (pD = pH meter reading +0.4)[24] by dotting with a glass stick and concentrated NaOD. The change in the chemical shift, i.e., Δ shift (Hz), of H(2) or H(8) of ATP was obtained by subtracting the chemical shift of the proton in the presence of 2,2′-bipyridyl and/or Zn^{2+} from that of the same proton in the free ligand. This means a positive value of Δ shift implies an upfield shift of the ^{1}H NMR signal.

Results

Potentiometric Determination of the Stability of the Binary and Ternary Metal Ion–Nucleotide Complexes. To obtain a sound basis the stability constants of the binary and ternary complexes containing either ATP[25] or ITP are assembled in Table I. The constants determined by us for $M(ITP)^{2-}$ are in part lower (for Co^{2+} and Ni^{2+}) and in part higher (Cu^{2+}, Zn^{2+}) than the values given by Taqui Khan and Reddy.[26] However, our stability constant for $Co(ITP)^{2-}$ agrees well with the one given by Walaas,[7] and all the values of the ITP systems compare well with those of the ATP systems as is to be expected.[2,4,7,8]

The stability constants of the mixed-ligand system are defined by eq 1-3. The overall stability constant $\beta^{M}_{M(bpy)(NTP)}$ is connected with $K^{M(bpy)}_{M(bpy)(NTP)}$ and $K^{M(NTP)}_{M(NTP)(bpy)}$ by eq 4 and 5, respectively.

$$M^{2+} + bpy + NTP^{4-} \rightleftharpoons M(bpy)(NTP)^{2-}$$

$$\beta^{M}_{M(bpy)(NTP)} = [M(bpy)(NTP)]/[M][bpy][NTP] \quad (1)$$

$$M(bpy)^{2+} + NTP^{4-} \rightleftharpoons M(bpy)(NTP)^{2-}$$

$$K^{M(bpy)}_{M(bpy)(NTP)} = [M(bpy)(NTP)]/[M(bpy)][NTP] \quad (2)$$

$$M(NTP)^{2-} + bpy \rightleftharpoons M(bpy)(NTP)^{2-}$$

$$K^{M(NTP)}_{M(NTP)(bpy)} = [M(bpy)(NTP)]/[M(NTP)][bpy] \quad (3)$$

$$\log K^{M(bpy)}_{M(bpy)(NTP)} = \log \beta^{M}_{M(bpy)(NTP)} - \log K^{M}_{M(bpy)} \quad (4)$$

$$\log K^{M(NTP)}_{M(NTP)(bpy)} = \log \beta^{M}_{M(bpy)(NTP)} - \log K^{M}_{M(NTP)} \quad (5)$$

The usual way to quantify the stability of mixed-ligand complexes, of the kind studied here, is according to eq 6,[13,19,25,27] i.e., by comparing the difference in stability, e.g., for the reaction between $M(bpy)^{2+}$ or $M(aq)^{2+}$ and NTP^{4-}.

$$\Delta \log K_M = \log K^{M(bpy)}_{M(bpy)(NTP)} - \log K^{M}_{M(NTP)} = \log K^{M(NTP)}_{M(NTP)(bpy)} - \log K^{M}_{M(bpy)} \quad (6)$$

This constant, Δ log K_M, is due to equilibrium 7.

$$M(NTP)^{2-} + M(bpy)^{2+} \rightleftharpoons M(bpy)(NTP)^{2-} + M^{2+} \quad (7)$$

In general, *negative* values for Δ log K_M (eq 6) are expected, since usually $K^{M}_{ML} > K^{ML}_{ML_2}$.[28] This is in accordance with the statistical values obtained for the coordination of two different bidentate ligands to a regular and to a distorted octahedral coordination sphere, i.e., $\Delta \log K_{oh} = -0.4$ and $\Delta \log K_{do} \simeq -0.9$, respectively.[13] In addition, it should be noted that, neglecting the coordination tendency of the purine moiety, Co^{2+} and Ni^{2+} coordinate to all three phosphate groups of the triphosphate chain, while Cu^{2+} and Zn^{2+} coordinate only bidentately, i.e., to the β and γ groups.[29] We may thus conclude from the results of Table I that all these ternary complexes are more stable than would be expected. The reasons for such increased stabilities have been discussed;[13] for the present it is important only that in the pH range where complexation occurs, the mixed-ligand complexes are by far the dominating species.

Spectrophotometric Evidence for Metal Ion Bridged Stacked Adducts with ATP or ITP. Formation of stacked adducts between the purine moiety of nucleosides or nucleotides and 2,2′-bipyridyl and the accompanying change of the UV-absorption have been observed before.[16] It should therefore be possible to determine the stability constants, K_{St}, of such adducts by plotting $1/[NTP]$ vs. $1/\Delta A$. Indeed, straight lines are obtained (Figure 1) which indicate that a Benesi-Hildebrand evaluation is justified and that 1:1 adducts are formed. The constants, obtained in this way, are apparent constants, valid only at the experimental pH, but by taking into account the protonation of the terminal phosphate group of NTP one obtains the constant which is due to eq 2.

$$\log K^{M(bpy)}_{M(bpy)(NTP)} = \log K_{St} + \log (1 + [H^+]/K^{H}_{H(NTP)}) \quad (8)$$

This proves that the stability of the stacked adducts between 2,2′-bipyridyl of $M(bpy)^{2+}$ and the purine moiety of ATP is dependent on the complex formation between $M(bpy)^{2+}$ and the phosphate chain. Indeed, the stability constants determined spectrophotometrically (fifth column of Table II) are, within experimental error, identical with the values obtained from the potentiometric titrations (Table I).

