JOURNAL

OF THE AMERICAN CHEMICAL SOCIETY

VOLUME 103, NUMBER 2 JANUARY 28, 1981

Macrochelate Formation in Monomeric Metal Ion Complexes of Nucleoside 5′-Triphosphates and the Promotion of Stacking by Metal Ions. Comparison of the Self-Association of Purine and Pyrimidine 5′-Triphosphates Using Proton Nuclear Magnetic Resonance

Kurt H. Scheller, Fritz Hofstetter, Paul R. Mitchell, Bernhard Prijs, and Helmut Sigel*

Contribution from the Institute of Inorganic Chemistry, University of Basel, CH-4056 Basel, Switzerland. *Received May 16, 1980*

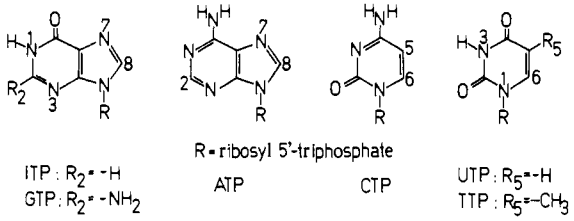
Abstract: The concentration dependence of the chemical shifts of the protons H-2, H-8, and H-1′ or H-5, H-6, and H-1′ of ATP^{4-}, ITP^{4-}, and GTP^{4-} or CTP^{4-} and UTP^{4-} (=NTP^{4-}), respectively, and of the corresponding nucleosides has been measured. The results are consistent with the isodesmic model of indefinite noncooperative stacking; the association constants for NTP^{4-} are between 1.3 (ATP^{4-}) and about 0.4 M^{-1} (UTP^{4-}) and for the nucleosides between 15 (adenosine) and 1.2 M^{-1} (uridine). The self-stacking tendency decreases within the series adenosine > guanosine > inosine > cytidine ~ uridine. Due to the repulsion of the negatively charged phosphate moieties, this trend is much less pronounced for the corresponding NTP^{4-} series. The charge effect also governs the series adenosine ≫ AMP^{2-} > ADP^{3-} ~ ATP^{4-}. Likewise the self-association tendency of ATP^{4-}, ITP^{4-}, and GTP^{4-} is promoted by a factor of about 3–5 by the coordination of Mg^{2+} to the phosphate moiety, which neutralizes part of the negative charge at this residue. However, the self-association tendency of $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$ is much larger than of $Mg(ATP)^{2-}$; this is explained by an increased tendency to form an *inter*molecular metal ion bridge in the dimeric stacks in which Zn^{2+} or Cd^{2+} is coordinated to the phosphate moiety of one ATP^{4-} and to N-7 of the adenine residue of the other ATP^{4-}. The shifts of H-8 for complete stacking (δ_∞) agree with this interpretation. There is no significant increase in stability in $Zn(ITP)^{2-}$ and $Zn(GTP)^{2-}$: i.e., the stability of these stacks is governed only by the charge neutralization—the effect of Zn^{2+} is the same as that of Mg^{2+}. Comparison of the shifts of H-8 at infinite dilution (δ_0) for several systems reveals that an M^{2+}/N-7 interaction exists in the monomeric Zn^{2+} and Cd^{2+} complexes of the purine 5′-triphosphates; i.e., a macrochelate is formed through an *intra*molecular coordination of the metal ion to the phosphate moiety and to N-7. The position of this concentration-independent equilibrium between the open isomer (with phosphate coordination only) and the macrochelated isomer is estimated by comparing δ_0 of $M(NTP)^{2-}$ with the shifts of H-8 for complete complex formation of the corresponding metal ion–nucleoside complexes, which were also determined. The NMR study gives no hint for such an N-7 interaction either for the corresponding $Mg(NTP)^{2-}$ complexes or for a base interaction in any of the pyrimidine 5′-triphosphate complexes. These NMR results prompted an evaluation of stability data (obtained earlier under conditions where no self-association occurs), which give further evidence that macrochelate formation also occurs in the $M(NTP)^{2-}$ complexes of purine nucleotides with Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}; no evidence for such an interaction is observed in the pyrimidine nucleotide complexes. Ionization of the base moiety in ITP^{4-}, GTP^{4-}, UTP^{4-}, and TTP^{4-} favors, however, the base–metal ion interaction and therefore also the formation of macrochelates in the $M(NTP\text{-}H)^{3-}$ complexes. The percentage of the macrochelated isomer is estimated for all these systems: the whole range from nearly 100% ring back-bonding to only insignificant traces is observed. The ambivalent coordinating properties of nucleotides and their structural versatility are discussed.

It is now well-known that metal ions are essential in a large variety of biological processes, including those with nucleic acids and their derivatives.[1-4] For example, DNA polymerase contains tightly bound Zn^{2+}, and there is evidence that this metal ion binds the enzyme to DNA.[5,6] To fulfill its function the enzyme must also be activated by a divalent cation such as Mg^{2+} or Mn^{2+}, and these metal ions bind the nucleoside triphosphate substrates to the enzyme.[7,8] Thus the interplay between metal ions and nucleotides, or their derivatives, is receiving much attention at present.[2-10]

(1) (a) Eichhorn, G. L., Ed. "Inorganic Biochemistry"; Elsevier: New York, 1973; Vol. 1, 2. (b) Sigel, H., Ed. "Metal Ions in Biological Systems"; Marcel Dekker: New York, 1973–1980; Vol. 1–11.
(2) Marzilli, L. G. *Prog. Inorg. Chem.* **1977**, *23*, 255–378.
(3) Gellert, R. W.; Bau, R. *Met. Ions Biol. Syst.* **1979**, *8*, 1–55.
(4) Martin, R. B.; Mariam, Y. H. *Met. Ions Biol. Syst.* **1979**, *8*, 57–124.
(5) Eichhorn, G. L. *Met. Ions Biol. Syst.* **1980**, *10*, 1–21.
(6) Slater, J. P.; Mildvan, A. S.; Loeb, L. A. *Biochem. Biophys. Res. Commun.* **1971**, *44*, 37–43.
(7) Slater, J. P.; Tamir, I.; Loeb, L. A.; Mildvan, A. S. *J. Biol. Chem.* **1972**, *247*, 6784–94.
(8) Mildvan, A. S. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1979**, *49*, 103–26.
(9) Howe-Grant, M.; Lippard, S. J. *Biochemistry* **1979**, *18*, 5762–9.

0002-7863/81/1503-247$01.00/0

Chart I



However, despite this interest several questions remain to be answered;[3,4] one of them is the extent of nucleic base stacking. Martin[4] concluded recently in this connection "the interplay of metal ion binding and stacking has not been fully recognized and more investigation along such lines appears to be in order". Indeed, such studies are very relevant to the properties of nucleotides in living systems. For example, the chromaffin granules, which store catecholamines in the adrenal medulla, contain both substantial amounts of metal ions and high concentrations of ATP ($\sim$0.1 M).[11-15]

Another interesting aspect is the metal-ion-promoted dephosphorylation of nucleoside 5'-triphosphates.[16,17] In the presence of Ni^{2+}, Cu^{2+}, or Zn^{2+} the hydrolysis of ATP proceeds via a dimeric complex which occurs in low concentration[17] and in which the bases seem to be stacked. In addition, a series of studies[18-23] has shown that an intramolecular aromatic-ring stacking occurs within the ternary complex in solutions containing tryptophanate, 2,2'-bipyridyl, or 1,10-phenanthroline/metal ion/ATP^{4-}, -ITP^{4-}, or -UTP^{4-}: thus this stacking causes new structural features. This intramolecular interaction involving the nucleic base has now also been confirmed by X-ray structural studies.[24,25] It seemed clear that a deeper understanding of the self-association of nucleotides would also promote the understanding of such heterostacks, which are probably important in nucleic acid/protein and enzyme/nucleotide interactions.[26,27]

Base stacking occurs in aqueous solutions of nucleic bases, nucleosides, and nucleotides, particularly with purines and to a lesser extent with pyrimidines.[28,29] An upfield shift due to stacking has been observed in NMR studies[30-33] of adenosine 2'-, 3'-, and 5'-monophosphates and also of IMP^{2-} and GMP^{2-};[34] the relaxation times of the protons H-2 and H-8 and the variation of the upfield chemical shift of the resonances H-1', H-2, and H-8 were said[31] to indicate "heat-to-tail" stacking, with the five-membered and six-membered rings alternating in the stack, but other geometries are also possible.[30,34] NMR studies,[30-34] ultracentrifuge studies,[35,36] and a kinetic study[37] show that AMP^{2-} and other adenine derivatives,[33,36-39] and IMP^{2-} and GMP^{2-} as well,[34] associate beyond the dimer stage. It is now generally accepted[34] that the self-association of these compounds occurs by stacking and that oligomers are formed.

Nucleoside 5'-triphosphates have been studied to a much lesser extent: difference ultraviolet spectroscopy[40] and difference circular dichroism[40,41] have been used to study the self-association of ATP and the effect of pH and temperature on this association. ^1H NMR studies confirmed the self-stacking[42-44] and it was also shown that stacking proceeds beyond dimers.[44] Stronger interactions between ATP molecules in the presence of Mg^{2+} and Ca^{2+} have been suggested on the basis of Raman spectra;[45] this is in accordance with other data[40,41] and also with a ^1H NMR study[44] of $Mg(ATP)^{2-}$. Apart from some preliminary data on UTP^{4-}, which indicated only very weak self-stacking,[44] there are no comparable ^1H NMR shift data on other nucleoside 5'-triphosphates. We have therefore now carried out such a study on the self-stacking tendency of purine and pyrimidine nucleoside 5'-triphosphates (see Chart I). Where possible, the influence of Mg^{2+}, Zn^{2+}, or Cd^{2+} on the stability of the stacks was also studied. The corresponding nucleosides, and AMP^{2-} and ADP^{2-}, were included in the measurements for comparison. Although the self-stacking is better at lower pH,[41] we carried out our measurements in the neutral pH range, as this is more relevant to the stacking of the nucleoside 5'-triphosphates in living systems.

There is one further important aspect: extrapolation of the ^1H NMR shift to zero concentration allowed the determination of the chemical shifts of the protons of the monomeric $M(NTP)^{2-}$ complexes; by comparing these chemical shifts with those obtained for the protons in the base moieties of the corresponding nucleoside complexes, we were able to estimate the extent of macrochelate formation in several $M(NTP)^{2-}$ complexes. These monomeric $M(NTP)^{2-}$ complexes exist in two isomeric forms:[4] in the one isomer the metal ion is coordinated only to the phosphate moiety, while in the other the metal ion forms a macrochelate by coordinating simultaneously to the phosphate moiety and to the nucleic base residue as well.

Experimental Section

Materials. The mono- or disodium salts of the nucleotides were from Sigma Chemical Co. (GTP, UTP, and ADP), from Serva Feinbiochemica GmbH, Heidelberg, FRG (ATP, ITP, and CTP), from Boehringer GmbH, Mannheim, FRG (ATP), and from Fluka AG, Buchs, Switzerland (AMP). The nucleosides were purchased from Serva (inosine),

(10) Marzilli, L. G.; de Castro, B.; Caradonna, J. P.; Stewart, R. C.; Van Vuuren, C. P. *J. Am. Chem. Soc.* **1980**, *102*, 916-24.

(11) Abbreviations: Ade, adenosine; AMP, ADP, and ATP, adenosine 5'-mono-, 5'-di-, and 5'-triphosphate; CTP, GTP, ITP, UTP, and TTP, cytidine, guanosine, inosine, uridine, and thymidine 5'-triphosphate; Gua, guanosine; Ino, inosine; M, general metal ion; Ns, nucleoside; NTP, nucleoside 5'-triphosphate.

(12) Kirshner, N.; Kirshner, A. G. *Philos. Trans. R. Soc. London, Ser. B* **1971**, *261*, 279-89.

(13) Winkler, H. *Neuroscience* **1976**, *1*, 65-80.

(14) Frausto da Silva, J. J. R.; Williams, R. J. P. *Nature (London)* **1976**, *263*, 237-9.

(15) Granot, J.; Rosenheck, K. *FEBS Lett.* **1978**, *95*, 45-8.

(16) Amsler, P. E.; Sigel, H. *Eur. J. Biochem.* **1976**, *63*, 569-81.

(17) Sigel, H.; Amsler, P. E. *J. Am. Chem. Soc.* **1976**, *98*, 7390-7400.

(18) Naumann, C. F.; Sigel, H. *J. Am. Chem. Soc.* **1974**, *96*, 2750-6.

(19) Sigel, H.; Naumann, C. F. *J. Am. Chem. Soc.* **1976**, *98*, 730-9.

(20) Chaudhuri, P.; Sigel, H. *J. Am. Chem. Soc.* **1977**, *99*, 3142-50.

(21) Fukuda, Y.; Mitchell, P. R.; Sigel, H. *Helv. Chim. Acta* **1978**, *61*, 638-47.

(22) Mitchell, P. R.; Sigel, H. *J. Am. Chem. Soc.* **1978**, *100*, 1564-70.

