

1:1 equilibrium constant expression, but *N*-methylimidazole does not. Thus, the calorimetric evidence confirms the lack of an isobestic point in the visible study and suggests that coordination to Co(saloph) is taking place through two different donor sites.

These are potentially very significant implications, the finding that both nitrogens of imidazole are fundamentally different types (*i.e.*, different *C/E* ratio) of donor centers. In electron transfer enzymes, coordination at different basic sites could stabilize different oxidation states and facilitate electron transfer. In other atom or group transfer enzymes, a change in donor center could ease the electron demands made on the metal in the transition state. These are possibilities

to consider in systems where binding of imidazole-type molecules is involved in enzyme function.

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Supplementary Material Available. A listing of experimental data will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for \$3.00 for photocopy or \$2.00 for microfiche, referring to code number JACS-74-2743.

Ternary Complexes in Solution. XVIII.^{1,2} The Stability Enhancement of Nucleotide-Containing Charge-Transfer Adducts through the Formation of a Metal Ion Bridge

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Abstract: The formation of a charge-transfer adduct between 2,2'-bipyridyl and the purine moiety of adenosine, inosine, AMP²⁻, IMP²⁻, ATP⁴⁻, and ITP⁴⁻ is connected with the appearance of an absorption that may be best measured by recording difference spectra (λ_{\max} 294 nm). With the aid of this absorption and Benesi-Hildebrand plots, it is possible to determine the stability of these charge-transfer adducts. Their stabilities are independent of the presence of phosphate groups, and they are all of the same order ($\log K_{CT} = 0.9-1.4$). In the presence of Cu²⁺ the absorption is shifted toward larger wavelength, λ_{\max} 313 nm. Based on this observation it is concluded that the purine moiety acts as the acceptor in the mentioned charge-transfer adducts. The coordination of Cu²⁺ to 2,2'-bipyridyl does not significantly influence the stability of the adducts formed with adenosine or inosine ($\log K_{CT} \approx 1.5$). However, the stability of the charge-transfer adducts formed with the nucleotides is dramatically increased in the presence of Cu²⁺ [Cu(bipy)(AMP) and Cu(bipy)(IMP), $\log K_{CT} \approx 3.7$; Cu(bipy)(ATP)²⁻ and Cu(bipy)(ITP)²⁻, $\log K_{CT} \approx 7.2$]. This stability increase is the result of the metal ion bridge formed between 2,2'-bipyridyl and the phosphate groups of the nucleotides, thus linking the two aromatic moieties together which form the charge-transfer adduct. A comparison of the mentioned stabilities with the stability constants, $K_{Cu(bipy)Cu(bipy)(NP)}$, due to Cu(bipy)(NP)^{0,2-} (where NP^{2-,4-} = AMP²⁻, IMP²⁻, ATP⁴⁻, ITP⁴⁻) reveals that both stabilities are about the same, $\log K_{CT} \approx \log K_{Cu(bipy)Cu(bipy)(NP)}$. Indeed, the mixed-ligand 2,2'-bipyridyl-Cu²⁺-nucleotide complexes exist in a folded form that allows a charge-transfer interaction between the pyridyl and purine moieties. The structure of these complexes is shortly discussed, and their biological implications are indicated by taking into account that certain side chains of amino acids (like indole and imidazole) are capable of forming charge-transfer adducts with nucleic bases. Hence, provided that steric conditions are favorable, the stability of these adducts may be considerably increased by the formation of a metal ion bridge between the two involved aromatic moieties.

Nucleotides and their derivatives are involved in many basic processes of life. To these belong the reactions where nucleoside di- and triphosphates participate. Virtually all enzymes requiring these phosphates as substrates need in addition a divalent metal ion;³ in fact, there are a number of examples showing that the metal ion-nucleotide complex rather than the free nucleotide itself is the true substrate in enzymatic reactions.⁴ Hence, it is not surprising that

metal ion-nucleotide interactions are studied rather intensively,^{5,6} but still the detailed roles of metal ions are not widely known.

Furthermore, up to now mainly simple binary metal ion-nucleotide complexes have been studied,^{5,6} but the reactions proceed within higher order species, like enzyme-metal ion-substrate complexes; hence, we believe that many of the unanswered questions may be approached by studying simple ternary complexes.^{7,8}

(1) Part XVII: Ch. F. Naumann, B. Prijs, and H. Sigel, *Eur. J. Biochem.*, **41**, 209 (1974).

(2) Part XVI: H. Sigel, R. Caraco, and B. Prijs, *Inorg. Chem.*, **13**, 462 (1974).

(3) M. Dixon and E. Webb, *Enzymes*, **1**, 1 (1964).

(4) W. Cleland, *Annu. Rev. Biochem.*, **36**, 77 (1967).

(5) A. T. Tu and M. J. Heller, in "Metal Ions in Biological Systems," Vol. 1, H. Sigel, Ed., Marcel Dekker, New York, N. Y., 1974, p 1.

(6) C. Miller Frey and J. Stuehr, ref 5, p 51.

(7) H. Sigel, ref 5, Vol. 2, 1973, p 63.

(8) H. Sigel and D. B. McCormick, *Accounts Chem. Res.*, **3**, 201 (1970).

So far, only some few studies deal with the stability,⁹⁻¹¹ formation,^{12,13} and reactivity^{14,15} of mixed-ligand complexes containing nucleotides.

Two aspects that are solely a quality of mixed-ligand complex formation are worth noting in this connection. (i) Ternary complexes may be considerably more stable than expected on a statistical basis.^{7,8,16} This is true, for example, for complexes formed by several of the divalent metal ions of the 3d transition series, an aromatic amine (like imidazole), and a second ligand having oxygen as donor atoms, *i.e.*, carboxylates, phosphates, etc.^{7,9,16} (ii) In ternary complexes, a direct electronic interaction between the two ligands is possible, *i.e.*, the complex may be folded and a charge-transfer interaction can occur.¹ Hence, such a species may be described as a metal ion bridged charge-transfer complex. Their potential importance in biological systems is evident, but nothing is yet known of their stabilities.

Features of the last mentioned kind have been detected by pmr to exist in the ternary complexes, 2,2'-bipyridyl-Cu²⁺-adenosine and -inosine 5'-triphosphate.¹ As the charge-transfer interaction within these mixed-ligand complexes manifests itself in a new absorption band occurring in the uv region, one may use this band to determine the stability constants of these charge-transfer complexes. To be able to evaluate the influence of a metal ion on the stability, first the simple systems consisting only of 2,2'-bipyridyl (bipy) and adenosine, adenosine 5'-monophosphate (AMP), and adenosine 5'-triphosphate (ATP) or inosine, inosine 5'-monophosphate (IMP), and inosine 5'-triphosphate (ITP) were studied. In all these cases the existence of a charge-transfer complex could be demonstrated. Next, experiments were carried out in the additional presence of Cu²⁺; again, charge-transfer adducts are formed. However, with the nucleotides, where due to the phosphate groups a metal ion bridge between the two aromatic partners of the charge-transfer interaction (*i.e.*, 2,2'-bipyridyl and the purine moiety) is formed, a considerable increase in stability of the adduct is observed.