The same experiments were carried out with the systems containing ITP, 2,2′-bipyridyl, and Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+}. However, in this case the apparent stability constants, K_{St}, of the stacked adducts did not show the expected dependence on pH,[30] and if the correction for the competition with the proton (cf. eq 8) is applied a constant value is not obtained; the data of column five in Table III decrease with increasing pH.

Table II. Logarithms of the Apparent Stability Constants, Log K_{St}, at the given pH, and of the pH-Independent Stability Constants, Log $K^{M(bpy)}{}_{M(bpy)(ATP)}$ (Eq 2), for the Stacking Adducts between M(2,2′-Bipyridyl)$^{2+}$ and the Purine Moiety of ATP as Determined by UV-Difference Spectra (25 °C; I = 0.1, $NaClO_4$)[a]

M^{2+}	pH[b]	Log K_{St}[c]	Log K_{St} + log (1 + $[H^+]/K^H{}_{H(ATP)}$)	Log $K^{M(bpy)}{}_{M(bpy)(ATP)}$[d]
Co^{2+}	3.80	2.30 ± 0.03	4.92	4.93 ± 0.13
	4.00	2.40 ± 0.02	4.82	
	4.50	3.04 ± 0.02	4.96	
	4.50	3.11 ± 0.02	5.03	
Ni^{2+}	4.00	2.06 ± 0.03	4.48	4.47 ± 0.07
	4.00	2.08 ± 0.05	4.50	
	4.50	2.50 ± 0.04	4.42	
Cu^{2+}	2.50	2.88 ± 0.02	6.80	6.80 ± 0.25[e] 6.96 ± 0.25[f]
Zn^{2+}	3.50	2.58 ± 0.04	5.50	5.31 ± 0.22
	3.54	2.43 ± 0.02	5.31	
	4.00	2.88 ± 0.04	5.30	
	4.00	2.72 ± 0.05	5.14	

[a] The experiments with Ni^{2+} were done in the presence of inorganic triphosphate; those for Co^{2+} were carried out under N_2 and in the presence of triphosphate but in the absence of Na^+ in the reference solution (for details see the Experimental Section and Table V). [b] Adjusted to ±0.02 log units. [c] Calculated from the intercept of the correlation line with the y axis (cf. Figure 1), together with the corresponding error. [d] The range of error given is three times the standard deviation. [e] This error is only estimated. [f] Result of ref 16.

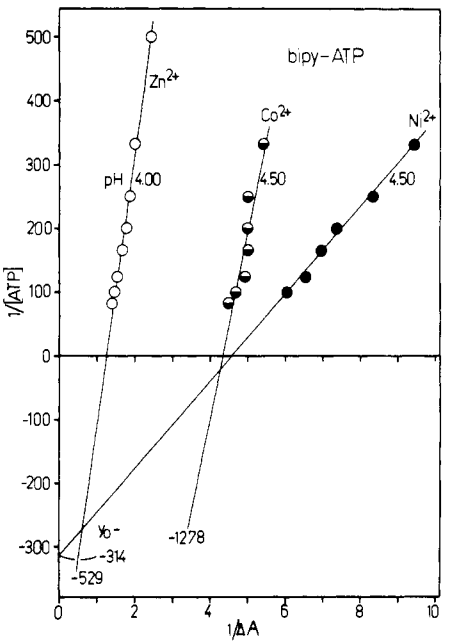


Figure 1. Determination of the apparent stability constants, K_{St}, at pH 4.00 (○) and 4.50 (◒, ●) for the stacking adducts between M(bpy)$^{2+}$([M^{2+}] = [bpy] = 4×10^{-4} M) and ATP, where M^{2+} = Co^{2+} (◒), Ni^{2+} (●), or Zn^{2+} (○) (25 °C; I = 0.1, $NaClO_4$). The UV-difference spectra evaluated for the Co^{2+} and Ni^{2+} systems were obtained in the presence of inorganic triphosphate (cf. Experimental Section and Table II). Intercepts with the y axis, i.e., $-K_{St}$: −1278 ± 42 (Co^{2+}), −314 ± 25 (Ni^{2+}), and −529 ± 64 (Zn^{2+}). Intercepts with the x axis, i.e., $1/\Delta A_{max}$: 4.34 ± 0.14 (Co^{2+}, 311 nm), 4.58 ± 0.36 (Ni^{2+}, 311 nm), and 1.26 ± 0.15 (Zn^{2+}, 313 nm).

Careful consideration of all the data in Table III reveals the following. Firstly, for Ni^{2+} and Zn^{2+}, the experiments at high pH could still be evaluated and the values corrected according to eq 8 are, within experimental error, identical with the stability constants $K^{M(bpy)}{}_{M(bpy)(ITP)}$ (eq 2) determined by the potentiometric titrations (Table I). The lower limit of the constant obtained for the Cu^{2+} system fits into the same picture. These results correspond to those obtained with ATP (Table II). Secondly, the values obtained for log K_{St} are, for example, with Co^{2+} identical at pH 2.50 and 3.50; this must mean that the stability of the stacked adduct is independent of $[H^+]$. Hence, the ITP species participating at the stacked adduct must be protonated and we attribute this pH-independent stability constant to eq 9.

$$M(bpy)^{2+} + HITP^{3-} \rightleftharpoons M(bpy)(HITP)^- \qquad K^{M(bpy)}{}_{M(bpy)(HITP)} = [M(bpy)(HITP)]/[M(bpy)][HITP] \qquad (9)$$

The differing behavior of the ATP and ITP systems in the lower pH range must be due to the differing affinities of their nucleic bases for protons;[17] this will be discussed later. Obviously, if only the Cu^{2+}-bpy-ITP system is considered, there is a hidden pitfall and misinterpretation[16] of the composition of the complex and its stability is possible. The existence of protonated complexes is only clear for the systems containing the three other metal ions (cf. Table III).