(23) Mitchell, P. R.; Prijs, B.; Sigel, H. *Helv. Chim. Acta* **1979**, *62*, 1723-35.

(24) Aoki, K. *J. Am. Chem. Soc.* **1978**, *100*, 7106-8.

(25) Aoki, K. *J. Chem. Soc., Chem. Commun.* **1979**, 589-91.

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

(27) Hélène, C. *Nucleic Acids Res.* **1975**, *2*, 961-9.

(28) Broom, A. D.; Schweizer, M. P.; Ts'o, P. O. P. *J. Am. Chem. Soc.* **1967**, *89*, 3612-22.

(29) Ts'o, P. O. P. In "Basic Principles in Nucleic Acid Chemistry"; Ts'o, P.O.P., Ed.; Academic Press: New York, 1974; Vol. 1, pp 453-584.

(30) Schweizer, M. P.; Broom, A. D.; Ts'o, P. O. P.; Hollis, D. P. *J. Am. Chem. Soc.* **1968**, *90*, 1042-55.

(31) Son, T.-D.; Chachaty, C. *Biochim. Biophys. Acta* **1973**, *335*, 1-13.

(32) Evans, F. E.; Sarma, R. H. *Biopolymers* **1974**, *13*, 2117-32.

(33) Cheng, D. M.; Kan, L. S.; Ts'o, P. O. P.; Giessner-Prettre, C.; Pullman, B. *J. Am. Chem. Soc.* **1980**, *102*, 525-34.

(34) Neurohr, K. J.; Mantsch, H. H. *Can. J. Chem.* **1979**, *57*, 1986-94.

(35) Rossetti, G. P.; Van Holde, K. E. *Biochem. Biophys. Res. Commun.* **1967**, *26*, 717-21.

(36) Bretz, R.; Lustig, A.; Schwarz, G. *Biophys. Chem.* **1974**, *1*, 237-41.

(37) Heyn, M. P.; Nicola, C. U.; Schwarz, G. *J. Phys. Chem.* **1977**, *81*, 1611-7.

(38) Schimmack, W.; Sapper, H.; Lohmann, W. *Biophys. Struct. Mechanism* **1975**, *1*, 113-120.

(39) Sapper, H. and Lohmann, W. *Biophys. Struct. Mech.* **1978**, *4*, 327-35.

(40) Heyn, M. P.; Bretz, R. *Biophys. Chem.* **1975**, *3*, 35-45.

(41) Gilligan, T. J.; Schwarz, G. *Biophys. Chem.* **1976**, *4*, 55-63.

(42) Fan, S.; Storer, A. C.; Hammes, G. G. *J. Am. Chem. Soc.* **1977**, *99*, 8293-8.

(43) Lam, Y.-F. Kotowycz, G. *Can. J. Chem.* **1977**, *55*, 3620-30.

(44) Mitchell, P. R.; Sigel, H. *Eur. J. Biochem.* **1978**, *88*, 149-54.

(45) Heyde, M. E.; Rimai, L. *Biochemistry* **1971**, *10*, 1121-8.

Fluka (guanosine), Sigma (uridine), and Merck AG, Darmstadt, FRG (adenosine and cytidine). The nitrate salts of sodium, magnesium, zinc, and cadmium (all p.a.), DNO_3 and NaOD (both with >99% D), and a 10% tetramethylammonium hydroxide solution (which was converted into the nitrate) were obtained from Merck. D_2O was from DRA-Bureau des Isotopes Stables, CEA-CEN, Saclay, France (99.8%), or from CIBA-Geigy AG, Basel, Switzerland (99.7%).

The sodium triphosphate was from the same source and also purified as before;[19] the content of orthophosphate was less than 1.3%.

Apparatus and Measurements. The ^1H NMR spectra were recorded with a Bruker WH-90 FT spectrometer (90.025 MHz) at 27 °C, using the center peak of the tetramethylammonium ion triplet as internal reference. All chemical shifts were converted to a (trimethylsilyl)-propanesulfonate reference by adding 3.188 ppm.

The pD of the solutions was obtained by adding 0.40 to the pH meter reading.[46] The pH was measured with a Metrohm potentiometer E 510 (Metrohm AG, Herisau, Switzerland), using a Metrohm glass electrode EA 125. The desired pD of a solution was adjusted by dotting with relatively concentrated DNO_3 or NaOD on a thin glass rod. On the basis of the known[47-49] acidity constants of $H(NTP)^{3-}$ and NTP^{4-} [to $(NTP-H)^{5-}$], the stability constants of $M(NTP)^{2-}$ and $M(NTP-H)^{3-}$, and the hydrolysis constants of $M(NTP)^{2-}$ [to $M(NTP)(OH)^{3-}$], the pD of each solution was selected such that either NTP^{4-} or $M(NTP)^{2-}$ were practically completely formed. As the degree of formation of $M(NTP)^{2-}$ is high (about 90% even at reactant concentrations of 10^{-3} M) over a rather broad pH interval,[47,48] this was no problem.[49]

The reactant concentrations in the self-stacking experiments varied typically from 0.005 to 0.4 M. $NaNO_3$ was added to increase the ionic strength to 0.1 M, when necessary,[50] although in the stronger nucleotide solutions the ionic strength was unavoidably higher. The individual experimental details for the systems are given in Table II and in Figures 1-4.

The experimental results were analyzed by using a Hewlett-Packard 9821A calculator connected to a 9862A calculator-plotter: the observed variation of the chemical shift with concentration (Figures 1-4) was fitted to eq 3 by using a Newton-Gauss nonlinear least-squares method.

On the Reliability of the Tetramethylammonium Ion as an Internal ^1H NMR Reference. Variations in the magnetic susceptibility of the strong solutions needed for the measurements on the $M(NTP)^{2-}$ complexes prevent the use of an external reference, as precise susceptibility corrections would be complicated.[54] The internal reference commonly used in aqueous solution, (trimethylsilyl)propanesulfonate, is unreliable in the presence of a ligand with an aromatic moiety, as hydrophobic interactions between the trimethylsilyl group and the aromatic moiety shift the trimethylsilyl resonance considerably upfield; this shift is increased by metal ions.[55,56] In fact, this upfield shift is known[44,57] to completely mask the upfield shifts due to the self-stacking of ATP^{4-}. Similarly, *tert*-butyl alcohol is also known to be shifted upfield in the presence of high concentrations of aromatic solutes,[58] owing to hydrophobic interactions which also occur[44] with ATP^{4-}.

Tetramethylammonium ion (TMA^+) has been found in earlier related work[22,44,55,56] to be unaffected by the presence of high concentrations of aromatic species (but see ref 59 and 60), and, as no variation in the chemical shift could be observed at concentrations of Na^+ or Zn^{2+} up to ~2 M, it may be presumed[54] that changes in ionic strength such as occur in this work have no effect. The use of the resonance of one proton in a molecule as reference for another proton in the same molecule in the determination of acidity constants by NMR has been recommended.[61,62]

Table I. Comparison of the Equilibrium Constants for Self-Stacking of ATP^{4-} as Calculated from the Chemical Shifts of H-2, H-8, and H-1′ Relative to the Internal Standard $(CH_3)_4N^+$ and by the Reference-Independent Shift Differences (H-8) − (H-2) and (H-2) − (H-1′)[a]

evaluated shift	K, M^{-1}	δ_0[b]	δ_∞[b]
H-2	1.28 ± 0.14	8.278 ± 0.002	6.95 ± 0.09
H-8	1.06 ± 0.28	8.562 ± 0.002	7.85 ± 0.12
H-1′	1.46 ± 0.48	6.165 ± 0.006	5.67 ± 0.08
(H-8) − (H-2)	1.52 ± 0.23		
(H-2) − (H-1′)	1.65 ± 0.22		

[a] 27 °C, I = 0.1 to about 2 M ($NaNO_3$), pD 8.4. The shifts were measured relative to $(CH_3)_4N^+$ and converted to values downfield from sodium (trimethylsilyl)propanesulfonate by adding 3.188 ppm. The range of error given is one standard deviation. [b] These shifts are the values obtained from calculations for each individual proton; the values differ therefore slightly from those listed in Table III, which have been calculated with K_{av} of Table II.

A similar procedure using one NMR resonance as a reference for another which is shifted more has proved useful in studies of the self-association of 1,10-phenanthroline in the presence of high concentrations of zinc ions[54] and in acid solution.[60]

Therefore we have checked the validity of TMA^+ as reference by measuring the chemical shift of a NTP^{4-} proton that is strongly concentration dependent vs. a proton that is less concentration dependent. The association constant calculated on the basis of this "intramolecular reference" should be the same as the constant calculated from the concentration dependence of the individual chemical shifts, measured against the TMA^+ resonance. As the probability of an ionic interaction, e.g., ion pair formation, between TMA^+ and an anion will increase with increasing negative charge of the anion, the results with the highly charged ATP^{4-} listed in Table I are especially interesting. It is clear (cf. also Figure 1) that the resonance of H-2 is shifted more than the resonances of H-8 and H-1′: indeed, an evaluation for the (H-8)-(H-2) and (H-2)-(H-1′) shift differences gives the two values of the association constant which agree well with each other; but more important these two values are within experimental error, identical with those calculated from the individual shifts. This then confirms the reliability of TMA^+ as a reference for the present study. Such an evaluation is not possible for the (H-8)-(H-2) difference, because the concentration dependence of the shifts of these two protons is too similar (see Figure 1). The association constant for the self-stacking of GTP^{4-} was not checked in this way, as the changes in the shifts of H-8 and H-1′ are again too similar (see Figure 3), but this test has also been carried out for $Mg(ATP)^{2-}$ and CTP^{4-}, and the agreement between the constants determined by the different methods was again satisfactory.

Results and Discussion

1. Definition of the Association Constant for Self-Stacking. The theory developed by Heyn and Bretz,[40] an isodesmic model for the indefinite noncooperative association of ATP^{4-}, studied by ultraviolet absorption spectra and circular dichroism, was adapted for NMR as described in detail recently.[44,54,63] It is assumed that the equilibrium constants (eq 1) for the equilibria (2) are all equal.

$$K = [(ATP^{4-})_{n+1}]/[(ATP^{4-})_n][ATP^{4-}] \quad (1)$$

$$(ATP^{4-})_n + ATP^{4-} \rightleftharpoons (ATP^{4-})_{n+1} \quad (2)$$

As the distance between stacked aromatic moieties of a nucleotide is in the order of 0.35 nm[3,24,25] and as the upfield shifts due to a ring current fall off very rapidly with increasing distance from the ring,[64,65] only the ring current in adjacent molecules in the stack is expected to have a significant effect on the upfield shift. As the upfield shifts caused by the two adjacent aromatic molecules within the stack are expected to be additive, the shift of an infinitely long stack (δ_∞) is then the same as the shift of a molecule within a shorter stack. This then leads[44,54] to the simplified expression (3), which is equivalent to the expression used by Dimicoli and Hélène[66] and which gives the relationship between the observed chemical shift (δ_{obsd}) in a solution of total concen-

(46) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188-9.
(47) Sigel, H. *J. Am. Chem. Soc.* **1975**, *97*, 3209-14.
(48) Sigel, H. *J. Inorg. Nucl. Chem.* **1977**, *39*, 1903-11.
(49) The isotope effect (e.g., $pK^H_{H(ATP)} = 6.42$ compared with $pK^D_{D(ATP)} = 6.96$)[21] was taken into account for deprotonations.
(50) Of course, $Na(ATP)^{3-}$ is present in these solutions.[51]
(51) As the stability constant[52,53] of $Na(ATP)^{3-}$, $K^{Na}_{Na(ATP)}$, is 15 M^{-1}, the proportion of $Na(ATP)^{3-}$ varies from 66% in 0.01 M solution to 95% in 0.4 M solution. However, in the presence of divalent metal ions, very little $Na(ATP)^{3-}$ is present (≤2%).
(52) O'Sullivan, W. J.; Perrin, D. D. *Biochemistry* **1964**, *3*, 18-26.
(53) Botts, J.; Chashin, A.; Young, H. L. *Biochemistry* **1965**, *4*, 1788-96.
(54) Mitchell, P. R. *J. Chem. Soc., Dalton Trans.* **1980**, *1079*-86.
(55) Mitchell, P. R.; Sigel, H. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 548.
(56) Mitchell, P. R. *J. Chem. Soc., Dalton Trans.* **1979**, 771-6.
(57) Bovey, F. A. "High Resolution NMR of Macromolecules"; Academic Press: New York, 1972; p 399.
(58) Jones, R. A. Y.; Katritzky, A. R.; Murrell, J. N.; Sheppard, N. *J. Chem. Soc.* **1962**, 2576-8.
(59) Orenberg, J. B.; Fischer, B. E.; Sigel, H. *J. Inorg. Nucl. Chem.* **1980**, *42*, 785-92.
(60) Mitchell, P. R. *J. Am. Chem. Soc.* **1980**, *102*, 1180-1.
(61) Lee, D. G. *Can. J. Chem.* **1970**, *48*, 1919-23.
(62) Cookson, R. F. *Chem. Rev.* **1974**, *74*, 5-28.
(63) A similar approach has been adopted by Lohmann and co-workers.[38]
(64) Johnson, C. E.; Bovey, F. A. *J. Chem. Phys.* **1958**, *29*, 1012-4.
(65) Rose, P. I. *Org. Magn. Reson.* **1973**, *5*, 187-9.
(66) Dimicoli, J.-L.; Hélène, C. *J. Am. Chem. Soc.* **1973**, *95*, 1036-44.