Experimental Section

Materials. All materials were reagent grade and used without further purification. Copper(II) (*purum*) and sodium (*purissimum*) perchlorate, inosine, adenosine, and the disodium salts of adenosine and inosine 5'-monophosphate (all *purissimum*) were obtained from Fluka AG, Buchs, Switzerland. The disodium salts of adenosine (*pro analysi*) and inosine 5'-triphosphate (*purissimum*) were purchased from Serva Feinbiochemica GMBH, Heidelberg, Germany. According to the latter company, the ATP contained about 1% ADP + AMP, while the ITP (>98%) had some IDP (1.1-1.3%) and IMP (0.2-0.3%). Additionally, we had determined the content of inorganic phosphate (*cf.* ref 15) for both nucleoside 5'-triphosphates and found that it was less than 3%. The exact metal ion concentration of the copper(II) perchlorate stock solution was determined with ethylenediamine-*N,N,N',N'*-tetraacetate.

(9) H. Sigel, K. Becker, and D. B. McCormick, *Biochim. Biophys. Acta*, **148**, 655 (1967).

(10) H. Sigel, *Eur. J. Biochem.*, **3**, 530 (1968).

(11) K. S. Rajan, J. M. Davis, R. W. Colburn, and F. H. Jarke, *J. Neurochem.*, **19**, 1099 (1972).

(12) D. N. Hague, S. R. Martin, and M. S. Zetter, *J. Chem. Soc., Faraday Trans. 1*, **68**, 37 (1972).

(13) M. S. Zetter, H. W. Dodgen, and J. P. Hunt, *Biochemistry*, **12**, 778 (1973).

(14) D. H. Buisson and H. Sigel, *Angew. Chem.*, **84**, 1103 (1973); *Angew. Chem., Int. Ed. Engl.*, **11**, 1025 (1972).

(15) D. H. Buisson and H. Sigel, *Biochim. Biophys. Acta*, **343**, 45 (1974).

(16) R. Griesser and H. Sigel, *Inorg. Chem.*, **10**, 2229 (1971).

Apparatus. The absorption spectra were recorded with a Beckman spectrophotometer DB, connected with a W + W Electronic Hi-speed Recorder 202. Some experiments were repeated on a Cary 14 spectrophotometer; the same results were obtained. The pH measurements were performed with a Metrohm potentiometer E 353 B with U micro glass electrodes. The potentiometric titrations were carried out with a Metrohm potentiograph E 336 and Metrohm UX glass electrode.

Stability Constants of the Charge-Transfer Adducts. Charge-transfer complexes formed between two aromatic systems very often exhibit a detectable absorption.¹⁷ This is also the case here; however, to obtain a clear-cut situation, uv-difference spectra had to be recorded (25°C). They were taken by placing in the reference beam one cuvette with [Cu(ClO₄)₂] = [bipy] and a second one with the nucleoside or nucleotide; the sample beam contained one cuvette with the mixed system and one with water. As the absorption of the reference solutions was high, the spectrophotometer had to be used on "manual" with the slit adjusted to 2.0 mm. The concentrations of solutions may be read from the figures and their legends. The desired pH of the solutions was adjusted by dotting with a glass stick and 2 N NaOH or 2 N HClO₄. Under these conditions the change in volume of the solutions was negligibly small. Within one series of measurements the change in pH was less than ±0.05 log unit.

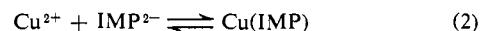
The stability constants of the charge-transfer complexes were determined according to the procedure given by Benesi and Hildebrand.¹⁸ In general, the concentrations of bipy, or Cu²⁺-bipy, were kept constant, and the concentrations of nucleoside or nucleotide (N) were varied. In this case, where [bipy] or [Cu(bipy)²⁺] < [N] holds

$$\frac{1}{[N]} = \Delta E_{\max} K^* \frac{1}{\Delta E} - K^* \quad (1)$$

Plots of 1/[N] vs. 1/ΔE resulted in straight lines that were calculated by a least-squares procedure. From the intercept with the y axis one obtains the stability constant, K* (M⁻¹), of the adduct (y₀ = -K*) and from the intercept with the x axis 1/ΔE_{max} (x₀ = 1/ΔE_{max}), from which the extinction coefficient, ε, may be calculated (ε = ΔE_{max}/d[bipy]_{tot}) (M⁻¹ cm⁻¹) (*cf.* the examples given in the figures).

Equilibrium Constants of the 2,2'-Bipyridyl-Cu²⁺-IMP System. The acidity constant, K^H_{H(IMP)}, due to the deprotonation of the phosphate group of IMP was determined from titrations of aqueous solutions containing 10% dioxane,¹⁹ 7.5 × 10⁻⁴ M HClO₄ and NaClO₄ (I = 0.1) in the presence and absence of Na₂IMP (5 × 10⁻⁴ M) under N₂ with 0.05 N NaOH (25°C). The difference of such a pair of titrations was evaluated and the acidity constant calculated from the respective degree of neutralization for 18 points, each, of four titration curves is pK^H_{H(IMP)} = 6.44 ± 0.01.²⁰

The stability constant, K^{Cu}_{Cu(IMP)}, of the binary complex, Cu(IMP) (*cf.* eq 2), was determined in solutions of the mentioned concen-



trations, but a part of the NaClO₄ was replaced by [Cu(ClO₄)₂] = 5 × 10⁻³ M, *i.e.*, the ratio, ligand to Cu²⁺, was 1:10 and, hence, the concentration of a possible species, Cu(IMP)₂²⁺, was negligible. The calculation of the stability constant was done by evaluating the part of the curves where no hydrolysis occurred and by taking into account the species, H⁺, H(IMP)⁻, IMP²⁻, Cu²⁺, and Cu(IMP)²⁺ (log K^{Cu}_{Cu(IMP)} = 3.50 ± 0.05).²⁰

The conditions for measuring the stability constants, β^{Cu}_{Cu(bipy)(IMP)} according to eq 3, of the ternary complex, Cu(bipy)(IMP), were the



same as for the binary one, but the solutions contained 2,2'-bipyridyl, Cu(ClO₄)₂, and IMP in the ratio 5:5:1 or 10:10:1 ([Na₂IMP] = 5 × 10⁻⁴ M). The evaluation²¹ of these titration curves was done by taking into account the species, H⁺, H(IMP)⁻,

(17) G. Briegleb, "Elektronen-Donator-Acceptor-Komplexe," Springer Verlag, Berlin, 1961.