Stability of the Stacked 2,2′-Bipyridyl–Nucleoside Adducts. For reasons of comparison, especially regarding eq 9 and the molar absorptivities, we decided to study also equilibrium 10.

$$M(bpy)^{2+} + NS \rightleftharpoons M(bpy)(NS)^{2+} \qquad (10)$$

The stabilities of the stacked adducts between 2,2′-bipyridyl and adenosine, inosine, ATP, or ITP were already known.[16] Those corresponding to eq 10 have now been determined (cf. Experimental Section and ref 16); the constants are independent of pH in the range studied (Table IV). It is obvious that the presence of metal ions has no significant effect on the stability of these stacked adducts, which is in the order found also for similar but metal ion free systems.[15,32,33a]

The molar absorptivities and the maxima of the absorption band of all the stacked adducts studied (eq 2, 9, and 10; Tables II–IV) are given in Table V. The molar absorptivities are in the order usually observed for such adducts involving nucleic bases.[32a,33,34] The variation of the absorption maxima could be taken as an indication that the adducts formed are charge-transfer complexes in which bipyridyl is the electron donor and the purine moiety the acceptor. In M(bpy)$^{2+}$ the electron density is increased on the amine, due to d_π-p_π back-donation,[35] and thus the charge transfer should occur in these adducts at lower energy, as is indeed the case (311–318 nm), compared with those formed by bipyridyl itself (293–295 nm). This agrees with other results,[32d,36] and especially with observations made on charge-transfer adducts between 8-hydroxyquinoline and electron-deficient aromatic compounds; coordination of Cu^{2+}, Pd^{2+}, or Ni^{2+} to 8-hydroxyquinoline lowered the energy of the charge-transfer transition.[37]

^{1}H NMR studies of the ATP- and ITP-Zn^{2+}–bpy systems were carried out to detect the intramolecular stacking in the mixed-ligand complexes by an independent method. The first evidence for stacking in these systems had been obtained by line-broadening studies[17] with Cu^{2+}. However, the following approach, studying the chemical shift, seems more satisfactory. When a stacking adduct is formed by two aromatic systems, the ^{1}H NMR resonances of those protons lying above or below a π system are shifted to higher field compared with the signals of the free molecule.[32c,38] As paramagnetic metal ions broaden the resonances the diamagnetic Zn^{2+} is best suitable for such experiments.

Indeed, the existence of an intramolecular stacking within Zn(bpy)(ATP)$^{2-}$ becomes evident when increasing concen-

Table III. Logarithms of the Apparent Stability Constants, Log K_{St}, at Different pH Values and of the Stability Constants, Log $K^{M(bpy)}{}_{M(bpy)(HITP)}$ (Eq 9) and Log $K^{M(bpy)}{}_{M(bpy)(ITP)}$ (Eq 2), for the Stacking Adducts between M(2,2′-Bipyridyl)$^{2+}$ and the Purine Moiety of ITP as Determined by UV-Difference Spectra (25 °C; I = 0.1, $NaClO_4$)[a]

M^{2+}	pH[b]	Log K_{St}[c]	Log $K^{M(bpy)}{}_{M(bpy)(HITP)}$[d]	Log K_{St} + log (1 + $[H^+]/K^H{}_{H(ITP)}$)	Log $K^{M(bpy)}{}_{M(bpy)(ITP)}$[d]
Co^{2+}	2.50	2.80 ± 0.08	2.78 ± 0.09	(6.76)	
	3.50	2.76 ± 0.05		(5.71)	
	4.50	*e*			
Ni^{2+}	2.80	2.64 ± 0.13	2.52 ± 0.19	(6.29)	
	3.50	2.39 ± 0.12		(5.34)	
	4.00	2.99 ± 0.10		(5.44)	
	4.50	3.03 ± 0.16		(4.98)	
	4.50	2.95 ± 0.15		(4.90)	
	4.90	3.09 ± 0.11		4.64	4.64 ± 0.20
	5.50	≥3.3		≥4.2	
Cu^{2+}	2.00	3.51 ± 0.15		(7.96)	
	2.30	3.25 ± 0.16		(7.40)	
	2.50	3.45 ± 0.24	3.46 ± 0.25	(7.40)	
	2.50	3.53 ± 0.16		(7.48)	
	2.50	3.53 ± 0.19		(7.48)	
	2.50	3.47[f]		(7.42)	
	3.00	3.93[g]		(7.38)	
				(7.43 ± 0.30)[h]	
	3.30	≥3.8		(≥6.95)	
	4.80	≥4.0		≥5.6	≥5.6
Zn^{2+}	2.50	2.52 ± 0.11		(6.47)	
	2.50	2.48 ± 0.17	2.46 ± 0.13	(6.40)	
	3.00	2.38 ± 0.04		(5.83)	
	4.20	3.04 ± 0.05		(5.29)	
	4.50	3.14 ± 0.05		5.09	5.09 ± 0.20
	5.00	≥3.5		≥4.9	

[a] Cf. footnote *a* of Table II. [b] Adjusted to ±0.02 log units. [c] Calculated from the intercept of the *correlation* line with the y axis (cf. Figures 2 and 3); the given error is the larger one, obtained from the correlation and the regression calculation. [d] These errors are estimated. [e] Several attempts were undertaken but despite working under N_2 the oxidation to Co(III) could not be prevented. [f] Value from Figure 5 of ref 16. [g] Value from Table I of ref 16. [h] Result of ref 16.