Table II. Equilibrium Constants for Self-Stacking of Several Nucleosides, Nucleotides, and Nucleotide-Metal Ion Complexes as Determined by ^{1}H NMR Measurements (27 °C)[a]

system	pD[b]	I	K (M^{-1}) determined from the shift of H-2	H-8	H-1'	K_{av}, M^{-1}
adenosine[c]	7.0	0.1	13.7 ± 3.4	18.7 ± 5.4	15.1 ± 4.9	15 ± 2
AMP^{2-}	8.9	0.1-~1.2	2.32 ± 0.21	1.77 ± 0.27	1.80 ± 0.36	2.1 ± 0.4
ADP^{3-}	8.9	0.1-~1.7	1.49 ± 0.34	1.02 ± 0.44	0.92 ± 0.39	1.3 ± 0.4
ATP^{4-} [c]	8.4	0.1-~2	1.28 ± 0.14	1.06 ± 0.28	1.46 ± 0.48	1.3 ± 0.2
$Mg(ATP)^{2-}$	7.4	0.1-~2	4.35 ± 0.52	3.50 ± 0.56	3.93 ± 0.62	4.0 ± 0.5
$Zn(ATP)^{2-}$	7.2	0.1-~2	10.30 ± 1.43	*d*	18.86 ± 6.40	11.1 ± 4.5[f]
$Cd(ATP)^{2-}$	7.2	0.1-~2	57.8 ± 7.4[e]	*d*	9.82 ± 0.84[e]	~17[f]
inosine	6.9	0.1	3.22 ± 0.56	3.59 ± 0.87	3.26 ± 0.84	3.3 ± 0.3
ITP^{4-}	8.25	0.1-~2	0.95 ± 0.17	0.51 ± 0.09	0.26 ± 0.03	0.4 ± 0.3
$Mg(ITP)^{2-}$	7.1	0.1-~2	2.81 ± 0.63	1.64 ± 0.28	1.95 ± 0.29	2.0 ± 0.6
$Zn(ITP)^{2-}$	6.6	0.1-~2	2.09 ± 0.57	*d*	3.26 ± 0.62	2.8 ± 1.2
guanosine[g]	6.9	0.1		10.2 ± 0.2[h] 5.7 ± 0.1[i]	10.6 ± 1.4[h] 6.5 ± 0.8[i]	8 ± 3
GTP^{4-}	8.45	0.1-~2		0.57 ± 0.11	1.34 ± 0.32	0.8 ± 0.6
$Zn(GTP)^{2-}$	6.6	0.1-~2		*d*	1.94 ± 0.58	1.9 ± 0.6

system	pD[b]	I	K (M^{-1}) determined from shift of H-5	H-6	H-1'	K_{av}, M^{-1}
cytidine	6.9	0.1	1.08 ± 0.25	1.19 ± 0.29	2.00 ± 0.44	1.4 ± 0.5
CTP^{4-}	8.9	0.1-~2	0.33 ± 0.26[e]	0.63 ± 0.28[e]	0.40 ± 0.33[e]	0.5 ± 0.2
$Mg(CTP)^{2-}$	7.4	0.1-~0.5				*j*
uridine	6.9	0.1	1.47 ± 0.45	*k*	1.00 ± 0.29	1.2 ± 0.5
UTP^{4-}	8.4	0.1-~2	0.60[l,m] 0.16[l,n]	0.20[k,n]	0.68[l,m] 0.26[l,n]	~0.4
$Mg(UTP)^{2-}$	7.4	0.1-~0.5				*j*

[a] The ionic strength was adjusted to 0.1 by adding $NaNO_3$ if necessary. The range of error given with the values for K of the individual protons corresponds to the standard deviation. K_{av} is the weighted mean (calculated by using log K) of the individual results; the range of error given here is *twice* the standard error. Several systems could not be studied due to precipitation under the following conditions: $[Cd^{2+}]$ = [ITP] = 0.07 M at pD 6.6; $[Mg^{2+}]$ = [GTP] = 0.05 M at pD 6.6; $[Cd^{2+}]$ = [GTP] = 0.009 M at pD 5.5; $[Zn^{2+}]$ = [CTP] = 0.02 M at pD 6.4; $[Cd^{2+}]$ = [CTP] = 0.05 M at pD 4.4; $[Zn^{2+}]$ or $[Cd^{2+}]$ = [UTP] = 0.01 M at pD 6.6. [b] pD of the solutions used. [c] Reference 44. [d] Due to a Zn^{2+} or Cd^{2+}/N-7 interaction the shift of H-8 deviates at low concentrations and cannot therefore be used to calculate K for the self-association; see also section 6 and Figure 1. [e] Average of two series of experiments. [f] Regarding the validity of this value, see section 4. [g] The solubility of guanosine is too low to obtain enough data for a curve-fitting procedure; however, estimates of K using the upfield shifts $\Delta\delta$ of the corresponding protons of inosine, adenosine, or GTP^{4-} lead to the values given.[h,i] [h] Calculated with the average value of $\Delta\delta$ for adenosine and inosine (cf. Table III). [i] Calculated with $\Delta\delta$ of GTP^{4-} (Table III). [j] As only solutions up to $[Mg^{2+}]$ = [CTP] or [UTP] = 0.1 M could be studied due to precipitation, no independent curve-fitting procedure was possible. The results give the same values of K, within experimental error, as those obtained for CTP^{4-} and UTP^{4-} alone. [k] The upfield shifts were too small (see Table III) for a curve-fitting procedure. [l] Estimates of K were obtained by using the upfield shifts $\Delta\delta$ of the corresponding protons of uridine or CTP^{4-} (cf. Figure 4).[m,n] [m] Calculated with $\Delta\delta$ of uridine (Table III). [n] Calculated with $\Delta\delta$ of CTP^{4-} (Table III).

tration [A] and the chemical shift of a free molecule, i.e., the shift at infinite dilution (δ_0), the chemical shift of an infinitely long stack (δ_∞), and the association constant K (eq 1).

$$\delta_{obsd} = \delta_\infty + (\delta_\infty - \delta_0)[1 - (4K[A] + 1)^{1/2}]/2K[A] \quad (3)$$

For completeness, it should be mentioned that if species larger than dimers are ignored, the relationship between the observed upfield shift and the total concentration becomes

$$\delta_{obsd} = \delta_D + (\delta_D - \delta_0)[1 - (8K_D[A] + 1)^{1/2}]/4K_D[A] \quad (4)$$

Equations 3 and 4 are identical except that δ_∞ is replaced by δ_D, the upfield shift in a dimer, and K is replaced by $2K_D$ (i.e., $K_D = 0.5K$), which is the equilibrium constant for dimerization:[44,54]

$$ATP^{4-} + ATP^{4-} \rightleftharpoons (ATP)_2^{8-} \quad (5)$$

$$K_D = [(ATP)_2^{8-}]/[ATP^{4-}]^2 \quad (6)$$

However, it must be emphasized that it is now generally agreed[34,44] that the self-association of purine derivatives[30-41] proceeds beyond the dimer stage.

2. Self-Stacking of Adenosine and Its 5'-Nucleotides. The variation of the upfield shifts of H-2, H-8, and H-1' of ATP^{4-} as a function of the concentration is shown in Figure 1. Computer-calculated least-squares fits of the variation of the upfield shifts of each of the protons with increasing concentration with eq 3 gave the same stability constant for each of the protons, within experimental error; the values are given in Table II. The association constants for AMP^{2-} and ADP^{3-} have been obtained in the same way. Our value for AMP^{2-}, $K = 2.1 \pm 0.4$ M^{-1}, is in excellent agreement with the values published recently by Neurohr and Mantsch,[34] $K = 1.9$ M^{-1} (30 °C), and by Imoto,[67] $K_D = 1.3$ M^{-1}, i.e., $K = 2.6$ M^{-1} (28 °C).

The decreasing tendency for self-stacking within the series adenosine ($K = 15 \pm 2$ M^{-1}) $\gg$ AMP^{2-} (2.1 ± 0.4) > ADP^{3-} (1.3 ± 0.4) $\simeq$ ATP^{4-} (1.3 ± 0.2) is to be expected due to the repulsion between the negatively charged phosphate groups: the effect of the introduction of one phosphate moiety to adenosine is especially striking. The association constant of adenosine itself is, as expected, between the values[54] for 1,10-phenanthroline ($K = 23.6 \pm 1.8$ M^{-1}) and 2,2'-bipyridyl ($K = 7.4 \pm 1.0$ M^{-1}). The aromatic system of phenanthroline is larger and would therefore be expected to associate more than adenosine, while, as bipyridyl is not only more flexible than adenosine but also usually skewed, it stacks less readily. That an increase in the aromatic system favors the stacking tendency may also be seen from *lin*-benzoadenine nucleotides: the association constants for aqueous solution are "at least one order of magnitude greater than those of the corresponding adenine nucleotides".[68]

The addition of another phosphate moiety to AMP^{2-} decreases the stacking tendency somewhat further, but the constants for ADP^{3-} and ATP^{4-} are identical within experimental error. This

(67) Imoto, T. *Biochim. Biophys. Acta* **1977**, *475*, 409-16.
(68) Barrio, J. R.; Liu, F.-T.; Keyser, G. E.; Van Der Lijn, P.; Leonard, N. J. *J. Am. Chem. Soc.* **1979**, *101*, 1564-9.

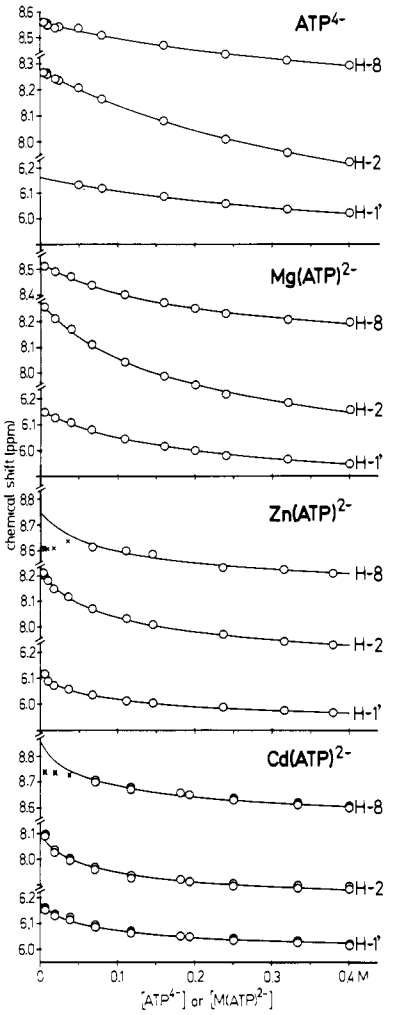


Figure 1. Variation of the chemical shift of H-2, H-8, and H-1′ of ATP with varying concentrations of ATP^{4-} or $M(ATP)^{2-}$. The spectra were measured on a Bruker FT 90 at 90.025 MHz (D_2O, 27 °C, I = 0.1 to ~2, $NaNO_3$), relative to internal $(CH_3)_4N^+$ and converted to values relative to sodium (trimethylsilyl)propanesulfonate by adding 3.188 ppm. The curves shown in this and in Figures 2–4 are the computer-calculated best fit of the experimental data (calculated with K_{av} of Table II), using the indefinite noncooperative stacking model; the corresponding average association constants and the corresponding shifts are listed in Tables II and III, respectively. From top to bottom: ATP^{4-}, pD 8.4; $Mg(ATP)^{2-}$, pD 7.4; $Zn(ATP)^{2-}$, pD 7.2; $Cd(ATP)^{2-}$, pD 7.2 (the points of two experimental series are shown). For the latter two complexes the curve shown through the experimental shifts of H-8 was also calculated with K_{av} given in Table II, but for the fit only concentrations greater than 0.06 M were used. The deviation between the calculated curve for H-8 and the experimental data in the Zn^{2+} and Cd^{2+} systems at lower concentrations is discussed in section 4.

is understandable: under the experimental conditions ATP^{4-} is present to a considerable extent as $Na(ATP)^{3-}$ in all of the solutions.[51] As at lower ATP^{4-} concentrations the upfield shift of ATP^{4-} is enhanced by increasing the concentration of Na^+, it appears that $Na(ATP)^{3-}$ self-stacks slightly better than ATP^{4-}. Therefore the association constant given above for ATP^{4-} must be considered an upper limit. As the stability of the other $Na(NTP)^{3-}$ complexes is certainly comparable, the above reasoning holds for all the association constants given in Table II for the self-association of the nucleoside 5′-triphosphates. The extent of complex formation between Na^+ and ADP^{3-} is not known, but it may be expected to be somewhat smaller than with ATP^{4-}.