(18) H. A. Benesi and J. H. Hildebrand, *J. Amer. Chem. Soc.*, **71**, 2703 (1949).

(19) The potentiograph was calibrated with commercial aqueous buffers (pH 4 and 7, from Metrohm AG) and the direct readings for pH were used in the calculations. No "corrections" were applied for the change in solvent (10% aqueous dioxane; I = 0.1, NaClO₄; 25°C).

(20) The range of error is three times the standard deviation.

(21) R. Griesser and H. Sigel, *Inorg. Chem.*, **9**, 1238 (1970).

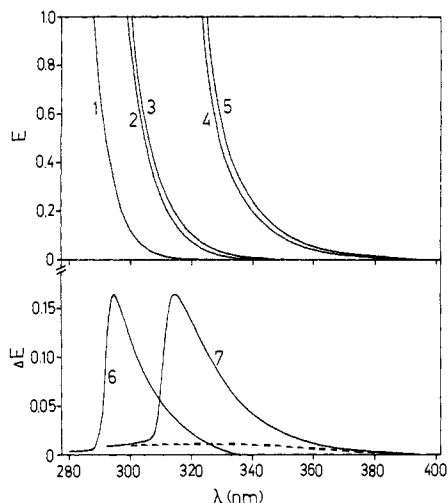


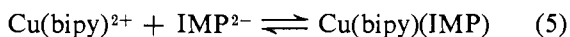
Figure 1. *Upper part.* Absorption spectra of adenosine (1), 2,2'-bipyridyl (2), adenosine and 2,2'-bipyridyl (3), $\text{Cu}(2,2'\text{-bipyridyl})^{2+}$ (4),²⁶ and adenosine and $\text{Cu}(2,2'\text{-bipyridyl})^{2+}$ (5) at $\text{pH } 6.50 \pm 0.05$ and 25° taken in 1-cm quartz cuvettes; $[\text{adenosine}] = 2 \times 10^{-2} \text{ M}$; $[2,2'\text{-bipyridyl}] = [\text{Cu}^{2+}] = 8 \times 10^{-4} \text{ M}$. *Lower part.* Charge-transfer absorpton of the adenosine-2,2'-bipyridyl (6) and of the adenosine- $\text{Cu}(2,2'\text{-bipyridyl})^{2+}$ (7; cf. \odot in Figure 3) adducts under the above conditions measured as difference absorption spectra. This means, the reference beam contained one cuvette with 2,2'-bipyridyl (or $[\text{Cu}^{2+}]:[\text{bipy}] = 1:1$)²⁶ and a second one with adenosine; the sample beam contained one cuvette with the mixed system and one with water (cf. Experimental Section). The broken-line portion is an "artificially" drawn base line; use was made of this in cases where the left-hand part of a difference absorption spectrum (cf., e.g., 7) did not exactly go back to zero absorption. (It must be mentioned that this behavior is not a quality of a given system but results from experimental "errors" (cuvettes, concentrations, etc.). This kind of correction with the aid of an "artificial" base line was always small, however.)

IMP^{2-} , $\text{H}_2(\text{bipy})^{2+}$, $\text{H}(\text{bipy})^+$, bipy , $\text{Cu}(\text{IMP})$, $\text{Cu}(\text{bipy})^{2+}$, $\text{Cu}(\text{bipy})_2^{2+}$, Cu^{2+} , and $\text{Cu}(\text{bipy})(\text{IMP})$ ($\log \beta_{\text{Cu}(\text{bipy})(\text{IMP})}^{\text{Cu}} = 11.72 \pm 0.04$).²⁰

Based on $\log \beta_{\text{Cu}(\text{bipy})(\text{IMP})}^{\text{Cu}}$ one may calculate with the aid of eq 4

$$\log K_{\text{Cu}(\text{bipy})\text{Cu}(\text{bipy})(\text{IMP})}^{\text{Cu}} = \log \beta_{\text{Cu}(\text{bipy})(\text{IMP})}^{\text{Cu}} - \log K_{\text{Cu}(\text{bipy})}^{\text{Cu}} \quad (4)$$

the stability constant, $K_{\text{Cu}(\text{bipy})\text{Cu}(\text{bipy})(\text{IMP})}^{\text{Cu}}$, which corresponds to eq 5 ($\log K_{\text{Cu}(\text{bipy})\text{Cu}(\text{bipy})(\text{IMP})}^{\text{Cu}} = 3.72$).²²



The evaluation of the titration curves of the mixed-ligand system may also be done by making the reasonable assumption²¹ that the complex, $\text{Cu}(\text{bipy})^{2+}$, is completely formed, i.e., in the calculation now only the following species are considered: H^+ , $\text{H}(\text{IMP})^-$, IMP^{2-} , $\text{Cu}(\text{bipy})^{2+}$, and $\text{Cu}(\text{bipy})(\text{IMP})$. In this way one obtains directly the stability constant of eq 4 ($\log K_{\text{Cu}(\text{bipy})\text{Cu}(\text{bipy})(\text{IMP})}^{\text{Cu}} = 3.69 \pm 0.04$).²⁰ The agreement with the above value is excellent.

The described preceding experiments had to be carried out in aqueous solution containing 10% dioxane to obtain the needed concentrations of 2,2'-bipyridyl. To learn how significant the influence of this solvent on the stability of the complexes is, the acidity constant and the stability constant of the binary complex (eq 2) were also determined in water ($I = 0.1$, NaClO_4 ; 25°) ($\text{p}K_{\text{H}(\text{IMP})}^{\text{H}} = 6.14 \pm 0.01$; $\log K_{\text{Cu}(\text{IMP})}^{\text{Cu}} = 3.26 \pm 0.04$).²⁰

Obviously, the change in solvent from 10% aqueous dioxane to water has a relatively small effect; the stability constant, $\log K_{\text{Cu}(\text{IMP})}^{\text{Cu}}$, decreases by about 0.2 log units, which is in accord with the earlier observation of the Cu^{2+} -AMP system.⁹ Hence, it may be surmised that the change in the case of the ternary complex, i.e., of $\log K_{\text{Cu}(\text{bipy})\text{Cu}(\text{bipy})(\text{IMP})}^{\text{Cu}}$, is of about the same size.

(22) $\log K_{\text{Cu}(\text{bipy})}^{\text{Cu}} = 8.00$; G. Anderegg, *Helv. Chim. Acta*, **46**, 2397 (1963).