Table IV. Logarithms of the Stability Constants, Log K_{St}, for the Stacking Adducts between 2,2′-Bipyridyl or M(2,2′-Bipyridyl)$^{2+}$ (Eq 10) and the Purine Moiety of Adenosine, Inosine, ATP, or ITP as Determined by UV-Difference Spectra (25 °C; I = 0.1, $NaClO_4$)[a]

System	Log K_{St}
Adenosine + bpy	1.36 ± 0.06[b]
+ $Co(bpy)^{2+}$	1.33 ± 0.14
+ $Ni(bpy)^{2+}$	1.25 ± 0.18
+ $Cu(bpy)^{2+}$	1.20 ± 0.24[c]
+ $Zn(bpy)^{2+}$	1.11 ± 0.27
Inosine + bpy	1.31 ± 0.19[b]
+ $Co(bpy)^{2+}$	1.37 ± 0.13
+ $Ni(bpy)^{2+}$	1.09 ± 0.31
+ $Cu(bpy)^{2+}$	1.32 ± 0.25[d]
+ $Zn(bpy)^{2+}$	0.87 ± 0.22
ATP + bpy	0.91 ± 0.22[b]
ITP + bpy	1.44 ± 0.10[b]

[a] The results are the average of at least four independent series of measurements, which were carried out in the pH range 5.5–6.5; the pH of the solutions of one series was adjusted to within ±0.02 log unit of the desired pH value. The additional presence of inorganic triphosphate in the solutions had no significant influence on K_{St} (cf. Experimental Section and Table V). The range of error given is three times the standard deviation. [b] Results taken from ref 16 (pH range 5.5–7.3). [c] Earlier value: 1.52 ± 0.04.[16] [d] Earlier value: 1.57 ± 0.25.[16]

trations of Zn^{2+} are added to solutions containing equimolar amounts of ATP and bpy (Figure 2, upper part). In the absence of Zn^{2+}, a small shift of the resonances of H(2) and H(8) of ATP (cf. Chart I) to higher field is observed, confirming stacking between the aromatic parts of the two ligands. However, as soon as both ligands are linked together by the metal ion, the upfield shift increases tremendously, indicating considerably increased stacking as a result of the stabilizing influence of Zn^{2+}. The known[39] downfield shift of the resonance of H(8) in the binary complex $Zn(ATP)^{2-}$ was confirmed.

For the ITP-Zn^{2+}-bpy system also positive shift values for H(2) and H(8) of ITP are observed (upper part of Figure 3), as well as a downfield shift for H(8) in the binary Zn^{2+}-ITP system (lower part of Figure 3). The spectrum of 2,2′-bipyridyl is a complex incompletely resolved pattern of lines which, under the influence of Zn^{2+}, are somewhat more separated and shifted toward lower field, whereas in the presence of Zn^{2+}-ATP or Zn^{2+}-ITP it appears that some of the lines are shifted upfield and some downfield.

The results obtained at pD = 2.8, where ITP exists mainly in its protonated form $HITP^{3-}$, are shown in the lower part of Figure 2. The curvature is probably due to the lower stability of $Zn(bpy)(HITP)^-$, compared with $Zn(bpy)(ATP)^{2-}$ or $Zn(bpy)(ITP)^{2-}$, which means that the concentration of this mixed-ligand complex is not really dominating in solution, but the spectrophotometric evidence for the intramolecular stacking in $M(bpy)(HITP)^-$ is still confirmed. With increasing $[Zn^{2+}]$, Δ shift also increases, indicating increased stacking due to the interaction between the two ligands. The larger upfield shifts of H(8) than of H(2) in all three systems (Figures 2 and 3) may possibly imply that the imidazole part of the purine moiety is more involved in the formation of the stacking adduct.

Table V. Molar Absorptivities, ϵ (M^{-1} cm^{-1}), and Maxima of the Absorption Bands, λ_{max} (nm), as Obtained by UV-Difference Spectra for the Stacking Adducts between 2,2′-Bipyridyl or M(2,2′-Bipyridyl)$^{2+}$ and the Purine Moiety of Adenosine, ATP, Inosine, or ITP (25 °C; I = 0.1, $NaClO_4$)[a]

	Adenine moiety		Hypoxanthine moiety		
Second reactant	Adenosine, ϵ (λ_{max})	ATP^{4-}, ϵ (λ_{max})	Inosine, ϵ (λ_{max})	ITP^{4-}, ϵ (λ_{max})	$H(ITP)^{3-}$, ϵ (λ_{max})
Bpy[b]	720 ± 160 (293)	420 ± 260 (295)	480 ± 130 (295)	210 ± 160 (294)	
$Co(bpy)^{2+}$	795 ± 107 (312)[c]	671 ± 160 (311)[d]	249 ± 69 (314)[e]	*f*	924 ± 350 (313)*[d]
$Ni(bpy)^{2+}$	1166 ± 339 (311)[c]	594 ± 152 (311)[d]	303 ± 84 (312)[d,g]	297 ± 100 (311)*[d]	461 ± 150 (311)*[d]
$Cu(bpy)^{2+}$	1409 ± 387 (318)[h]	1188 ± 300 (318)*[h]	1281 ± 250 (318)[h]	886 ± 150 (318)*	857 ± 200 (318)*[h]
$Zn(bpy)^{2+}$	1678 ± 547 (312)	2120 ± 285 (313)	1342 ± 343 (313)	1683 ± 250 (313)*	857 ± 500 (315)*

[a] The range of error corresponds to three times the standard deviation, with the exception of the cases marked with an asterisk where an estimation is given (cf. Tables III and IV). [b] These data are from ref 16; they hold for the pH range 5.5–7.3; i.e., protonation of the phosphate chain of ATP or ITP has no significant influence. [c] The additional presence of inorganic triphosphate had no significant influence on the ϵ values. [d] Cf. footnote *a* of Table II. [e] The influence of triphosphate was not studied because the corresponding ITP system could not be measured; cf. footnote *f*. [f] Cf. footnote *e* of Table III. [g] Without triphosphate one obtains ϵ_{312} = 890 ± 342. [h] Evaluation of the measurements at the wavelengths used in ref 16 gave results which agree well with these former ones: for adenosine ϵ_{314} = 421 ± 90, for ATP^{4-} ϵ_{313} = 326 ± 150, for inosine ϵ_{314} = 280 ± 76, and for $H(ITP)^{3-}$ ϵ_{313} = 213 ± 52.