The chemical shifts of the protons in monomeric ATP^{4-} and in completely stacked ATP^{4-} are given in Table III. The upfield shifts, $\Delta\delta = \delta_0 - \delta_\infty$, especially for H-2 of ATP^{4-} are much higher than would be expected for the shift due to a single adjacent molecule, as in the dimer. This then confirms the expectation that stacking proceeds beyond the dimer stage. A comparison

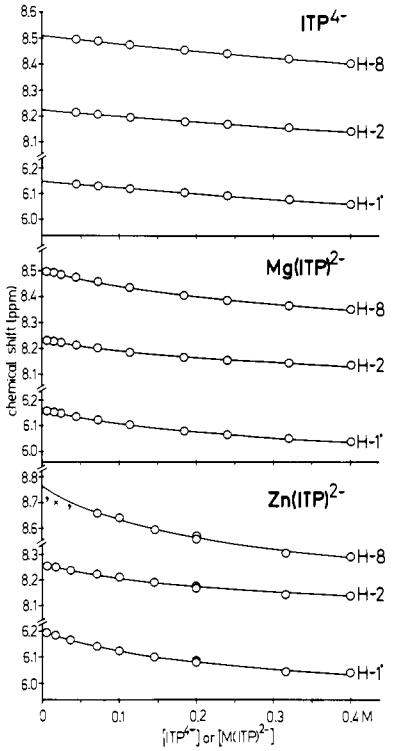


Figure 2. Variation of the chemical shift of H-2, H-8, and H-1′ of ITP with varying concentrations of ITP^{4-} or $M(ITP)^{2-}$; for details see legend to Figure 1. From top to bottom: ITP^{4-}, pD 8.25; $Mg(ITP)^{2-}$, pD 7.1; $Zn(ITP)^{2-}$, pD 6.6. The curve shown through the experimental shifts of H-8 of $Zn(ITP)^{2-}$ was also calculated with K_{av} = 2.8 M^{-1} (Table II), using concentrations greater than 0.06 M; see text in section 5.

of the upfield shifts for all the adenine derivatives indicates that for H-2, H-8, and H-1′ the upfield shifts increase in the series adenosine < $AMP^{2-} \lesssim ADP^{3-} \sim ATP^{4-}$. It is noticeable, however, that for all these substrates δ_0 of H-2 is identical within experimental error, while δ_∞ decreases with increasing negative charge. These differences are probably due to variations in the relative orientation of the molecules in the stack,[54] which result from changes in the repulsion between the differently charged phosphate moieties; in adenosine there is of course no such repulsion.

3. Comparison of the Self-Stacking Tendency of Nucleoside 5′-Triphosphates. The concentration dependence of the upfield shifts of H-2, H-8, and H-1′ of ITP^{4-} is shown in Figure 2; the same is shown for H-8 and H-1′ of GTP^{4-} in Figure 3, and for H-5, H-6, and H-1′ of CTP^{4-} and UTP^{4-} in Figure 4: evaluation of these experimental data gives the association constants listed in Table II. It appears that the self-stacking tendency of ATP^{4-} (K = 1.3 ± 0.2 M^{-1}) is about 3 times as large as that of ITP^{4-} (K = 0.4 ± 0.3 M^{-1}). The association constant for GTP^{4-} (K = 0.8 ± 0.6 M^{-1}) appears to be between those of ATP^{4-} and ITP^{4-}. The known[34] association tendency of GMP^{2-} (K = 1.3 M^{-1} at 30 °C) and of IMP^{2-} (K = 1.4 M^{-1} at 30 °C) is smaller than the one of AMP^{2-} (K = 1.9 M^{-1} at 30 °C/2.1 M^{-1} at 27 °C; Table II) but larger compared with that of the corresponding 5′-triphosphates and fits therefore into the general picture.

The association tendency of the pyrimidine 5′-triphosphates is smaller than that of the purine 5′-triphosphates, as expected, for the smaller ring systems. However, the association constants for CTP^{4-} and UTP^{4-} are very similar.

The high charge of the nucleoside 5′-triphosphates and the resulting rather low association constants hinder a detailed comparison of the self-stacking. However, the trend observed with the 5′-triphosphates is seen much clearer with the nucleosides themselves. Indeed, the more pronounced association tendency of the adenine moiety compared with the hypoxanthine residue is then quite clear: K = 15 ± 2 M^{-1} for adenosine and K = 3.3 ± 0.3 M^{-1} for inosine. Unfortunately, the stacking tendency of the guanine moiety could not be measured directly, as guanosine

Table III. Chemical Shifts (ppm) of Several Monomeric (δ_0) and Self-Stacked (δ_∞) Nucleosides, Nucleotides, and Nucleotide-Metal Ion Complexes, Together with the Corresponding Upfield Shifts ($\Delta\delta = \delta_0 - \delta_\infty$)[a]

	H-2			H-8			H-1′		
system	δ_0	δ_∞	$\Delta\delta$	δ_0	δ_∞	$\Delta\delta$	δ_0	δ_∞	$\Delta\delta$
adenosine[b]	8.278 ± 0.009	7.77 ± 0.08	0.51 ± 0.07	8.350 ± 0.006	8.07 ± 0.04	0.28 ± 0.04	6.087 ± 0.005	5.86 ± 0.04	0.23 ± 0.03
AMP^{2-}	8.265 ± 0.011	7.25 ± 0.11	1.01 ± 0.11	8.622 ± 0.006	8.17 ± 0.05	0.45 ± 0.05	6.145 ± 0.005	5.77 ± 0.05	0.38 ± 0.05
ADP^{3-}	8.277 ± 0.013	6.98 ± 0.30	1.30 ± 0.30	8.553 ± 0.008	7.93 ± 0.16	0.62 ± 0.16	6.159 ± 0.007	5.63 ± 0.13	0.53 ± 0.14
ATP^{4-} [b]	8.278 ± 0.004	6.93 ± 0.10	1.35 ± 0.11	8.563 ± 0.004	7.92 ± 0.06	0.64 ± 0.07	6.163 ± 0.006	5.63 ± 0.05	0.53 ± 0.05
$Mg(ATP)^{2-}$	8.275 ± 0.014	7.35 ± 0.07	0.93 ± 0.07	8.527 ± 0.009	8.02 ± 0.04	0.51 ± 0.04	6.158 ± 0.008	5.70 ± 0.04	0.46 ± 0.04
$Zn(ATP)^{2-}$	8.230 ± 0.022	7.75 ± 0.06	0.48 ± 0.06	[c]	8.36 ± 0.06[d]		6.115 ± 0.017	5.88 ± 0.04	0.24 ± 0.04
$Cd(ATP)^{2-}$	8.104 ± 0.088	7.77 ± 0.14	0.33 ± 0.08	[c]	8.49 ± 0.08[d]		6.181 ± 0.006	5.95 ± 0.10	0.23 ± 0.06
inosine	8.234 ± 0.002	7.89 ± 0.03	0.34 ± 0.03	8.346 ± 0.002	8.06 ± 0.03	0.29 ± 0.03	6.106 ± 0.002	5.81 ± 0.03	0.30 ± 0.03
ITP^{4-}	8.225 ± 0.005	7.51 ± 0.48	0.72 ± 0.48	8.511 ± 0.006	7.59 ± 0.60	0.92 ± 0.60	6.151 ± 0.005	5.39 ± 0.48	0.76 ± 0.48
$Mg(ITP)^{2-}$	8.236 ± 0.005	7.93 ± 0.06	0.31 ± 0.06	8.506 ± 0.006	8.05 ± 0.08	0.46 ± 0.08	6.164 ± 0.005	5.79 ± 0.06	0.37 ± 0.06
$Zn(ITP)^{2-}$	8.266 ± 0.010	7.95 ± 0.08	0.32 ± 0.08	[c]	8.08 ± 0.14[e]		6.203 ± 0.012	5.79 ± 0.10	0.41 ± 0.10
guanosine				8.006 ± 0.003	[f]		5.921 ± 0.003	[f]	
GTP^{4-}				8.164 ± 0.010	7.70 ± 0.29	0.46 ± 0.29	5.945 ± 0.009	5.53 ± 0.27	0.42 ± 0.27
$Zn(GTP)^{2-}$				[c]	7.81 ± 0.26[g]		5.998 ± 0.015	5.49 ± 0.19	0.51 ± 0.19

	H-5			H-6			H-1′		
system	δ_0	δ_∞	$\Delta\delta$	δ_0	δ_∞	$\Delta\delta$	δ_0	δ_∞	$\Delta\delta$
cytidine	6.056 ± 0.004	5.86 ± 0.05	0.20 ± 0.05	7.842 ± 0.002	7.73 ± 0.03	0.11 ± 0.03	5.906 ± 0.002	5.81 ± 0.03	0.10 ± 0.03
CTP^{4-}	6.146 ± 0.002	5.84 ± 0.11	0.31 ± 0.12	8.006 ± 0.005	7.34 ± 0.24	0.67 ± 0.24	6.012 ± 0.002	5.78 ± 0.09	0.23 ± 0.09
$Mg(CTP)^{2-}$	6.131 ± 0.004[i]	[h]		7.927 ± 0.004[i]	[h]		5.996 ± 0.004[i]	[h]	
uridine	5.900 ± 0.002	5.79 ± 0.03	0.11 ± 0.03	7.874 ± 0.002	7.82 ± 0.02	0.05 ± 0.02	5.919 ± 0.002	5.81 ± 0.03	0.11 ± 0.03
UTP^{4-}	5.975 ± 0.002	[h]		7.993 ± 0.004	[h]		5.996 ± 0.002	[h]	
$Mg(UTP)^{2-}$	5.976 ± 0.004[i]	[h]		7.942 ± 0.004[i]	[h]		5.996 ± 0.004[i]	[h]	

[a] The experimental conditions are the same as given in Table II. The chemical shifts were measured relative to internal $(CH_3)_4N^+$ and converted to values downfield from sodium (trimethylsilyl)propanesulfonate by adding 3.188 ppm. The shifts were calculated by using the values of K_{av} given in Table II; the errors are *twice* the standard deviation. [b] Taken from ref 44. [c] See Figures 1–3, Table V, and section 6. [d] See legend to Figure 1. [e] See legend to Figure 2. [f] The solubility of guanosine is too low for significant stacking to occur even in saturated solution. [g] See legend to Figure 3. [h] See footnotes *j–n* of Table II (cf. also Figure 4). [i] Estimated error.

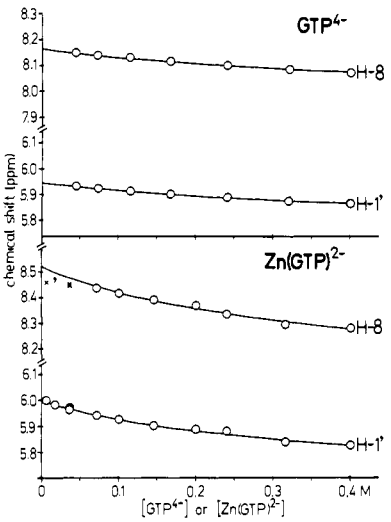


Figure 3. Variation of the chemical shift of H-8 and H-1′ of GTP with varying concentrations of GTP^{4-} (pD 8.45) or $Zn(GTP)^{2-}$ (pD 6.6); for details see legend to Figure 1. The curve shown through the experimental shifts of H-8 of $Zn(GTP)^{2-}$ was also calculated with $K_{av} = 1.9\ M^{-1}$ (Table II), using concentrations greater than 0.06 M; see text in section 5.

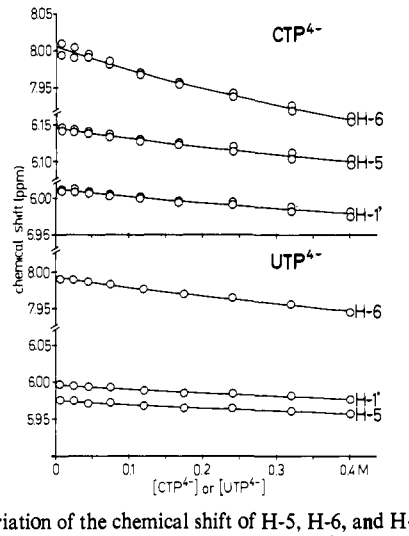


Figure 4. Variation of the chemical shift of H-5, H-6, and H-1′ of CTP^{4-} (pD 8.9; the points of two experimental series are shown) or UTP^{4-} (pD 8.4) with varying concentrations; for details see legend to Figure 1 (see also Table II).

is too insoluble and even in a saturated solution (~0.006 M) no significant stacking occurs. However, using the limiting shifts of either GTP^{4-} or the average of adenosine/inosine,[69] one can estimate the association constant ($K = 8 \pm 3\ M^{-1}$). The lower stacking ability of the pyrimidine moiety compared with the purine residue also becomes very clear-cut if the data for the nucleosides are compared (Table II). However, the association constants for cytidine and uridine are very similar.

Thus taking everything together it is evident that the association tendency of the base moieties of the nucleic acids decreases in the series adenine > guanine > hypoxanthine > cytosine ~ uracil.