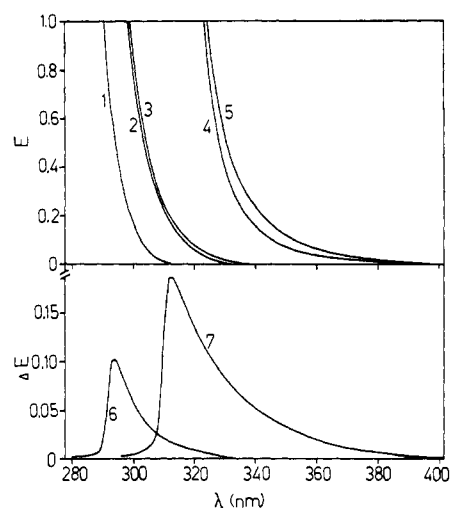


Figure 2. *Upper part.* Absorption spectra of inosine (1), 2,2'-bipyridyl (2), inosine and 2,2'-bipyridyl (3), $\text{Cu}(2,2'\text{-bipyridyl})^{2+}$ (4),²⁶ and inosine and $\text{Cu}(2,2'\text{-bipyridyl})^{2+}$ (5) at $\text{pH } 6.50 \pm 0.05$ and 25° taken in 1-cm quartz cuvettes; $[\text{inosine}] = 2 \times 10^{-2} \text{ M}$; $[2,2'\text{-bipyridyl}] = 8 \times 10^{-4} \text{ M}$ in (2 and 3); $[2,2'\text{-bipyridyl}] = [\text{Cu}^{2+}] = 6.4 \times 10^{-4} \text{ M}$ (in 4 and 5). *Lower part.* Charge-transfer absorption of the inosine-2,2'-bipyridyl (6; combination of 1, 2, and 3; cf. \odot in Figure 3) and the inosine- $\text{Cu}(2,2'\text{-bipyridyl})^{2+}$ (7; combination of 1, 4, and 5; cf. \odot in Figure 3) adducts under the above conditions measured as difference absorption spectra (cf. legend to Figure 1 and Experimental Section).

Results

The formation of charge-transfer adducts between purines or pyrimidines and other aromatic systems is well known.²³⁻²⁵ Usually, the formation of such charge-transfer complexes exhibits a detectable absorption,¹⁷ and, indeed, by recording difference spectra (cf. Experimental Section), a new absorption band, which is due to the interaction between the purine moiety and 2,2'-bipyridyl, could be observed in the near-uv region. Examples of such spectra are shown in Figures 1 and 2 for the systems containing 2,2'-bipyridyl or $\text{Cu}(2,2'\text{-bipyridyl})^{2+}$ (cf. ref 26) and adenosine or inosine, respectively.

Based on the described difference spectra, it is possible to determine the stability constants, K_{CT} , of these charge-transfer adducts according to the method given by Benesi and Hildebrand.¹⁸ In general, the concentrations of 2,2'-bipyridyl or $\text{Cu}(\text{bipy})^{2+}$ were kept constant while increasing concentrations of the nucleosides or nucleotides (N) were used. Plots of $1/[\text{N}]$ vs. $1/\Delta E$ resulted in straight lines, thus indicating that eq 1 is valid and that 1:1 adducts are formed.²⁷⁻²⁹ Ex-

(23) A. Fulton and L. E. Lyons, *Aust. J. Chem.*, **21**, 419 (1968).

(24) T. Montenay-Garestier and C. Hélène, *Biochemistry*, **10**, 300 (1971); C. Hélène, T. Montenay-Garestier, and J. L. Dimicoli, *Biochim. Biophys. Acta*, **254**, 349 (1971); J. L. Dimicoli and C. Hélène, *J. Amer. Chem. Soc.*, **95**, 1036 (1973).

(25) K. G. Wagner and R. Lawaczek, *J. Magn. Resonance*, **8**, 164 (1972).

(26) Under the experimental conditions in a 1:1 mixture of Cu^{2+} and 2,2'-bipyridyl, the 1:1 complex is practically formed completely.²¹

(27) With the exceptions of $\text{Cu}(\text{bipy})^{2+}$ and ATP or ITP the adduct formation is independent of the reactant which is in excess. In one case when ATP or ITP is in excess, compared to $\text{Cu}(\text{bipy})^{2+}$, 1:1 adducts are formed, while with $\text{Cu}(\text{bipy})^{2+}$ in excess the absorption of a new species appears (at about 268 nm). This latter was not studied in detail but a Job's series indicates $[\text{Cu}(\text{bipy})]_2\text{ATP}$.

(28) The weak self-association of the nucleosides ($K_{\text{adenosine}} = 4.5$; $K_{\text{inosine}} = 1.8$)²⁹ was not taken into account in the calculation of K_{CT} .

(29) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **89**, 3612 (1967).

amples of such plots are shown in Figure 3 for systems containing 2,2'-bipyridyl and one of the hypoxanthine derivatives, inosine, IMP²⁻, or ITP⁴⁻; in addition, the results obtained for the systems containing Cu(bipy)²⁺ and adenosine or inosine are also given.

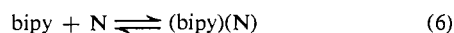
Already from a comparison of the data shown in Figure 3 and more clearly from the results listed in Table I, it follows that the stability for all the mentioned

Table I. Logarithms of the Apparent Stability Constants, $\log K_{CT}$, for the Charge-Transfer Adducts of 2,2'-Bipyridyl or Cu(2,2'-Bipyridyl)²⁺ and the Purine Moiety of the Nucleosides or Nucleotides Determined in Aqueous Solution (25°)^{a,20,28}

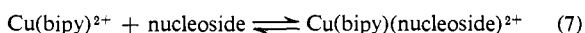
System ^b	$\log K^*$ (pH) ^c	$\log K_{CT}$	I^d
Adenosine + bipy	1.36 ± 0.06	Nat. (~0)	
Inosine + bipy	1.31 ± 0.19	Nat. (~0) and 0.7	
AMP ²⁻ + bipy	1.41 ± 0.09	Nat. (0.001-0.2)	
IMP ²⁻ + bipy	1.13 ± 0.08	Nat. (0.001-0.2)	
ATP ⁴⁻ + bipy	0.91 ± 0.22	Nat. (0.04-0.8)	
ITP ⁴⁻ + bipy	1.44 ± 0.10	Nat. (0.03-0.7)	
Adenosine + Cu-(bipy) ²⁺	1.52 ± 0.04	Nat. (~0.003)	
Inosine + Cu-(bipy) ²⁺	1.57 ± 0.25	Nat. (0.003-0.007)	
AMP ²⁻ + Cu-(bipy) ²⁺	3.65 (6.50)	3.78 ± 0.27	Nat. (0.002-0.02)
IMP ²⁻ + Cu-(bipy) ²⁺	3.72 (6.50)	3.61 ± 0.46	Nat. (0.001-0.02) and 0.5
ATP ⁴⁻ + Cu-(bipy) ²⁺	3.46 (3.00)	6.96 ± 0.25	0.5
ITP ⁴⁻ + Cu-(bipy) ²⁺	3.93 (3.00)	7.43 ± 0.30 ^e	0.5