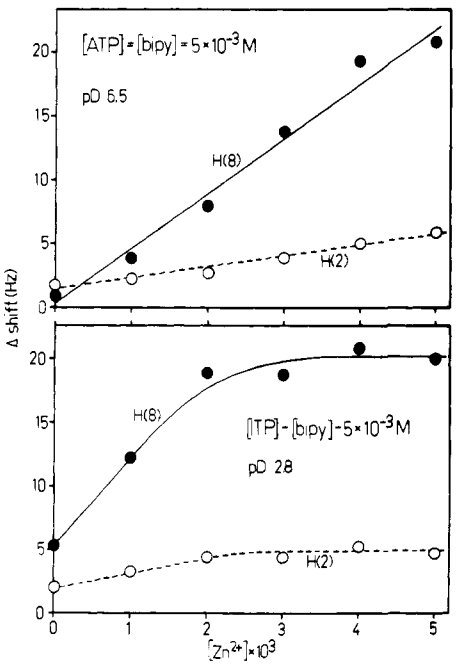


Figure 2. Change in chemical shift, Δ shift, of the ^1H NMR resonances for H(2) and H(8). Upper part: for ATP in the presence of 2,2′-bipyridyl in D_2O at pD 6.5 in dependence on $[Zn(ClO_4)_2]$; [ATP] = [bpy] = 5 × 10^{-3} M; 25 °C. The chemical shifts of free ATP at pD 6.5 are 767 Hz for H(8) and 743 Hz for H(2). Lower part: for ITP in the presence of 2,2′-bipyridyl in D_2O at pD 2.8 in dependence on $[Zn(ClO_4)_2]$; [ITP] = [bpy] = 5 × 10^{-3} M; 25 °C. The chemical shifts of free ITP at pD 2.8 are 793 Hz for H(8) and 745 Hz for H(2).

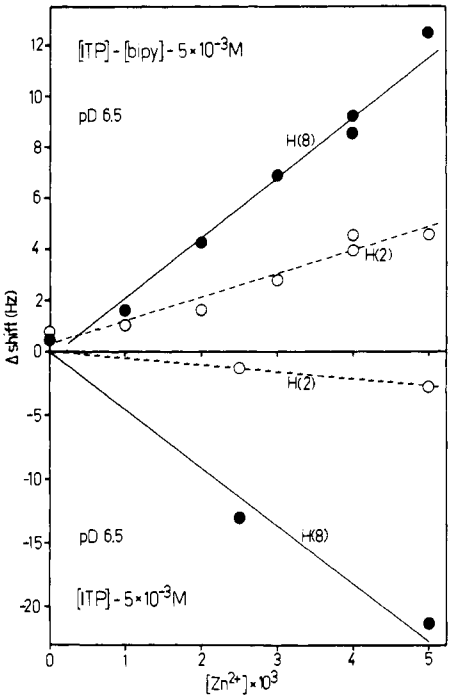


Figure 3. Change in chemical shift, Δ shift, of the ^1H NMR resonances for H(2) and H(8) of ITP in the presence (upper part) and absence (lower part) of 2,2′-bipyridyl in D_2O at pD 6.5 in dependence on $[Zn(ClO_4)_2]$; [ITP] = 5 × 10^{-3} M (=[bpy], if present); 25 °C. The chemical shifts of free ITP at pD 6.5 are 762 Hz for H(8) and 739 Hz for H(2).

Discussion

Protonated ITP Complexes. The first problem to be discussed is the structure of the protonated nucleotide complexes. A comparison of the stability constants, $K^{M(bpy)}{}_{M(bpy)(HITP)}$, of Table III with those of Table IV shows that the former are larger. Hence, the adduct between 2,2′-bipyridyl and the purine moiety of $HITP^{3-}$ must be metal ion bridged, because in the adducts with the nucleosides the metal ion has no significant influence on the stability; the adduct stability is about the same with bpy and with $M(bpy)^{2+}$. From this comparison it follows that the metal ion in $M(bpy)(HITP)^-$ must be coordinated to the phosphate chain, because these phosphate groups are the only difference between inosine and ITP. The enhanced stability of these metal ion bridged stacked adducts is also in accord with the ^1H NMR results (Figure 2, lower part).

For the $M(bpy)(HITP)^-$ complexes there remains then a further question. Where is the proton located? With eq 11

$$pK^{H}_{M(bpy)(HITP)} = pK^{H}_{H(ITP)} + \log K^{M(bpy)}{}_{M(bpy)(HITP)} - \log K^{M(bpy)}{}_{M(bpy)(ITP)} \quad (11)$$

and the results of Tables I and III one may calculate the acidity constants of the complexes $M(bpy)(HITP)^-$ (cf. Table VI). N(7) with pK_a = 2.1 (cf. ref 17) as the only basic position of the hypoxanthine moiety cannot be the site of protonation in these mixed-ligand complexes. Hence, the only remaining possibility is the phosphate chain. As $pK_a \leq 2.2$,[4a,31] of the primary phosphate protons only the γ-phosphate group is a possible site for protonation in these complexes. Indeed, this group is rather basic with pK_a = 6.45 (Table I) for deprotonation of free $HITP^{3-}$.