4. Influence of Divalent Metal Ions on the Self-Association Tendency of Adenosine 5′-Triphosphate. Comparison of the association constant for $Mg(ATP)^{2-}$ with the corresponding value for uncomplexed ATP^{4-} clearly shows that the addition of Mg^{2+} to solutions of ATP^{4-} favors self-stacking (see Figure 1); K is increased by a factor of about 3 (cf. Table II). This promotion of the self-stacking tendency is certainly due to partial neutralization of the negative charge at the phosphate moiety by formation of a Mg^{2+} complex. Charge neutralization by Mg^{2+} also stabilizes stacked poly(adenylic acid).[70]

(69) Guanine and hypoxanthine have similar ring currents: Giessner-Prettre, C.; Pullman, B. *C. R. Hebd. Seances Acad. Sci.* **1965**, *261*, 2521–3.

(70) Dewey, T. G.; Turner, D. H. *Biochemistry* **1979**, *18*, 5757–62.

As the size of an upfield shift is dependent on the geometric arrangement of the stacks,[54] among several other factors, it is interesting to compare the upfield shifts $\Delta\delta$ of H-2 and H-1′ for the various ATP stacks listed in Table III. The values of $\Delta\delta$ for ATP^{4-} and $Mg(ATP)^{2-}$ are both rather large and also very similar to each other, thus confirming the expectation that stacking proceeds beyond dimers in both these cases.[44]

Zn^{2+} also clearly promotes the self-association, but the situation is more complicated with this metal ion. In case of $Zn(ATP)^{2-}$ only the shifts of H-2 and H-1′ behave "normally" (Figure 1), while the shifts of H-8 deviate downfield at low concentrations (<0.07 M). However, evaluation of the experimental data for H-2 and H-1′ with eq 3 leads to two values for the association constants of $Zn(ATP)^{2-}$ which are similar to each other, although apparently not identical (Table II).[71] The average constant, K = 11.1 M^{-1}, is significantly larger than the value obtained for $Mg(ATP)^{2-}$, K = 4.0 M^{-1}; this indicates that in $Zn(ATP)^{2-}$ an additional effect, besides charge neutralization, must be operating. The deviation of the shifts of H-8 at low concentrations confirms this and suggests that an additional equilibrium must be considered.

The situation with $Cd(ATP)^{2-}$ is similar and it is clear that Cd^{2+} promotes the self-association of ATP^{4-} at least as effectively as Zn^{2+}. H-8 of $Cd(ATP)^{2-}$ also deviates at lower concentrations (<0.07 M) although the deviation is smaller than with $Zn(ATP)^{2-}$ (see Figure 1). Moreover, the shifts of H-2 and H-1′ lead to quite different association constants (Table II). This again indicates that the indefinite noncooperative model used is inadequate: as with $Zn(ATP)^{2-}$, an additional effect must occur. It is also noteworthy that for $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$ the values of $\Delta\delta$ (Table III) are only about half the size of those for $Mg(ATP)^{2-}$.

The interaction between N-7 of adenine derivatives with metal ions such as Zn^{2+} or Cd^{2+} is well documented.[3,4] Such an interaction has already been shown to occur[72] in $Zn(ATP)^{2-}$ (see also section 6). It is therefore to be expected that an *inter*molecular interaction of this type will occur within the stacks, i.e., that the metal ion coordinates to the phosphate moiety of one ATP^{4-} and to N-7 of the adenine of the next—a conclusion which has independently been reached from a kinetic study[17] of the metal-ion-promoted dephosphorylation of ATP^{4-} which proceeds via dimer $[M(ATP)]_2^{4-}$ intermediates. Such an *inter*molecular interaction is expected to shift the resonance of H-8 downfield.

However, if the *inter*molecular interaction influenced only the chemical shift of the stacked species and did not also affect the association constant, the observed deviation of the shift of H-8 at low concentration cannot be explained. Likewise a metal ion/N-7 interaction in monomeric $Zn(ATP)^{2-}$ or $Cd(ATP)^{2-}$ would only influence δ_0, and thus also $\delta_0 - \delta_\infty$ (see eq 3), and again cannot account for the observed deviation.

This downfield deviation of the resonance of H-8 can be explained, however, if the association constant of dimeric $M_2(ATP)_2^{4-}$ is higher than the association constant for stacking between the dimeric units. The *inter*molecular metal ion/N-7 interaction would be expected to increase the dimerization constant and also simultaneously to cancel out the upfield shift, caused by stacking in the dimer, because coordination of a diamagnetic metal ion will deshield a neighboring proton and thus initiate a downfield shift.[72,73] The experimental data for H-8 of $Zn(ATP)^{2-}$ in Figure 1 indicate the variation of the chemical shift to be expected for the following model:

$$2Zn(ATP)^{2-} \rightleftharpoons Zn_2(ATP)_2^{4-} \quad (7)$$

$$K_D^* = [Zn_2(ATP)_2^{4-}]/[Zn(ATP)^{2-}]^2 \quad (8)$$

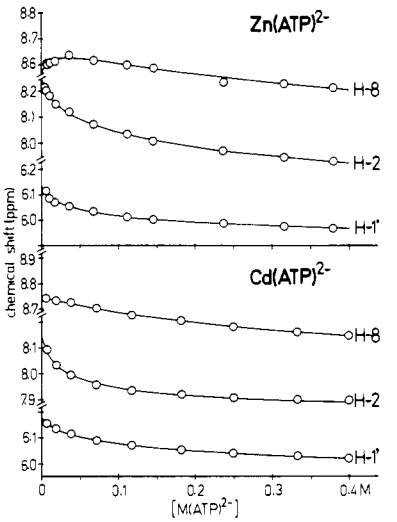


Figure 5. Variation of the chemical shift of H-2, H-8, and H-1′ of $Zn(ATP)^{2-}$ (upper part) and of $Cd(ATP)^{2-}$ (lower part) with varying concentration of $M(ATP)^{2-}$. The experimental data are those of Figure 1, where the experimental conditions are also given. The curves shown are the computer-calculated best fit of the experimental data, using the model given by eq 7–10 and the constants[74] K_{St} = 4 M^{-1} and K_D^* = 20 M^{-1} for both the $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$ systems. Although it proved impossible to perform a least-squares fit of the experimental data by allowing all parameters to vary, it is evident that the model used may at least qualitatively explain the experimental results (see text in section 4).

These *inter*molecular metal-ion-bridged dimers of eq 7 may stack with each other, as well as with monomeric $Zn(ATP)^{2-}$; the association constants for such nonbridged stacks are again expected to be equal. This may be expressed in a general form by eq 9.

$$Zn_2(ATP)_2^{4-} + Zn_n(ATP)_n^{2n-} \rightleftharpoons Zn_{2+n}(ATP)_{2+n}^{(4+2n)-} \quad (9)$$

$$K_{St} = [Zn_{2+n}(ATP)_{2+n}^{(4+2n)-}]/([Zn_2(ATP)_2^{4-}][Zn_n(ATP)_n^{2n-}]) \quad (10)$$

The results of a least-squares curve fit with the experimental data of $Zn(ATP)^{2-}$ and of $Cd(ATP)^{2-}$ of Figure 1 and the described model (eq 7–10) are given in Figure 5. However, due to the increased number of variable parameters (K_D^*, K_{St}, δ_0, δ_D^*, and δ_∞) and the scatter of the experimental results, it proved impossible to perform a least-squares fit of the experimental data using *all* parameters. Nevertheless, this model is able to explain at least qualitatively the initial *down*field shift of H-8 in $Zn(ATP)^{2-}$ followed by the usual upfield shift at higher concentrations. Several least-squares calculations indicated that the equilibrium constant for stacking, K_{St}, is about 4–5 M^{-1} and that the dimerization constant K_D^* must be within the range 10–50 M^{-1}, with a most-probable value between 20–30 M^{-1}, for both $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$ (cf.[74]).

The shift difference $\delta_0 - \delta_D^*$ was found to be negative for H-8 of $Zn(ATP)^{2-}$ (i.e., H-8 is shifted *down*field on dimerization) and approximately zero for H-8 of $Cd(ATP)^{2-}$, as was expected from the shapes of the curves of the variation of the shift of H-8 for $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$. Thus the curves plotted in Figure 5 are the least-squares fits calculated with K_{St} and K_D^* fixed at 4 and 20 M^{-1}, respectively.[74]

The smaller deviation observed for H-8 of $Cd(ATP)^{2-}$ (Figure 1) compared with H-8 of $Zn(ATP)^{2-}$ may also be explained. It

(71) It should be noted that the omission of a single experimental value (at 0.0061 M; see Figure 1) changes the individual constants drastically: for H-2 K = 9.90 ± 1.53 M^{-1} and for H-1′ K = 9.45 ± 1.23 M^{-1}, while the resulting value for K_{av}, 9.6 ± 0.8 M^{-1} (twice the standard error is given), is quite similar to the value given in Table II; its standard error is much smaller.

(72) Cohn, M.; Hughes, T. R., Jr. *J. Biol. Chem.* **1962**, *237*, 176–81.

(73) Sigel, H.; Scheller, K. H.; Rheinberger, V. M.; Fischer, B. E. *J. Chem. Soc., Dalton Trans.* **1980**, 1022–8.

(74) K_{St} = 4–5 M^{-1} is reasonable because for $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$ this constant should be of the order of K = 4.0 ± 0.5 M^{-1}, as was found for $Mg(ATP)^{2-}$ (Table II) using the noncooperative stacking model; in both cases only the charge effects of M^{2+} are operating. Moreover, by using K_{St} = 4 M^{-1} (i.e., the value of $Mg(ATP)^{2-}$; Table II) and K_D^* = 20 M^{-1} for the $Zn(ATP)^{2-}$ system, the variation of the proportions of $Zn(ATP)^{2-}$ present in the monomer, dimer, trimer, etc. have been calculated (see Figure 6); the concentrations of monomeric $Zn(ATP)^{2-}$ obtained with these values are very similar to the concentrations obtained by using K = 11.1 M^{-1} (noncooperative model) of Table II; this indicates again that the values of K_D^* and K_{St} are of the correct order.

Table IV. Importance of the M^{2+}/N-7 Interaction for the Self-Association of $M(NTP)^{2-}$ Complexes, as Judged from a Comparison of the Chemical Shifts (ppm) of H-8 in Stacked (δ_∞) Purine 5'-Triphosphates with the Corresponding Shifts of Purine 5'-Triphosphate-Metal Ion Complexes (27 °C, $I \simeq 2$)[a]

system	δ_∞ of H-8[b]	downfield shift, $\Delta\delta_\infty$[c]
adenosine	8.07 ± 0.04	
ATP^{4-}	7.92 ± 0.06	
$Mg(ATP)^{2-}$	8.02 ± 0.04	
$Zn(ATP)^{2-}$	8.36 ± 0.06	0.34 ± 0.10
$Cd(ATP)^{2-}$	8.49 ± 0.08	0.47 ± 0.12
inosine	8.06 ± 0.03	
ITP^{4-}	7.59 ± 0.60	
$Mg(ITP)^{2-}$	8.05 ± 0.08	
$Zn(ITP)^{2-}$	8.08 ± 0.14	~0
GTP^{4-}	7.70 ± 0.29	
$Zn(GTP)^{2-}$	7.81 ± 0.26	~0[d]

[a] The corresponding shifts of the nucleosides are given for comparison (27 °C, $I = 0.1$, $NaNO_3$). [b] Values from Table III. [c] Shift difference $\Delta\delta_\infty$ for H-8 between $Zn(NTP)^{2-}$ or $Cd(NTP)^{2-}$ and $Mg(NTP)^{2-}$. The errors given are the sum of the errors of the values used for the calculation of $\Delta\delta_\infty$. [d] The data of the systems for ATP^{4-} and ITP^{4-} with and without Mg^{2+} indicate that δ_∞ of GTP^{4-} may be used as a basis for the comparison with δ_∞ of $Zn(GTP)^{2-}$.

has already been suggested[4] that a metal ion/N-7 interaction occurs in monomeric $Zn(ATP)^{2-}$ and there are clear indications (see section 6 and Table V) that such an interaction is stronger in monomeric $Cd(ATP)^{2-}$. Therefore the *intra*molecular, concentration-independent equilibrium (vide infra: eq 11) of monomeric $M(ATP)^{2-}$ between an open (phosphate binding only) and a macrochelated form (simultaneous coordination of M^{2+} to the phosphate moiety and to N-7)[4] must also be considered when estimated the effect of dimer formation on the chemical shift of H-8. Owing to the increased extent of metal ion/N-7 interaction in monomeric $Cd(ATP)^{2-}$, the resonance of H-8 would be expected to change less on dimerization. Indeed, as we have seen, the model of eq 7–10 also fits the $Cd(ATP)^{2-}$ system (Figure 5, lower part).