^a The results are the average of usually four independent series of measurements. ^b The stability constants of those systems that contain either no Cu²⁺ or no phosphate group are independent of pH; the experiments were carried out at a certain pH (±0.05 log units; cf. Experimental Section) in the range 5.5-7.3. The stability constants of the bipy-Cu²⁺-nucleotide systems were dependent on pH; the given constants are corrected for the competition between Cu²⁺ and the proton for the coordination at the phosphate groups; cf. text and c. ^c Apparent stability constant, K^* , at a certain pH (given in parentheses). Only the value of one or the average of two experiments is given, while $\log K_{CT}$ is the average of more experiments done at different pH values (cf. footnote a). ^d Natural ionic strength = Nat. In several cases it was proved that a change in ionic strength had no remarkable influence on the stability of the adducts, e.g., the systems inosine + bipy or ATP + bipy, were also measured at $I = 0.7$. In all these cases the desired ionic strength was maintained constant by the addition of certain amounts of NaClO₄. ^e Calculated with $pK_{H(ITP)}^0 = 6.42$ (cf. ref 31).

charge-transfer adducts is about of the same order. Furthermore, it is important to note that the stability constant, K_{CT} , as defined by eq 6 and 7, is independent



N = nucleoside or nucleotide



of pH within the studied range of 5.5-7.3.

However, the described independence of K_{CT} on pH holds only for those systems that do not contain both a phosphate group and Cu²⁺ at the same time. This means, in the case of the 2,2'-bipyridyl-Cu²⁺-nucleotide systems, only apparent stability constants, K^* , could be determined which are valid only at the pH of the corresponding experiment. In Figure 4, examples are

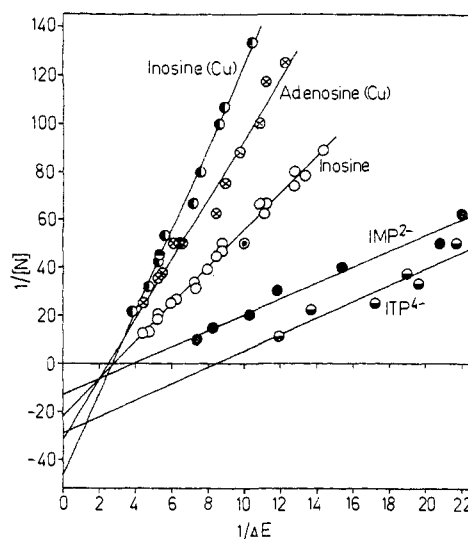


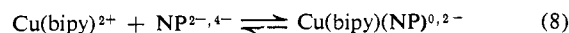
Figure 3. Graphical determination (cf. Experimental Section) of the apparent stability constants, K_{CT} , at pH 6.50 ± 0.05 and 25° for the charge-transfer adduct formation of the purine moiety of inosine (○, ⊙), IMP²⁻ (●, ⊙), and ITP⁴⁻ (⊖, ⊙) with 2,2'-bipyridyl (●, ⊙, ⊖, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙) or of the purine part of inosine (●, ⊙) and adenosine (⊗, ⊙) with 2,2'-bipyridyl and Cu²⁺ (1:1) (●, ⊙, ⊖, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙, ⊗, ⊙) (M^{-1}) (cf. Table I) and those with the x axis, i.e., $1/\Delta E_{max}$, are 2.83 (○, ⊙), 3.94 (●, ⊙), 8.48 (⊖, ⊙), 2.67 (●, ⊙), and 2.50 (⊗, ⊙) (cf. Experimental Section and Table III). The points, ⊗ or ⊙ and ⊙, are the evaluation for the corresponding parts of Figures 1 and 2, respectively.

shown for nucleotide-containing systems at pH 6.5. It is immediately obvious that these charge-transfer adducts are considerably more stable than those of Figure 3. In fact, for the systems containing Cu(bipy)²⁺ and ATP or ITP, the adduct is so stable under these conditions that only a lower limit for its stability can be given ($\log K^* > 4$ at pH 6.5).

To be able to determine an apparent stability constant, K^* , for the 2,2'-bipyridyl-Cu²⁺-nucleoside triphosphate systems, it is necessary to perform the experiments at pH ≤ 3 (cf. Figure 5). These pH-dependent apparent stability constants, K^* (cf. Table I), are becoming independent of pH if one takes into account the protonation of the terminal phosphate group, i.e.

$$\log K_{CT} = \log K^* + \log (1 + [\text{H}^+]/K_{H(NP)})$$

From this observation it follows unequivocally that the stability of the charge-transfer adducts between Cu(bipy)²⁺ and the purine moieties of the nucleoside phosphates (NP^{2-,4-}), AMP, IMP, ATP, and ITP, is dependent on the complex formation between Cu(bipy)²⁺ and the phosphate chains. Hence, K_{CT} holds for eq 8.



In excellent accord with this conclusion is the fact that the potentiometrically determined apparent stability constants, $K_{Cu(bipy)Cu(bipy)(NP)}$, due to eq 8 are within experimental error identical with the values of K_{CT} . The corresponding constants are listed in Table

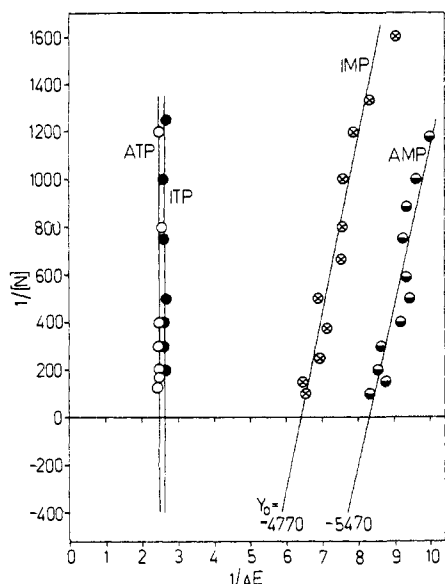


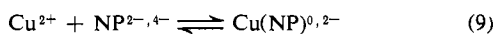
Figure 4. Graphical determination of the apparent stability constants, K^* , at $\text{pH } 6.50 \pm 0.05$ and 25° for the charge-transfer adduct formation of the purine moiety of AMP^{2-} (\ominus), IMP^{2-} (\otimes), ATP^{4-} (\circ), and ITP^{4-} (\bullet) with 2,2'-bipyridyl and Cu^{2+} (1:1) (\ominus , $10^{-4} M$; \otimes , $1.6 \times 10^{-4} M$; \circ , \bullet , $8 \times 10^{-4} M$)²⁶ by plotting $1/[N]$ (N = nucleotide) vs. $1/\Delta E$. The straight lines were drawn according to the least-squares method. The intercepts with the y axis, i.e., $-K^*$, are -5470 (\ominus), -4770 (\otimes), and approximately $-\infty$ (\circ , \bullet) (M^{-1}) (cf. Table I), and those with the x axis, i.e., $1/\Delta E_{\text{max}}$, are 8.32 (\ominus), 6.42 (\otimes), 2.48 (\circ), and 2.64 (\bullet) (cf. Experimental Section and Table III).