This agrees with an infrared study by Khalil and Brown,[12] in which binary $M(HATP)^-$ complexes with a protonated

Table VI. Comparison of the Acidity Constants, $K^{H}_{M(bpy)(HITP)}$, for the Ternary M(bpy)(HITP)$^-$ Complexes (Eq 11) with the Corresponding Data, $K^{H}_{M(HATP)}$, for Binary M(HATP)$^-$ Complexes, and of the Acidity Constants, $K^{H}_{H_2(NTP)}$, for the Diprotonated Uncomplexed $H_2(ITP)^{2-}$ and $H_2(ATP)^{2-}$ Species

		$pK^{H}_{M(HATP)}$	
M^{2+}	$pK^{H}_{M(bpy)(HITP)}$ (25 °C; I = 0.1, $NaClO_4$)	25 °C; I = 0.1, KNO_3[c]	30 °C; I = 0.1, $(CH_3)_4NBr$[d]
Co^{2+}	4.50	4.19	4.25
Ni^{2+}	4.53	4.23	4.47
Cu^{2+}	3.88	3.52	3.95
Zn^{2+}	3.87	4.35	4.20
$pK^{H}_{H_2(NTP)}$	2.1[a]	4.06[b,c]	3.83[b,d]

[a] $I \sim 0.2$; 37 °C. The site of deprotonation is at N(7).[17] [b] The site of deprotonation is at N(1).[17] [c] See ref 40. [d] See ref 41.

phosphate group were observed, and also agrees with the acidity constants of these complexes (columns 3 and 4 of Table VI),[40,41] which are in part somewhat larger than the acidity constant for the deprotonation at N(1) of uncomplexed H_2ATP^{2-}. The existence of $M_2(NTP)$ complexes,[6,18] with two metal ions bound to one nucleotide, can also only be rationalized by assuming that both metal ions interact with the phosphate chain (and possibly to some extent also with the nucleic base).[18]

The existence of M(bpy)(HITP)$^-$ is at the same time strong evidence for the existence of the corresponding binary complexes, i.e., M(HITP)$^-$, because in these the γ-phosphate group is certainly not less basic. This is also true for those nucleoside 5′-triphosphates with nucleic bases of low basicity, i.e., GTP, UTP, and TTP. These protonated species will be difficult to detect by normal potentiometric titrations; because no proton is liberated their formation is pH independent (eq 9), and thus titration methods are insensitive and the species can only be observed indirectly by coupling to H^+-dependent equilibria.

Problem of Protonation in the ATP Systems. Considering the results obtained for the ITP systems (cf. Table III) one wonders why the results of Table II for the ATP systems were obtained so easily. Here, the apparent stability constants, K_{St}, gave, by taking into account the competition with the proton (eq 8), the pH-independent stability constants. With hindsight it seems in a way fortuitous that these results were obtained so simply. The explanation is probably that under the conditions employed part of ATP and part of the resulting complexes were protonated at N(1) of the adenine moiety.

This becomes more obvious by taking into account the infrared study of Khalil and Brown[12] (cf. Table VII). These data were obtained in 0.1 M solution of the reactants, and $M(H_2ATP)$ was probably the dominating species in solution.[12] Under our conditions (e.g., 10^{-3} M)[41] this is certainly not so; here $M(H_2ATP)$ will be completely dissociated,[40] i.e., "diprotonated complexes of the type $M(H_2ATP)$ are insignificant beyond pH 3";[41] in fact Perrin and Sharma[41] state in addition, "the species $M(H_2ATP)$ was negligible, so that no estimate of K_1 could be obtained". This means for the present case, we have rather to look at the data of Table VII having the complex M(HATP)$^-$ in mind and asking for the location of the proton in this species. Also, there is an isotope effect[12] in addition to some self-association[22] which is certainly present under these experimental conditions. But still, these data evidence clearly that the stability constants[40,41] determined for M(HATP)$^-$ are global constants in the sense that they cover both species, one where the proton is at N(1) of the adenine moiety and one

Table VII. Values of pD Corresponding to Half-Removal of Protons from Partially Protonated M^{2+}–ATP 1:1 Systems (0.1 M) as Determined by Khalil and Brown[12] in an Infrared Study

M^{2+}	D^+ at N(1)	D^+ at γ-(P)
	4.65	6.5
Mg^{2+}	4.4	4.7
Ni^{2+}	4.3	4.3
Zn^{2+}	4.25	3.9
Cu^{2+}	4.0	3.0

where it is at the γ-phosphate group. The ratio of these two species changes from metal ion to metal ion, being about 1:1 in Ni(HATP)$^-$, while for Cu(HATP)$^-$ the N(1) protonated species dominates.

It appears that under our experimental conditions part of the existing M(bpy)(HATP)$^-$ is protonated at N(1) and that the resulting positive charge at the adenine moiety prevents stacking with bipyridyl due to the repulsion with the dipositively charged metal ion, coordinated to bipyridyl, and does therefore not show up in the spectrophotometric experiments. Contrary, the other part of M(bpy)(HATP)$^-$, where the γ-phosphate group is protonated, forms the stacking adduct and contributes to the absorption. Obviously, both effects diminish each other in so far that within experimental error the constants $K^{M(bpy)}_{M(bpy)(ATP)}$ turn out correctly (cf. Tables I and II). In ITP no corresponding protonation site is available and in M(bpy)(HITP)$^-$, protonated at the γ-phosphate group, stacking is possible and was in fact the source for the discovery of these species.