As it is well-known that the coordination of a diamagnetic metal ion to a binding site deshields neighboring protons and therefore the resonance signal of such protons is shifted *down*field,[72,73] we have compiled the values of δ_∞ for H-8 on the different ATP-stacks in Table IV. If Zn^{2+} and Cd^{2+} coordinate in the dimeric stacks to N-7 forming the *inter*molecular metal ion bridge discussed earlier, this then should also affect the values of δ_∞ even though these were calculated for the noncooperative stacking model. Hence, δ_∞ of H-8 for $Zn(ATP)^{2-}$ should be shifted downfield compared to the shift (δ_∞) of H-8 for the $Mg(ATP)^{2-}$ stacks in which the effect of charge neutralization operates but practically no N-7 interaction should occur,[4,75,76] and this is indeed observed: The values of δ_∞ for ATP^{4-} and $Mg(ATP)^{2-}$ are the same within experimental error while the downfield shifts $\Delta\delta_\infty$ for the stacks of $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$, compared with $Mg(ATP)^{2-}$, are 0.34 and 0.47 ppm, respectively (Table IV), values which are of the expected order (see section 6). Hence, these data are also consistent with an *inter*molecular metal ion bridge.

5. Influence of Divalent Metal Ions on the Self-Association Tendency of the Other Nucleoside 5'-Triphosphates. As indicated in the introduction, one of the main aims of this work was to learn more about the influence of metal ions on the self-stacking tendency of nucleotides. Unfortunately, due to precipitation on the addition of divalent metal ion, only a limited number of complexes with ITP, GTP, CTP, and UTP could be studied (cf. footnote *a* in Table II). Nevertheless, several general conclusions can be drawn from these systems.

Comparison of the association constant for $Mg(ITP)^{2-}$ with the constant for uncomplexes ITP^{4-} (Table II) confirms the observations made with the corresponding ATP systems: the self-stacking tendency is promoted by a factor of about 5, which again is due to partial neutralization of the negative charge at the phosphate moiety by coordination of Mg^{2+}.

The shifts of H-8 in $Zn(ITP)^{2-}$ (Figure 2) and $Zn(GTP)^{2-}$ (Figure 3) also deviate somewhat at low concentrations, but the effects are much smaller than with $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$. It may thus be concluded that the interaction with N-7 is much less pronounced than with ATP; i.e., the enhancement of the stability of the dimers $Zn_2(ITP)_2^{4-}$ and $Zn_2(GTP)_2^{4-}$ is much less than that of $Zn_2(ATP)_2^{4-}$. Indeed, comparison of the association constant of $Mg(ITP)^{2-}$ with the corresponding apparent constant of $Zn(ITP)^{2-}$ (Table II) shows that they are identical within experimental error although the constant for $Zn(ITP)^{2-}$ may be slightly larger. The values of δ_∞ for H-8 given in Table IV also indicate that there is little or no *inter*molecule interaction in the stacks of $Zn(ITP)^{2-}$. Moreover, the size of the association constant of $Zn(GTP)^{2-}$ (Table II) can clearly be explained by a charge effect alone; there is thus also no evidence for an *inter*molecular stacking promotion by Zn^{2+} in $Zn(GTP)^{2-}$ stacks.[77] This overall picture is in excellent accordance with the Zn^{2+} promotion of the dephosphorylation of NTP^{4-} which decreases at pH 6.5 within the series ATP ≫ ITP ≳ GTP > CTP.[16]

Practically no data are available on the influence of metal ions on the self-association tendency of pyrimidine 5'-triphosphates. The Mg^{2+} systems of CTP^{4-} and UTP^{4-} could only be studied in the concentration range up to 0.1 M (Table II), and the association constants estimated for these limited data are the same within experimental error as those of the corresponding nucleotides alone. This is not surprising because the influence of charge neutralization is small in these systems: e.g., the association constants of cytidine and CTP^{4-} are 1.4 ± 0.5 and 0.5 ± 0.2 M^{-1}, respectively (Table II).

It appears that promotion of the self-stacking tendency of UTP^{4-} (and TTP^{4-}) by an *inter*molecular interaction with a metal ion is not very likely, as the base has only oxygen binding sites if N-3 is not deprotonated (cf. Chart I).[47,48] Moreover, it has been concluded[4] that "the pyrimidine rings of cytosine and uracil posses little aromaticity,[69,79,80] and their tendency to form mixed phosphate complexes [cf. ref 81–83] may be expected to be weaker than of the more aromatic purines" (see also sections 6 and 7). Hence an *inter*molecular promotion, as observed for the stacks of $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$, is unlikely to occur in the corresponding complexes with UTP^{4-}, (TTP^{4-}), and CTP^{4-}; however, owing to precipitation, this could not be confirmed experimentally.

6. Intramolecular Metal Ion/N-7 Interaction in Monomeric Nucleoside 5'-Triphosphate Complexes of Zn^{2+} and Cd^{2+}. The possibility that for certain metal ions an *intra*molecular macrochelate formation between the phosphate residue and the base moiety may occur has fascinated chemists for many years,[75,84] and several examples are now known.[4,47,48,85,86] We have seen that

(75) Schneider, P. W.; Brintzinger, H.; Erlenmeyer, H. *Helv. Chim. Acta* **1964**, *47*, 992–1002.
(76) Happe, J. A.; Morales, M. *J. Am. Chem. Soc.* **1966**, *88*, 2077–8.

(77) However N-7 of the hypoxanthine and guanine moieties *does* coordinate to metal ions (see also sections 6 and 7).[3,4,47] For example, the coordination tendency of guanosine to Cu^{2+} (log $K^{Cu}_{Cu(Gua)} = 2.15$) is even larger than that of adenosine (log $K^{Cu}_{Cu(Ade)} = 0.70$).[78] One explanation of the above results could therefore be that, due to the different substituents at the purine moiety in ITP and GTP, compared with ATP (Chart I), the orientation of the aromatic rings within the stacks may be somewhat different: thus in $Zn(ITP)^{2-}$ and $Zn(GTP)^{2-}$ coordination at N-7 of the neighboring ITP or GTP may be incompatible with the optimal stacking geometry, whereas in $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$ an interaction with N-7 and formation of a good stack are simultaneously possible. Another, less important, aspect is that *inter*molecular metal ion bridging in the dimers of $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$ may also occur to some extent via N-1 (the values for the chemical shifts given in Table III may indicate this); this type of interaction is, of course, impossible with $M(ITP)^{2-}$ and $M(GTP)^{2-}$.
(78) Fiskin, A. M.; Beer, M. *Biochemistry* **1965**, *4*, 1289–94.
(79) Schweizer, M. P.; Chan, S. I.; and Ts'o, P. O. P. *J. Am. Chem. Soc.* **1965**, *87*, 5241–7.
(80) Giessner-Prettre, C.; Pullman, B. *J. Theor. Biol.* **1970**, *27*, 87–95.
(81) Sigel, H. *Met. Ions Biol. Syst.* **1973**, *2*, 63–125.
(82) Sigel, H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 394–402.
(83) Sigel, H.; Fischer, B. E.; Prijs, B. *J. Am. Chem. Soc.* **1977**, *99*, 4489–96.
(84) Szent-Györgyi, A. In "Enzymes: Units of Biological Structure and Function", Gaebler, O. H., Ed.; Academic Press: New York, 1956; p 393.

Table V. Evidence for a Zn^{2+} or Cd^{2+}/N-7 Interaction in $M(NTP)^{2-}$ Complexes, from a Comparison of the Chemical Shifts (δ_0, ppm) of H-8 for Monomeric Purine 5'-Triphosphates with the Corresponding Shifts of Monomeric Purine 5'-Triphosphate-Metal Ion Complexes[a]

system	nucleotide δ_0 of H-8	downfield shift,[b] $\Delta\delta_0$	downfield shift, $\Delta\delta'$, for the corresponding nucleoside system[c]	estimated % $M(NTP)^{2-}_{cl}$ (eq 11, 12)
ATP^{4-}	8.563 ± 0.004[d]			
$Mg(ATP)^{2-}$	8.527 ± 0.009[d]			0
$Zn(ATP)^{2-}$	8.61[e,f]	0.08	~0.4[g]	~20
$Cd(ATP)^{2-}$	8.74[e,f]	0.21	0.39	54
ITP^{4-}	8.511 ± 0.006[d]			
$Mg(ITP)^{2-}$	8.506 ± 0.006[d]			0
$Zn(ITP)^{2-}$	8.72[e,h]	0.21	0.44	48
$Cd(ITP)^{2-}$	8.79[e,i]	0.28	0.29	97
GTP^{4-}	8.164 ± 0.010[d]			
$Mg(GTP)^{2-}$	8.15[e,i]			0
$Zn(GTP)^{2-}$	8.47[e,j]	0.32	0.36	89
$Cd(GTP)^{2-}$	8.48[e,i]	0.33	0.27	~100
CTP^{4-}	6.146 ± 0.002[d,k]			
$Mg(CTP)^{2-}$	6.131 ± 0.004[d,k]			0
$Zn(CTP)^{2-}$	6.14[e,i,k]	0		~0
$Cd(CTP)^{2-}$	6.14[e,i,k]	0	*l*	~0
UTP^{4-}	5.975 ± 0.002[d,k]			
$Mg(UTP)^{2-}$	5.976 ± 0.004[d,k]			0
$Zn(UTP)^{2-}$	5.98[e,i,k]	0		0
$Cd(UTP)^{2-}$	5.98[e,i,k]	0		0

[a] The corresponding data (δ_0 of H-5) for the pyrimidine 5'-triphosphates are given for comparison (27 °C, $I = 0.1$, $NaNO_3$). The percentage of the macrochelated isomers, $M(NTP)^{2-}_{cl}$, is estimated from the downfield shifts $\Delta\delta'$ observed for H-8 in the corresponding nucleoside/metal ion systems. [b] Shift difference $\Delta\delta_0$ for H-8 (or H-5) between $Cd(NTP)^{2-}$ or $Zn(NTP)^{2-}$ and $Mg(NTP)^{2-}$. [c] Downfield shifts for H-8 from Table VI: $\Delta\delta' = \delta_\infty' - \delta_0'$. With these $\Delta\delta'$ values and $\Delta\delta_0$, the percentage of $M(NTP)^{2-}_{cl}$ may now be calculated [% closed isomer = $(\Delta\delta_0/\Delta\delta') \times 100$]; this then allows calculation of the intramolecular equilibrium constant K_I (eq 12). [d] Value from Table III. [e] Graphically extrapolated; the estimated error is ±0.01 ppm. [f] See Figure 1. [g] Estimation based on the Cd^{2+}/Ade system (see Table VI); the downfield shifts $\Delta\delta_\infty$ of Table IV are of the same order. [h] See Figure 2. [i] These were determined from dilute solutions (~0.002–0.005 M); see also footnote *a* of Table II. [j] See Figure 3. [k] δ_0 of H-5. [l] Even though there is no indication for a metal ion interaction with the cytosine moiety in $M(CTP)^{2-}$, $M(Cyt)^{2+}$ complexes exist.[87,88]

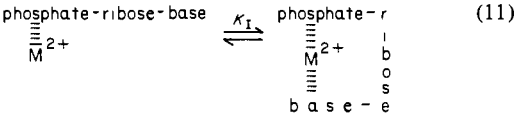
the $Mg(ATP)^{2-}$ and $Mg(ITP)^{2-}$ complexes behave "normally"; i.e., the shifts of H-2, H-8, and H-1′ vary continuously with increasing concentration (Figures 1 and 2) and, for each complex, curve fitting of all three protons results in the same value of the association constants (Table II). The Zn^{2+} complexes of ATP^{4-}, ITP^{4-}, and GTP^{4-}, and also of $Cd(ATP)^{2-}$, behave differently: the shifts of H-8 deviate at low complex concentration from the curve expected on the basis of the shifts of H-2 and H-1′ (Figures 1–3).

In order to learn more about the extent of *intra*molecular chelate formation in these $M(NTP)^{2-}$ complexes, we have extrapolated the shifts of H-8 to infinite dilution (δ_0); the shifts of H-8 in the monomeric $M(NTP)^{2-}$ complexes are thus obtained. This was also done for the shifts of H-5 of the pyrimidine systems. These values are listed in Table V together with the corresponding values of δ_0 for the uncomplexed nucleoside 5′-triphosphates. Even the δ_0 values of those systems which formed precipitates at higher concentrations (Table II) were obtained from measurements in diluted solutions and are also given in Table V.