Table II. Comparison of the Potentiometrically Determined Apparent Stability Constants³⁰ of the Binary ($\log K^{\text{CuCu(NP)}}$) and Ternary ($\log K^{\text{Cu(bipy)Cu(bipy)(NP)}}$) Nucleotide (NP) Complexes ($I = 0.1$; 25°) with the Apparent Stability Constants ($\log K_{\text{CT}}$) of the Corresponding Charge-Transfer Adducts (25°)

Nucleotide (NP)	log		
	$\log K^{\text{CuCu(NP)}}$	$K^{\text{Cu(bipy)Cu(bipy)(NP)}}$	$\log K_{\text{CT}}$
AMP^{2-}	$3.22 \pm 0.05^{a,b}$	$3.72 \pm 0.05^{a,c}$	3.78 ± 0.27
IMP^{2-}	3.50 ± 0.05^b	$3.72 \pm 0.04^{b,c}$	3.61 ± 0.46
ATP^{4-}	6.38 ± 0.09^a	6.91 ± 0.15^a	6.96 ± 0.25
ITP^{4-}			7.43 ± 0.30

^a Data from ref 9. ^b These values were determined for experimental reasons in aqueous solution containing 10% dioxane (cf. Experimental Section).⁹ ^c In water as solvent, these constants would be smaller by about 0.2 log units as may be concluded from a comparison of the values for $\log K^{\text{CuCuL}}$ in water (3.04 ,⁹ AMP^{2-} ; 3.26 IMP^{2-}) and in 10% aqueous dioxane (3.22 ,⁹ AMP^{2-} ; 3.50 , IMP^{2-}).

II together with the apparent stability constants, $K^{\text{CuCu(NP)}}$, of the binary systems due to eq 9.³⁰⁻³³



(30) The stability constants for eq 8 and 9 with $\text{NP} = \text{ITP}^{4-}$ can be assumed to be approximately the same as for $\text{NP} = \text{ATP}^{4-}$ (cf. Table II). This is based on the experience that the kind of nucleic base has no influence on the stability of the corresponding complexes (cf. also ref 31 and 32).^{8,33}

(31) Acidity constants: $\text{p}K_{\text{H}}^{\text{H(AMP)}} = 6.14$;⁹ $\text{p}K_{\text{H}}^{\text{H(IMP)}} = 6.14$ (cf. Experimental Section); $\text{p}K_{\text{H}}^{\text{H(ATP)}} = 6.42$;⁹ $\text{p}K_{\text{H}}^{\text{H(ITP)}} \approx \text{p}K_{\text{H}}^{\text{H(ATP)}} = 6.42$. This latter assumption is justified, as the kind of nucleic base has no influence on the basicity of the phosphate group;³² this fact can also be observed from the preceding values for AMP and IMP.

(32) R. Phillips, P. Eisenberg, P. George, and R. J. Rutman, *J. Biol. Chem.*, **240**, 4393 (1965); R. M. Izatt, J. J. Christensen, and J. H. Rytting, *Chem. Rev.*, **71**, 439 (1971).

(33) E. Walaas, *Acta Chem. Scand.*, **12**, 528 (1958); P. W. Schneider, H. Brintzinger, and H. Erlenmeyer, *Helv. Chim. Acta*, **47**, 992 (1964).

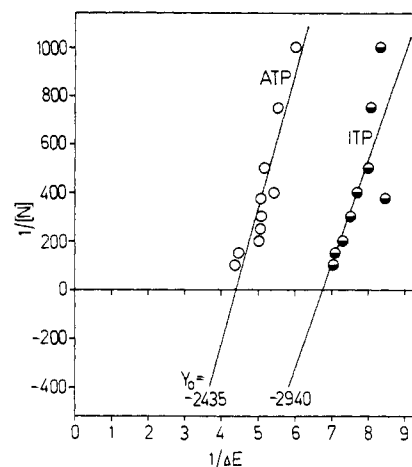


Figure 5. Graphical determination of the apparent stability constants, K^* , at 25° for the charge-transfer adduct formation of the purine moiety of ATP (\circ) at $\text{pH } 3.00 \pm 0.05$ and ITP (\circ) at $\text{pH } 2.50 \pm 0.05$ with 2,2'-bipyridyl and Cu^{2+} (1:1) (\circ , \bullet , $6 \times 10^{-4} M$)²⁸ by plotting $1/[N]$ (N = nucleotide) vs. $1/\Delta E$. The straight lines were drawn according to the least-squares method. The intercepts with the y axis, i.e., $-K^*$, are -2435 (\circ) and -2940 (\bullet) (M^{-1}) (cf. Table I), and those with the x axis, i.e., $1/\Delta E_{\text{max}}$, are 4.41 (\circ) and 6.77 (\bullet) (cf. Experimental Section and Table III).

From the Benesi-Hildebrand plots (cf. Experimental Section) as shown in Figures 3 through 5, it is also possible to calculate the extinction coefficients, ϵ ($M^{-1} \text{ cm}^{-1}$), which are due to the absorption of the charge-transfer adducts. These data are assembled in Table III together with the peaks, λ_{max} , of the uv-dif-

Table III. Extinction Coefficients, ϵ ($M^{-1} \text{ cm}^{-1}$), and Maxima of the Absorption Bands, λ_{max} (nm), as Obtained by Uv-Difference Spectra for the Charge-Transfer Adducts of 2,2'-Bipyridyl or $\text{Cu}(2,2'\text{-bipyridyl})^{2+}$ and the Purine Moiety of the Nucleosides or Nucleotides (25°)^a

System	ϵ	λ_{max}
Adenosine + bipy	720 ± 160	293
Inosine + bipy	480 ± 130	295
AMP^{2-} + bipy	630 ± 100	293
IMP^{2-} + bipy	590 ± 150	294
ATP^{4-} + bipy	420 ± 260	295
ITP^{4-} + bipy	210 ± 160	294
Adenosine + $\text{Cu}(\text{bipy})^{2+}$	430 ± 220	314
Inosine + $\text{Cu}(\text{bipy})^{2+}$	370 ± 190	314
AMP^{2-} + $\text{Cu}(\text{bipy})^{2+}$	1350 ± 450	313
IMP^{2-} + $\text{Cu}(\text{bipy})^{2+}$	1010 ± 180	310
ATP^{4-} + $\text{Cu}(\text{bipy})^{2+}$	520 ± 340	313
ITP^{4-} + $\text{Cu}(\text{bipy})^{2+}$	290 ± 160	313

^a Cf. footnotes 20 and a of Table I. The ionic strength, I , is the same as given in Table I.

ference spectra. The extinction coefficients are in the order usually observed for charge-transfer complexes involving nucleic bases.³⁴⁻³⁷

The direction of the charge transfer in the adducts between the purine moieties and 2,2'-bipyridyl or $\text{Cu}(\text{bipy})^{2+}$ may be deduced from the different values

(34) S. Shifrin, in "Molecular Associations in Biology," B. Pullman, Ed., Academic Press, New York, N. Y., 1968, p 323.