In this connection it is of interest to compare the acidity constants in Table VI. To a first approximation one may say that for a given metal ion $pK^{H}_{M(bpy)(HITP)}$ and $pK^{H}_{M(HATP)}$ are similar. The fact that the acidity constant for the deprotonation at N(1) is of the same order as the acidity constants $K^{H}_{M(HATP)}$ confirms our conclusion about the variability of the proton binding site in M(HATP)$^-$. Some of the constants are above and others below the value of $pK_{a/N(1)}$ ($=pK^{H}_{H_2(ATP)}$); this must mean that some of the protons are located at the γ-phosphate group, otherwise larger values would not be possible. On the other hand, the "acidification" of the proton at N(1) in the opened phosphate complexes will be small due to the great distance between M^{2+} at the phosphate chain and H^+ at N(1), while in the ring back-bound chelates "acidification" should be significant. The amounts of these ring back-bound forms differ from metal ion to metal ion,[42] and the exact ratios and structures are still somewhat uncertain.[4a,6]

As the acidity constant for the deprotonation of N(3) in the cytosine moiety of CTP is $pK_a = 4.37$,[43] one may extrapolate from the ATP systems and postulate that in M(HCTP)$^-$ and M(bpy)(HCTP)$^-$, the H^+ will also be located in part at the base and in part at the γ-phosphate group; i.e., again two isomers of the protonated complexes exist. Similarly, for UTP, TTP, and GTP having no or only a weak basic site at the nucleic base moiety, the same situation is expected as with the ITP species. The acidity constants of all these complexes (Table VI) are so that this protonation could be of biological importance, especially together with a change of the dielectric constant by the creation of a structured water region in the vicinity of a protein; this might well allow such a species to participate in a reaction.

General Considerations. For all the nucleotide systems the stability constants for the stacking adducts determined spectrophotometrically (Tables II and III) are identical, within experimental error, with the stability constants determined by potentiometric titrations for the ternary complexes M(bpy)-(NTP)$^{2-}$ (Table I). This proves that in these latter complexes

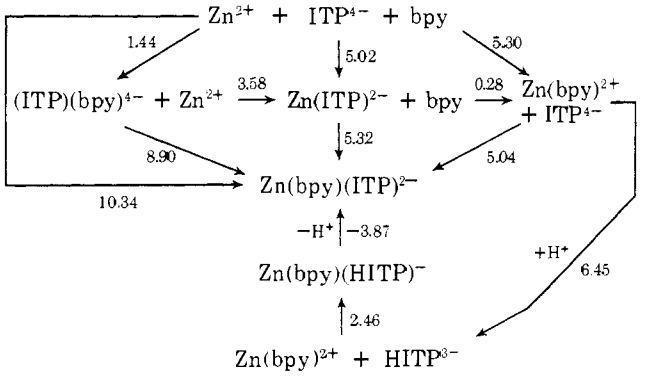
intramolecular stacking occurs between the aromatic moieties of the two coordinated ligands. However, this does not mean that the intramolecular equilibrium 12

$$\text{bpy-M}^{2+}\text{-NTP} \overset{K'}{\rightleftharpoons} \begin{matrix}\text{bpy}\\ \diagup\\ \text{M}^{2+}\\ \diagdown\\ \text{NTP}\end{matrix} \qquad (12)$$

between an opened and stacked form, is toward the right side, because the corresponding constant K' is dimensionless and independent of the concentration. One may try to reach a conclusion about the favored side of this equilibrium based on the relative magnitudes of the molar absorptivities of the stacked adducts. The basis for this evaluation is the molar absorptivities of the nucleoside adducts, $M(bpy)(NS)^{2+}$, because they must exist as stacked adducts; no other linkage between the two ligands is possible. A comparison between the corresponding ϵ values in Table V indicates that those of $M(bpy)(NTP)^{2-}$ are between 50 and 100% of those of $M(bpy)(NS)^{2+}$. Hence, we conclude that the mixed-ligand complexes, $M(bpy)(NTP)^{2-}$, exist mainly in the stacked form, i.e., $K' \gtrsim 1$. The same may be concluded from the molar absorptivities for the protonated complexes $M(bpy)(HITP)^{-}$.

The different reaction paths leading to the formation of $M(bpy)(NTP)^{2-}$ may be summarized as is shown for the case of the Zn^{2+}-bpy-ITP system in Chart II. The logarithms of

Chart II



the equilibrium constants are given for each step; these data were either taken or calculated from the results given in Tables I and III, using also the stability constant of the binary complex, $Zn(bpy)^{2+}$.[21] The data for all the other metal ion-nucleotide systems may be obtained in the same way. In this connection it is further of interest to consider the formation of the complex species in dependence on pH. Again the Zn^{2+}-bpy-ITP system is used as an example (Figure 4); the binary Zn^{2+}-ITP and the ternary Zn^{2+}-bpy-ATP systems are shown for comparison. Obviously, the mixed-ligand complexes dominate over a large pH range.

For the nucleotides we have seen that the formation of the stacked adduct is governed by the coordination tendency of the phosphate groups, i.e., the stacked adducts are stabilized by the metal ion bridge. Hence, it is the metal ion which facilitates a specific orientation of a ligand. On the other hand, a ligand may also specifically orientate a metal ion, as is demonstrated by the nucleoside adducts. The stability constants of the binary complexes $M(adenosine)^{2+}$, with all metal ions studied here, are smaller ($\log K^{M}_{M(Ado)} \leq 0.84$)[42] than those observed for the stacking adducts between $M(bpy)^{2+}$ and adenosine ($\log K_{St} \simeq 1.2$; Table IV). Even though in the latter species, M^{2+} does not directly interact with the adenine residue, it is a ternary complex with the unique feature that a coordinatively "unsaturated" metal ion is held in a distinct position. From the constants in Table IV it must be concluded that this holds also for the inosine systems. It should again be emphasized that the ternary complexes formed in this way differ considerably from