It is clear from these data in Table V that the shifts of NTP^{4-} and $Mg(NTP)^{2-}$ are rather similar, and no downfield shift can be observed for any of the Mg^{2+} complexes. This is in accordance with the low coordination tendency of Mg^{2+} toward N-7 of the adenine residue.[4,75,85,86] which has also been confirmed by an ^{15}N NMR study.[76]

Moreover, an *intra*molecular macrochelate formation in $Zn(CTP)^{2-}$, $Cd(CTP)^{2-}$, $Zn(UTP)^{2-}$, and $Cd(UTP)^{2-}$ appeared rather unlikely, as, so far, all evidence[4,48,85,86] is against it, including our results of Table V. Indeed it has already been concluded[86] from a comparison of the properties of $Ni(CTP)^{2-}$ and $Ni(ATP)^{2-}$ complexes that macrochelate formation occurs only rarely, if at all, in cytosine nucleotides. However, it must be pointed out that the N-3 atom of cytidine is well able to form complexes[87] in contrast to N-3 or uridine (as long as it is not deprotonated),[47] and, therefore, at least for $M(CTP)^{2-}$, it is *not* a reduced coordination ability of the cytosine residue which is responsible for the lack of macrochelate formation.[88]

However, for all purine nucleotide complexes with Zn^{2+} or Cd^{2+}, i.e., $Zn(ATP)^{2-}$, $Cd(ATP)^{2-}$, $Zn(ITP)^{2-}$, $Cd(ITP)^{2-}$, $Zn(GTP)^{2-}$, and $Cd(GTP)^{2-}$, there is clearly a downfield shift of H-8 ($\Delta\delta_0$, Table V). This provides convincing evidence that in these unstacked complexes an *intra*molecular metal ion/N-7 interaction occurs, and that an intramolecular, and therefore concentration independent, equilibrium between an "open" isomer, $M(NTP)^{2-}_{op}$, and a "closed" species, $M(NTP)^{2-}_{cl}$, exists: In order to learn



(11)

$$K_I = [M(NTP)^{2-}_{cl}]/[M(NTP)^{2-}_{op}] \quad (12)$$

(85) Frey, C. M.; Stuehr, J. E. *J. Am. Chem. Soc.* **1972**, *94*, 8898–904.
(86) Mariam, Y. H.; Martin, R. B. *Inorg. Chim. Acta* **1979**, *35*, 23–8.

(87) For example, the Cu^{2+} complex of cytidine ($\log K^{Cu}_{Cu(Cyt)} = 1.59$)[78] is even more stable than the one with adenosine ($\log K^{Cu}_{Cu(Ade)} = 0.70$),[78] and $Ni(Cyt)^{2+}$ is also known to exist.[86] In addition we observed in preliminary measurements that the ^1H NMR resonances of H-5, H-6, and H-1′ of cytidine (0.05 M in H_2O; pH 5.75; 34 °C; $I = 0.1$, $NaNO_3$; measured on a Varian Anaspect EM-360 spectrometer) are shifted downfield by 0.131, 0.036, and 0.039 ppm, respectively, in the presence of Cd^{2+} (1.67 M; $I = 5$). The downfield shift of H-5 is quite large and clearly indicates that a $Cd(Cyt)^{2+}$ complex is formed.

(88) This observation may possibly be rationalized by the conformation which nucleotides adopt in aqueous solution, i.e., by the relative orientation of the base and sugar rings about the N-1/C-1′ bond in pyrimidines and about the N-9/C-1′ bond in purines.[4] Usually two main conformations can occur—syn and anti; the latter is favored in aqueous solution. In this anti conformation the N-1/C-6 bond of pyrimidines and the N-9/C-8 bond of purines project onto or near the sugar ring.[4] This explains the observations of Table V ($\Delta\delta_0$ values) in a simple manner because in the anti conformation, N-3 of pyrimidine nucleotides is directed away from the phosphate moiety while in purine nucleotides N-7 is orientated toward the phosphate residue.

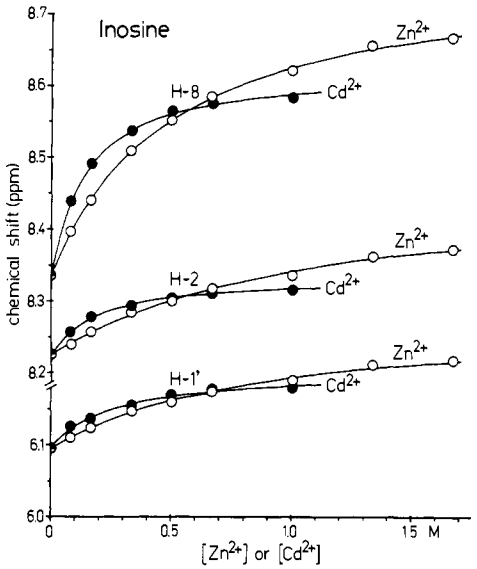


Figure 6. Variation of the chemical shift of H-2, H-8, and H-1′ of inosine (0.01 M) with increasing concentrations of Zn^{2+} (○) or Cd^{2+} (●). The spectra were measured on a Bruker FT 90 at 90.025 MHz (D_2O, pD 4.8, 27 °C, I = 0.1–5, $NaNO_3$) relative to internal $(CH_3)_4N^+$ and converted to values relative to sodium (trimethylsilyl)propanesulfonate by adding 3.188 ppm. The curves shown are the computer-calculated best fit of the experimental data, using eq 1–6 of ref 73 (cf. also ref 89).

more about the position of equilibrium 11, we have tried to assess a value for the dimensionless equilibrium constant K_I (eq 12) by determining the downfield shift for complete complexation of Zn^{2+} or Cd^{2+} at N-7. Measurement of the shift of H-8 of adenosine (0.005 M), inosine (0.01 M), and guanosine (0.004 M) in dependence on the concentration of zinc or cadmium hydrogentriphosphates failed, due to precipitation. Therefore we carried out the experiments with Zn^{2+} and Cd^{2+} alone. Although hydrogentriphosphate increases the stability of the $Ni(Ade)^{2+}$ complex,[86] we expect the extent of the downfield shift of H-8 for complete complexation to be nearly independent of the presence of hydrogentriphosphate, and the stability constants (eq 14) for equilibrium 13 are less important in the present connection.

$$M^{2+} + \text{nucleoside} \rightleftharpoons M(\text{nucleoside})^{2+} \qquad (13)$$

$$K^{M}_{M(Ns)} = [M(Ns)^{2+}]/([M^{2+}][Ns]) \qquad (14)$$

The variation of the downfield shifts of H-2, H-8, and H-1′ as a function of increasing concentration of Zn^{2+} or Cd^{2+} is shown, as an example, for inosine in Figure 6. Computer-calculated least-squares fits of the variation of the downfield shifts of each of the protons with increasing metal ion concentration gave the stability constant and, more important, the downfield shift (δ_∞') for complete complexation. The results are summarized in Table VI for the purine nucleoside systems.

It should be mentioned that the ionic strength in these experiments varied from 0.1 to 5 M, but curve fitting without the data at high ionic strength gave the same results within experimental error, although the stability constants (K', Table VI) obtained from the shifts of H-2 and H-1′ increased then somewhat and became more equal to those obtained from the shifts of H-8.[90] The stability constants $K^{M}_{M(Ns)}$ (eq 14) given in Table VI for equilibrium 13 are reasonable estimates and compare favorably with values available in the literature: $\log K^{Ni}_{Ni(Ade)} = -0.15$ (cf. ref 75) or 0.3 (cf. ref 86 and 91), $\log K^{Cu}_{Cu(Ade)} = 0.70$ (cf. ref 78) or 0.84 (cf. ref 75), $\log K^{Zn}_{Zn(Ade)} = -0.28$ (cf. ref 75), and

Table VI. Chemical Shifts (ppm) for Several Nucleoside/Metal Ion Systems, Together with the Stability Constants (K', M^{-1}) Calculated from the Shift Data (e.g., Figure 6) of each Individual Proton (See Text) and an Estimate for the Stability Constant, $\log K^{M}_{M(Ns)}$ (eq 13, 14), of the M(nucleoside)$^{2+}$ Complexes[a] in D_2O at 27 °C

system	H-2			H-8			H-1′			$\log K^{M}_{M(Ns)}$[b] (eq 14)
	δ_0'	δ_∞'	K'	δ_0'	δ_∞'	K'	δ_0'	δ_∞'	K'	
$Ade^{c,d}/Zn^{2+}$	8.245			8.332		0.47[e]	6.071			−0.3 ± 0.2[f]
$Ade^{c,g}/Cd^{2+}$	8.246 ± 0.002	8.536 ± 0.042	0.38 ± 0.08	8.332 ± 0.002	8.723 ± 0.018	0.77 ± 0.08	6.072 ± 0.004	6.363 ± 0.072	0.34 ± 0.12	−0.11 ± 0.06
$Ino^{g,h}/Zn^{2+}$	8.224 ± 0.005	8.468 ± 0.029	0.93 ± 0.24	8.333 ± 0.005	8.771 ± 0.012	2.02 ± 0.18	6.093 ± 0.006	6.281 ± 0.024	1.18 ± 0.36	0.31 ± 0.06
$Ino^{h,i}/Cd^{2+}$	8.221 ± 0.004	8.335 ± 0.006	5.79 ± 1.24	8.334 ± 0.007	8.624 ± 0.009	7.29 ± 0.96	6.094 ± 0.006	6.205 ± 0.011	4.03 ± 1.36	0.86 ± 0.09
$Gua^{d,h}/Zn^{2+}$				8.002 ± 0.006	8.362 ± 0.007	6.37 ± 0.54	5.916 ± 0.011	6.047 ± 0.027	2.46 ± 1.54	0.80 ± 0.06
$Gua^{d,h}/Cd^{2+}$				8.013 ± 0.006	8.280 ± 0.004	14.82 ± 1.43	5.916 ± 0.008	6.022 ± 0.010	5.20 ± 1.07	1.17 ± 0.06

[a] The errors are *twice* the standard deviation.[b] [b] The values of $\log K^{M}_{M(Ns)}$ are those obtained from the shifts of H-8, as we feel that this is the best measure of the interaction with N-7. The error given is *three* times the standard deviation. For $Cd(Cyt)^{2+}$ see footnote 87. [c] pD 5.75. [d] I = 0.1–4 ($NaNO_3$). [e] This value was calculated by using a shift difference $\Delta\delta' = \delta_\infty' - \delta_0'$ of 0.4 ppm, i.e., the value of the $Cd(Ade)^{2+}$ complex. [f] This error range covers $\Delta\delta'$ values between 0.29 and 0.54 ppm. [g] I = 0.1–5 ($NaNO_3$). [h] pD 4.8. [i] I = 0.1–3 ($NaNO_3$).

(89) Fischer, B. E.; Sigel, H. *J. Am. Chem. Soc.* **1980**, *102*, 2998–3008.
(90) The shifts of H-2 and H-1′ are influenced not only by the metal ion coordination at N-7 but also by a competing coordination at N-1 (or O-6) and at the hydroxy groups of the ribose moiety, especially at high concentrations of metal ion.
(91) Taylor, R. S.; Diebler, H. *Bioinorg. Chem.* **1976**, *6*, 247–64.

log $K^{Cu}_{Cu(Gua)}$ = 2.15 (cf. ref 78).

The position of the intramolecular equilibrium 11 can be estimated from the downfield shifts, $\Delta\delta_0$, observed for H-8 of the monomeric purine nucleotide complexes of Zn^{2+} or Cd^{2+} (Table V, column 3) and the values obtained for complete complexation ($\Delta\delta'$) at N-7 (Table VI and column 4 of Table V) from the experiments with the corresponding nucleosides. The resulting estimates of the percentages of the "closed" isomers, $M(NTP)^{2-}_{cl}$, are given in Table V. It is evident that both the "open" and the "closed" isomers occur in appreciable concentrations. The concentration of the "closed" isomer is also somewhat larger for the $Cd(NTP)^{2-}$ complexes, in agreement with the slightly larger stability observed for the Cd(nucleoside)$^{2+}$ complexes (Table VI).

The macrochelated isomers of $Zn(ITP)^{2-}$, $Cd(ITP)^{2-}$, $Zn(GTP)^{2-}$, and $Cd(GTP)^{2-}$ are formed by coordination of the metal ion to the phosphate moiety and to a single position at the base,[4] namely, mainly to N-7,[92] just as in $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$. In $Cd(Gua)^{2+}$ O-6 coordination is expected to be somewhat more frequent than in $Cd(GTP)^{2-}$ because mixed-ligand complex formation [81-83,94] and the steric conditions (phosphate–base distance) favor N-7 coordination in $Cd(GTP)^{2-}$. Thus $\Delta\delta_0$ of $Cd(GTP)^{2-}$ is slightly larger (0.33 ppm) than the $\Delta\delta'$ value of $Cd(Gua)^{2+}$ (0.27 ppm), which was used as a basis for the evaluation of the position of the isomeric equilibrium 11 for $Cd(GTP)^{2-}$ (see footnote *b* of Table V). This also means that the estimated percentages given in Table V for the "closed" isomers of $M(ITP)^{2-}$ and $M(GTP)^{2-}$ are possibly slightly too large.

7. Comparison of the Extent of Macrochelate Formation in Monomeric Nucleoside 5′-Triphosphate Complexes. Even though the position of the intramolecular equilibrium 11 could be estimated only for the Zn^{2+} and Cd^{2+} complexes of the purine 5′-triphosphates, the results described in section 6 clearly show that both the "open" isomers exist in aqueous solution. This provides a solid basis for the additional estimation of the position of equilibrium 11 for nucleoside 5′-triphosphate complexes from stability data which were determined earlier[48] by potentiometric pH titrations under conditions ([NTP] = 6×10^{-4} M) in which no significant self-association occurs.