(35) H. Yoshino, F. Morita, and K. Yagi, *J. Biochem. (Tokyo)*, **72**, 1227 (1972).

(36) G. Cilento and S. Schreier, *Arch. Biochem. Biophys.*, **107**, 102 (1964).

(37) B. M. Anderson and M. L. Reynolds, *Arch. Biochem. Biophys.*, **114**, 299 (1966).

of λ_{\max} for these systems. In the adducts formed with $\text{Cu}(\text{bipy})^{2+}$ the charge transfer occurs at lower energy (313 nm) than in those formed with 2,2'-bipyridyl alone (294 nm). As in $\text{Cu}(\text{bipy})^{2+}$ the electron density is increased on the amine, due to $d_{\pi}-p_{\pi}$ back-donation,³⁸ compared with the electron density in the uncomplexed 2,2'-bipyridyl, one has to conclude that bipyridyl acts as electron donor and, hence, the purine moiety as electron acceptor. This conclusion is in accord with other results,^{24,39} especially with the observations made at charge-transfer adducts between 8-hydroxyquinoline and electron deficient aromatic compounds; the coordination of Cu^{2+} , Pd^{2+} , or Ni^{2+} to 8-hydroxyquinoline lowered the energy of the charge-transfer transition.⁴⁰

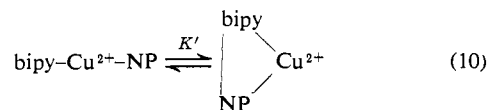
Discussion

The stability of the charge-transfer adducts between adenosine or inosine and 2,2'-bipyridyl (*cf.* Table I) is on the larger side of the order observed for the corresponding adducts between purine (or pyrimidine) compounds and indole derivatives ($K = 1-15 M^{-1}$).^{24,25} The same order of stability holds for the charge-transfer complexes formed by purine derivatives with other aromatic substances.^{37,41}

As one would expect, the stability of the nucleotide-2,2'-bipyridyl charge-transfer adducts is within experimental error identical with those containing the nucleosides (Table I). This means the formation of the charge-transfer adducts is independent of the presence and size of a phosphate chain. More surprising is the fact that the stability of the nucleoside-2,2'-bipyridyl adducts is also not significantly altered by the coordination of Cu^{2+} to 2,2'-bipyridyl. However, it must be pointed out that the ternary complexes formed in this way differ considerably from those commonly described;^{7-9,16,38} in the present cases the two ligands are not metal ion bridged but interact directly with each other.

A completely new situation occurs if the two aromatic reactants that form the charge-transfer adduct own suitable donors that may become metal ion bridged. This is the case in the 2,2'-bipyridyl- Cu^{2+} -nucleotide systems, where the formation of the ionic bridge leads to an increase of the stability of the charge-transfer adducts by a factor of about 2.5×10^2 for the monophosphates and of about 10^6 for the triphosphates (Table I).⁴² In addition, it is important to note again that the stability constants, K_{CT} , of the charge-transfer adducts are (within experimental error) identical with the potentiometrically determined stability constants, $K^{\text{Cu}(\text{bipy})}_{\text{Cu}(\text{bipy})(\text{NP})}$ (*cf.* eq 8), of the corresponding mixed-ligand complexes (Table II). Hence, one has to conclude that the coordination of $\text{Cu}(\text{bipy})^{2+}$ to the phosphate group(s) leads also to an immediate interaction be-

tween the purine moiety and 2,2'-bipyridyl. Furthermore, the identity of the extinction coefficients (Table III) of the charge-transfer adducts formed between $\text{Cu}(\text{bipy})^{2+}$ and the nucleosides or the nucleoside 5'-triphosphates means that the intramolecular equilibrium (10), between an opened and stacked form, is on



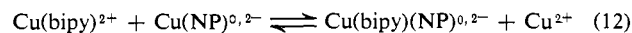
its right side and the dimensionless constant, K' , is rather large. The same conclusion probably holds for the AMP and IMP containing charge-transfer adducts, although their extinction coefficients are different (this point is discussed below).

That the stability of the charge-transfer adducts is governed by the coordination tendency of the phosphate groups is also obvious from a comparison of the stability constants, K_{CT} , of the corresponding mono- and triphosphates (Table I and II); the complexes formed by the latter ligands are considerably more stable. In this connection another comparison is of interest; from the data in Table II it follows that the ternary complexes are more stable than the corresponding binary ones. This means, for the constant $\Delta \log K$ as defined by eq 11, positive values are ob-

$\Delta \log K =$

$$\log K^{\text{Cu}(\text{bipy})}_{\text{Cu}(\text{bipy})(\text{NP})} - \log K^{\text{Cu}}_{\text{Cu}(\text{NP})} \quad (11)$$

tained. From this it follows that equilibrium 12, to



which $\Delta \log K$ corresponds,⁷ is displaced toward its right side. Knowing that the ternary complexes, $\text{Cu}(\text{bipy})(\text{NP})^{0,2-}$, exist in a stacked form, one might be tempted to suggest that the described increased stability ($\Delta \log K \simeq 0.4$; *cf.* Table II) is due to the observed charge-transfer interaction between the purine moiety and 2,2'-bipyridyl, because this interaction is not possible, of course, in the binary complexes, $\text{Cu}(\text{NP})^{0,2-}$. However, such a conclusion is not justified, because positive values for $\Delta \log K$ are also observed in mixed-ligand systems (containing, *e.g.*, HPO_4^{2-} (*cf.* ref 9) or oxalate⁴³), where no stacking between the two ligands is possible.⁴⁴ Hence, the charge-transfer interaction between 2,2'-bipyridyl and the purine moiety within the complexes, $\text{Cu}(\text{bipy})(\text{NP})^{0,2-}$, does not manifest itself significantly in the overall stability of these ternary complexes.

The different reaction paths that finally lead to the formation of the mixed-ligand complexes, $\text{Cu}(\text{bipy})(\text{NP})^{0,2-}$, may be summarized as is shown for the case of $\text{NP} = \text{ATP}^{4-}$ in the Scheme I. The logarithms of the equilibrium constants are given for each step at the arrows. The constants given in parentheses are due to the 2,2'-bipyridyl- Cu^{2+} -AMP²⁻ system. Both sets of constants were calculated with the aid of the results of Table II and the literature.^{9,22} For the inosine phosphates, the analog data may easily be calculated on the same basis.

(43) H. Sigel, P. R. Huber, and R. F. Pasternack, *Inorg. Chem.*, **10**, 2226 (1971).