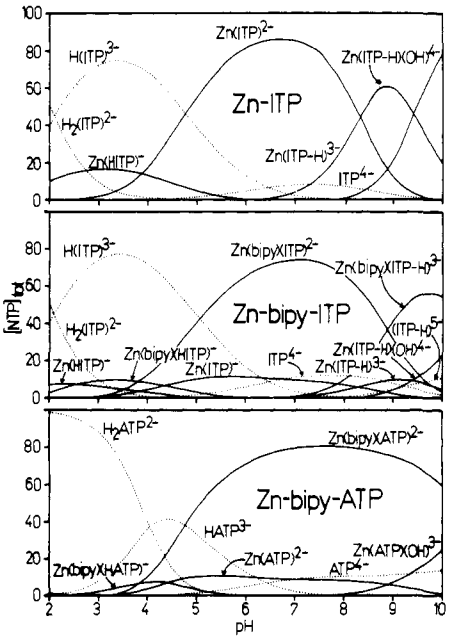


Figure 4. Effect of pH on the concentrations of the species present in an aqueous solution (25 °C; I = 0.1) of Zn^{2+}-ITP, Zn^{2+}-bpy-ITP, or Zn^{2+}-bpy-ATP, given as the percentage of the total NTP (or Zn^{2+}) present; computed for concentrations of 10^{-3} M for each reactant with constants given in Tables I and III, in ref 9 and 21, and with $pK^{H}_{H_3(NTP)} = 4.06$ for ATP[40] and 2.1 for ITP,[17] $\log K^{Zn}_{Zn(HATP)} = 2.67$,[40] $\log K^{Zn}_{Zn(HITP)} \simeq \log K^{Zn(bpy)}_{Zn(bpy)(HITP)} = 2.46$ (Table III), and $\log K^{Zn}_{Zn(HATP)} = 2.67 \simeq \log K^{Zn(bpy)}_{Zn(bpy)(HATP)}$. The dotted lines indicate the free NTP species and the solid lines the NTP complexes. Doubly protonated complex species were ignored in the calculations as the appropriate constants are unknown. However, such species could only exist in the lowest pH range.[41] Loss of the primary protons from the triphosphate chain occurs approximately with $pK_a \leq 2.2 - 1.0$.[4a,31] Upper part: Zn^{2+} and ITP ($[(ITP\text{-}H)^{5-}] \leq 1.7\%$). Middle part: Zn^{2+}, 2,2′-bipyridyl, and ITP. Lower part: Zn^{2+}, 2,2′-bipyridyl, and ATP ($[Zn(HATP)^{-}] < 2.8\%$).

those commonly described,[8,13,19,25,27,35] where a metal ion bridges two ligands which do not directly interact with each other.

Such effects of "specificity" are certainly of importance in nature. In fact, it is clear that specific interactions between aromatic moieties play an important role in biological systems.[44] Stacking complexes appear to be important for the synthesis of proteins and the determination of the amino acid sequence.[45] Also, aromatic amino acid residues are often in the active center of enzymes, e.g., a tryptophanyl group in heavy meromyosin[33] (cf. also ref 15). For microsomal (Na^+ and K^+) ATPase it was suggested that the adenine moiety of ATP is necessary for binding to the enzyme.[46] Furthermore, a study of the helical interactions poly[N^6-(Δ^2-isopentenyl)adenylic acid] showed an increased stacking and base pairing in the presence of Mg^{2+}.[47] Similarly, metal ions may mediate specific interactions between nucleic bases of polynucleotides and amino acid side chains of polypeptides; e.g., in the ternary complex $(Glu\text{-}Tyr\text{-}Glu)_n\text{-}Zn^{2+}$-poly(A), tyrosine-adenine interactions are induced.[48a] Finally, results similar to those outlined here must be expected for systems containing other purine or pyrimidine nucleotides, metal ions, and, e.g., aromatic amino acid residues. The tryptophanyl,[15,33a] the tyrosinyl,[48] or the histidinyl[49] moieties are known to form stacking adducts with nucleotides.

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(11) Abbreviations: ATP, adenosine 5′-triphosphate; bpy, 2,2′-bipyridyl; ITP, inosine 5′-triphosphate; M^{2+} = Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+}; NTP = nucleoside 5′-triphosphate = ATP or ITP; NS = nucleoside = adenosine or inosine; TP, tripolyphosphate. The phosphate groups of the triphosphate chains are labeled in the usual way as α, β, and γ, where the latter refers to the terminal phosphate group.
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Model Compounds for Protein–Nucleic Acid Interactions. 5.[1a] 5-*S*-Cysteinyluracil Monohydrate, a Photoaddition Product between an Amino Acid and Pyrimidine Base

Graheme J. B. Williams,[1b] A. J. Varghese,[1c] and Helen M. Berman*[1d]

Contribution from the Department of Chemistry, Brookhaven National Laboratory, Upton, New York 11973, Ontario Cancer Institute, Toronto, Ontario, M4X 1K9 Canada, and The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111. *Received August 25, 1976*

Abstract: The molecular and crystal structure of a pyrimidine–amino acid irradiation product, 5-*S*-cysteinyluridine, has been determined by x-ray crystallography. The cell dimensions are $a = 14.59$ (3) Å, $b = 6.82$ (1) Å, $c = 5.05$ (1) Å, $\beta = 95.1$ (1)°, space group $P2_1$ with two formula units of $C_7H_9N_3O_4S \cdot H_2O$ per cell. The pyrimidine rings are linked together in ribbons by four hydrogen bonds. Seven other hydrogen bonds interconnect the other polar functions and indicate how the unusual conformation of the cysteinyl group is stabilized. The irradiation product in its observed conformation can be fit into a RNA helix and proposed structures of intermediates in the in vivo covalent coupling of cysteine to uracil are described.

The cross-linking of nucleic acids to protein is one of the consequences of radiation in biological systems.[2] The importance of these cross-links in aging, carcinogenesis, and radiation biology has been recently reviewed.[3] Results of studies with model systems suggest that addition of cysteine residues to pyrimidine bases is one mechanism for the formation of cross-links in biological systems. One model reaction is the addition of cysteine to uracil under the influence of radiation.[4,5]