(44) For a discussion of the reasons why for certain mixed-ligand systems positive values of $\Delta \log K$ are observed see ref 7. (*Cf.* also ref 8, 16, 21, and 38.)

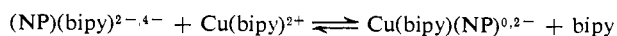
(38) P. R. Huber, R. Griesser, and H. Sigel, *Inorg. Chem.*, **10**, 945 (1971); F. A. Walker, H. Sigel, and D. B. McCormick, *ibid.*, **11**, 2756 (1972).

(39) H. Hanssum and W. Lohmann, *Z. Naturforsch. B*, **28**, 82 (1973).

(40) A. S. Bailey, R. J. P. Williams, and J. D. Wright, *J. Chem. Soc.*, 2579 (1965).

(41) J. L. Fox and G. Tollin, *Biochemistry*, **5**, 3873 (1966); M. W. Hanna and A. Sandoval, *Biochim. Biophys. Acta*, **155**, 433 (1968).

(42) These factors, 2.5×10^2 and 10^6 , are identical with the constants due to the equilibrium



where NP = monophosphate or triphosphate, respectively.

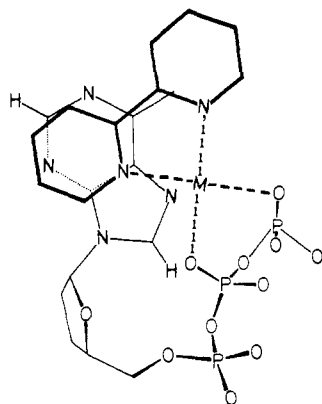
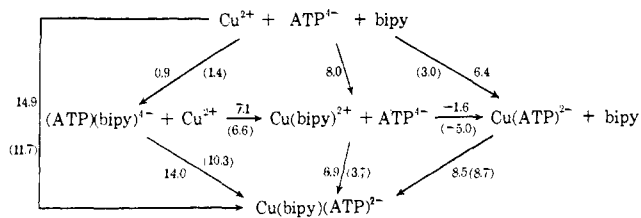


Figure 6. Tentative and simplified structure for the ternary complexes formed between $\text{Cu}(2,2'\text{-bipyridyl})^{2+}$ and ATP^{4-} or ITP^{4-} .¹

Scheme I



At this point it appears desirable to discuss briefly the structure of these stacked 2,2'-bipyridyl- Cu^{2+} -nucleotide complexes. Recently, on the basis of pmr data the tentative structure, which is shown in Figure 6, has been suggested for $\text{Cu}(\text{bipy})(\text{ATP})^{2-}$ and $\text{Cu}(\text{bipy})(\text{ITP})^{2-}$.¹ The intramolecular charge transfer is assumed to occur between the pyrimidine part of the purine moiety and one of the pyridyl rings of 2,2'-bipyridyl. Already in the pmr study some hints were observed suggesting small differences between the ATP and ITP complexes. It seems possible, that the differences in the extinction coefficients, ϵ (Table III), result from the same effect; all the charge-transfer adducts containing hypoxanthine derivatives show a smaller ϵ than the corresponding adenine containing adducts. Maybe, this signifies a somewhat different orientation (torsion) of the aromatic reactants within these complexes.

The results of Table III show an additional point of interest; the extinction coefficients of the two monophosphate-containing ternary complexes are significantly larger, compared with the ϵ values of all the other charge-transfer adducts. Possibly, this is an indication that the two planes of the aromatic rings, which form the charge-transfer adduct, are not quite parallel to each other. As the phosphate-metal ion bridge which connects the two planes is rather short, it appears understandable that the angle between these is not zero (as it is usually the case). Additionally, extinction coefficients of similar order are observed in intramolecular adducts of pyridinium salts, where the rings are bridged by an ethylene group; here, the planes of the two aromatic rings form an angle that is significantly different from zero.³⁴

General Conclusions

Today it is unequivocally clear that the specific interactions between aromatic moieties play an important role in biological systems (see Pullman, ref 34). Such charge-transfer complexes appear to be important for the synthesis of proteins and the determination of the amino acid sequence.⁴⁵ Usually, the stability of these complexes is weak, but, as we have seen in the preceding part of this paper, charge-transfer adducts may be stabilized by orders of magnitude through the formation of a metal ion bridge between the interacting aromatic moieties. Possibly, during the hydrolysis of ATP by meromyosin, Ca^{2+} plays such a role; Yoshino, *et al.*,³⁵ could demonstrate that a charge-transfer interaction between the indole moiety of a tryptophan in the active center of the enzyme and ATP is observable only in the presence of Ca^{2+} or Mg^{2+} . In this connection it should be mentioned that the stability of the charge-transfer adduct between tryptophan and ATP^{4-} may also be increased by a metal ion bridge between these two ligands, thus leading to the formation of a ternary complex.⁴⁶

Ribonuclease has an affinity for 3'-CMP and for Cu^{2+} (or Zn^{2+}), as well. The point of interest, however, is that the binding of 3'-CMP to ribonuclease increases the affinity of this protein for Cu^{2+} (or Zn^{2+}); similarly binding of Cu^{2+} to ribonuclease increases its affinity for 3'-CMP.⁴⁷ Among other factors,⁷ a charge-transfer interaction between a suitable amino acid side chain and the nucleotide may be responsible for this observation. In fact, there is evidence for a possible stacking between the adenine moiety of 5'-AMP and histidine (119) of ribonuclease.⁴⁸

Along similar lines is the quality of pyruvate kinase to bind Mn^{2+} tightly only in the presence of nucleotides.⁴⁹ Again, one is tempted to predict that this is due to a charge-transfer interaction between the nucleotides and suitable aromatic moieties of the enzyme. Another example, where a charge transfer might be of importance is in the storage and transport of neurotransmitters.¹¹ In this case mixed-ligand complexes formed by Mg^{2+} , ATP, and biogenic amines appear to be involved.

Acknowledgments. The potentiometric titrations and their evaluations were performed with the skillful technical assistance of Miss R. Baumbusch and Mrs. J. Buisson. The computer, IBM 1620, was made available by the Rechenzentrum der Universität Basel. This work was supported by a research grant from the Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung and by a grant from the CIBA-Stiftung (Basel).

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(47) E. Breslow and A. W. Girotti, *J. Biol. Chem.*, **245**, 1527 (1970); E. Breslow, ref 5, Vol. 3, 1974, p 133.

(48) P. H. Haffner and J. H. Wang, *Biochemistry*, **12**, 1608 (1973).

(49) M. Cohn, J. S. Leigh, Jr., and G. H. Reed, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 533 (1971); E. J. Milner-White and D. C. Watts, *Biochem. J.*, **122**, 727 (1971); G. H. Reed and M. Cohn, *J. Biol. Chem.*, **247**, 3073 (1972).