Mariam and Martin[86] have recently shown that the intramolecular equilibrium constant K_I (eq 11 and 12) can be deduced from the experimentally accessible overall stability constant, $K^{M}_{M(NTP)}$ (eq 15), using eq 16 (cf. ref 73):

$$K^{M}_{M(NTP)} = [M(NTP)^{2-}]/([M^{2+}][NTP^{4-}]) \quad (15)$$

$$K_I = \left(K^{M}_{M(NTP)}/K^{M}_{M(NTP)_{op}}\right) - 1 \quad (16)$$

$K^{M}_{M(NTP)_{op}}$ is the stability constant of the "open" isomer $M(NTP)^{2-}_{op}$ (eq 11 and 12). The accuracy of the logarithm of the ratio from eq 16

$$\log \Delta = \log K^{M}_{M(NTP)} - \log K^{M}_{M(NTP)_{op}} \quad (17)$$

depends very much on the experimental error of the constants and this error becomes more important the more similar the two constants are. This means that the values calculated for K_I can only be considered as estimates. Moreover, $K^{M}_{M(NTP)_{op}}$ is usually not directly accessible by experimental determinations; in the present case the stability constants determined for pyrimidine nucleotide complexes (which exist only in the "open" form as we have seen in section 6) provide estimates for $K^{M}_{M(NTP)_{op}}$, which may therefore be compared (eq 17) with the stability constants determined for purine nucleotide complexes. On this basis the intramolecular equilibrium constant K_I (eq 11 and 12) and the percentage of the "closed" isomer $M(NTP)^{2-}_{cl}$ may be estimated for purine 5′-triphosphate complexes; the results are listed in Table VII.

The values of Table VII confirm the macrochelation for $Zn(ATP)^{2-}$, $Zn(ITP)^{2-}$, and $Zn(GTP)^{2-}$, which was already proved to occur in section 6, and they give evidence for a "closed" isomer in the purine nucleotide complexes with Co^{2+}, Ni^{2+}, or Cu^{2+} and to a lesser extent also in the Mn^{2+} complexes. There is no indication for a base–metal ion interaction in $Mg(ITP)^{2-}$, $Mg(GTP)^{2-}$, $Ca(ITP)^{2-}$, and $Ca(GTP)^{2-}$ (in accordance with the NMR results; section 6). However, the percentage of the "closed" isomers is apparently throughout somewhat larger for the $M(ATP)^{2-}$ species than for the $M(ITP)^{2-}$ and $M(GTP)^{2-}$ complexes.[95]

All available data on the extent of the macrochelate formation in purine 5′-triphosphate complexes are summarized in Table VIII. Considering the crudeness of the available methods and the different assumptions inherent in the evaluation of the different experimental determinations, the agreement of the data is amazingly good, and the existence of equilibrium 11 must now be accepted as a reality in nucleotide complex chemistry. Indeed, a third isomer has recently been suggested by Mariam and Martin:[86] an isomer with the metal ion both outer-sphere coordinated to the adenine moiety and inner-sphere coordinated to the phosphate residue (see also footnote 95 and footnote *c* of Table VIII). It can be seen from Table VIII that the percentages of the "closed" isomer calculated for the $M(ATP)^{2-}$ complexes from the potentiometrically determined stability constants are apparently always somewhat larger than the percentages based on spectrophotometric or NMR measurements. This could be taken to indicate the presence of such an isomer,[95] in which the interaction of the metal ion with the base moiety via a water molecule would probably not show up in spectrophotometric[86] or in ^1H NMR shift experiments.

There is one further aspect which must be considered in this connection: ITP^{4-} and GTP^{4-} or UTP^{4-} and TTP^{4-} carry a proton at the base position N-1 or N-3, respectively (Chart I), which is ionized at higher pH values ($pK_A \sim 9.7$)[47] and this then leads to an increased coordination tendency of the corresponding base moieties, which results in a depression of the pK_A values. We have therefore used the stability constants determined earlier[47] in 8×10^{-4} M solutions (with no significant self-association) by potentiometric pH titrations to calculate the extent of macrochelate formation, which should be favored by this deprotonation. From the results summarized in Table IX it is evident that the formation of macrochelates is indeed more pronounced: base deprotonation clearly favors the base–metal ion interaction (cf. with Tables VII and VIII). Moreover, under conditions with ionized N-3 $(UTP-H)^{5-}$ and $(TTP-H)^{5-}$ are now also able to undergo macrochelate formation, at least with metal ions such as Zn^{2+}. The exact site of metal ion coordination to the base cannot be identified unequivocally at present: possibilities are the N-1, O-6, or N-7 sites for purines and the N-3 or O-4 sites for pyrimidines.[47] However, some metal ions (e.g., Cu^{2+} and Zn^{2+}) form the base-

(92) Another possibility is that macrochelation occurs independently to O-6: e.g., it has been shown[93] that about 11% of $Mn(ITP)^{2-}$ exists as a closed isomer and that Mn^{2+} interacts independently with N-7 and O-6 of the hypoxanthine moiety (i.e., Mn^{2+} cannot bind simultaneously to N-7 and O-6). This agrees with the conclusion[4] that "not only is conclusive evidence for direct chelation between N-7 and O-6 lacking, but the weight of evidence indicates that it does not occur to an appreciable extent".

(93) Kuntz, G. P. P.; Kotowycz, G. *Biochemistry* **1975**, *14*, 4144–50.

(94) The combination of a heteroaromatic N ligand and an O donor (like a purine and a phosphate residue) favors the formation of mixed-ligand complexes of Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}.[81-83]

(95) The values obtained for $Mg(ATP)^{2-}$ and $Ca(ATP)^{2-}$ (nos. **4** and 10 in Table VII) appear anomalous, as spectrophotometric studies[86] and our own ^1H NMR shift measurements (see section 6) give no indication for a Mg^{2+}/N-7 interaction. However, Frey and Stuehr[85] also found (15 °C; I = 0.1) a similar, albeit smaller difference, $\log \Delta = \log K^{Mg}_{Mg(ATP)} - \log K^{Mg}_{Mg(CTP)} = 4.05 - 3.97 = 0.08$ (corrected for the difference in basicity as described in footnote *a* of Table VII), which corresponds to 17% of the "closed" isomer. An explanation for these apparently contradictory results, if the stability differences are indeed real, would be to assume that Mg^{2+} (and Ca^{2+}) is *not* coordinated *directly* to N-7 of the adenine moiety but that a water molecule is in between; such a species has recently been suggested for $Ni(ATP)^{2-}$ by Mariam and Martin.[86] An interaction with the base moiety via a water molecule would not show up in spectrophotometric experiments[86] and would be difficult to detect by ^1H NMR shift experiments.

(96) Lam, Y.-F.; Kuntz, G. P. P.; Kotowycz, G. *J. Am. Chem. Soc.* **1974**, *96*, 1834–9.

Table VII. Evidence for Intramolecular Macrochelate Formation in Metal Ion Complexes of Purine 5′-Triphosphates: Estimations of the Intramolecular Dimensionless Equilibrium Constant K_I and of the Percentage of the "Closed" Isomer $M(NTP)^{2-}_{cl}$ ($I = 0.1$, $NaClO_4$, 25 °C)

no.	complex	$\log K^{M}_{M(NTP)}$[a] (eq 15)	$\log K^{M}_{M(NTP)_{op}}$	log Δ (eq 17)	K_I (eq 12, 16)	estd % $M(NTP)^{2-}_{cl}$ (eq 11)
1	$Mg(CTP)^{2-}$	4.02				
2	$Mg(UTP)^{2-}$	4.00	4.05[b]			
3	$Mg(TTP)^{2-}$	4.13				
4	$Mg(ATP)^{2-}$	4.27	4.05	(0.22)	(0.66)[c]	(40)[c]
5	$Mg(ITP)^{2-}$	4.08	4.05	0[d]	~0	~0
6	$Mg(GTP)^{2-}$	4.13	4.05	0[d]	~0	~0
7	$Ca(CTP)^{2-}$	3.66				
8	$Ca(UTP)^{2-}$	3.66	3.68[b]			
9	$Ca(TTP)^{2-}$	3.73				
10	$Ca(ATP)^{2-}$	3.91	3.68	(0.23)	(0.70)[c]	(41)[c]
11	$Ca(ITP)^{2-}$	3.73	3.68	0[d]	~0	~0
12	$Ca(GTP)^{2-}$	3.73	3.68	0[d]	~0	~0
13	$Mn(CTP)^{2-}$	4.68				
14	$Mn(UTP)^{2-}$	4.58	4.63[b]			
15	$Mn(ATP)^{2-}$	4.84[e]	4.63	0.21	0.62	38
16	$Mn(ITP)^{2-}$	4.66	4.63	0.03	~0.07	~7
17	$Mn(GTP)^{2-}$	4.64	4.63	0[d]	~0	≲20[d]
18	$Co(UTP)^{2-}$	4.53	4.53[b]			
19	$Co(ATP)^{2-}$	4.89	4.53	0.36	1.3	57
20	$Co(ITP)^{2-}$	4.81	4.53	0.28	0.91	48
21	$Ni(UTP)^{2-}$	4.29	4.29[b]			
22	$Ni(ATP)^{2-}$	4.88	4.29	0.59[f]	2.9	74[f]
23	$Ni(ITP)^{2-}$	4.73	4.29	0.44	1.8	64
24	$Cu(CTP)^{2-}$	5.68				
25	$Cu(UTP)^{2-}$	5.53	5.62[b]			
26	$Cu(TTP)^{2-}$	5.65				
27	$Cu(ATP)^{2-}$	6.24[e]	5.62	0.62	3.2	76
28	$Cu(ITP)^{2-}$	5.99	5.62	0.37	1.3	57
29	$Cu(GTP)^{2-}$	5.93	5.62	0.31	1.0	50
30	$Zn(CTP)^{2-}$	4.73				
31	$Zn(UTP)^{2-}$	4.75	4.77[b]			
32	$Zn(TTP)^{2-}$	4.84				
33	$Zn(ATP)^{2-}$	5.19[e]	4.77	0.42	1.6	62
34	$Zn(ITP)^{2-}$	5.02	4.77	0.25	0.78	44
35	$Zn(GTP)^{2-}$	4.96	4.77	0.19	0.55	35

[a] These data were determined by potentiometric pH titrations.[48] The acidity constants of $H(ITP)^{3-}$, $H(GTP)^{3-}$, and $H(UTP)^{3-}$ are identical ($pK^{H}_{H(NTP)} = 6.45$), while those of the other nucleotides differ slightly: $pK^{H}_{H(ATP)} = 6.42$, $pK^{H}_{H(CTP)} = 6.51$, $pK^{H}_{H(TTP)} = 6.50$. Therefore all the constants given above were "normalized" to $pK_A = 6.45$ by adding 0.03 log units to the constants listed for $M(ATP)^{2-}$ in ref 48; those of $M(CTP)^{2-}$ and $M(TTP)^{2-}$ were reduced by 0.06 and 0.05 log units, respectively. [b] The constants of the pyrimidine 5′-triphosphate complexes are considered as reasonable estimates for $\log K^{M}_{M(NTP)_{op}}$ because in these complexes no macrochelate formation occurs (see text and section 6). [c] See footnote 95. [d] The constants of the pyrimidine 5′-triphosphate complexes are usually identical within 0.1 log unit; therefore only differences ≥0.1 log unit are listed with the exception of no. 16 because there is already NMR evidence[93] for an intramolecular Mn^{2+}/base interaction for $Mn(ITP)^{2-}$; log Δ = 0.1 corresponds to about 20% of the "closed" isomer. [e] This is the average of the two values given for this constant in ref 48. [f] Using the results obtained by Frey and Stuehr[85] log Δ can be calculated: $\log \Delta = \log K^{Ni}_{Ni(ATP)} - \log K^{Ni}_{Ni(CTP)} = 4.79 - 4.35$ (corrected for the difference in basicity)[a] = 0.44; i.e., $K_I = 1.8$ and $Ni(ATP)^{2-}_{cl} = 64\%$.

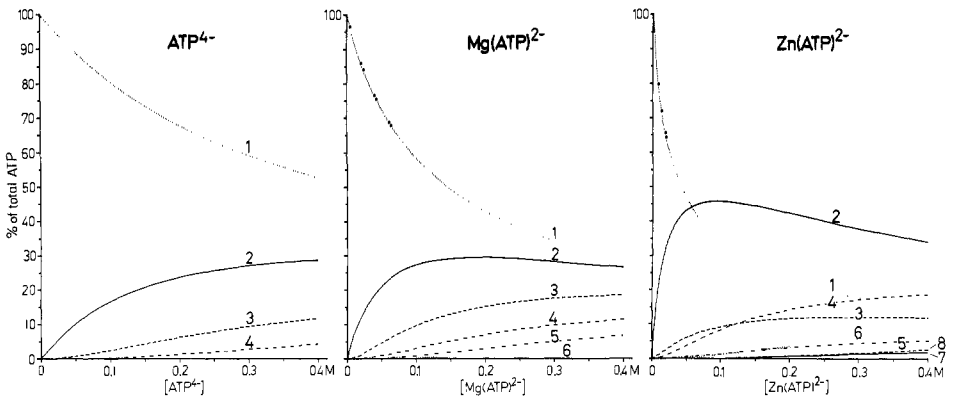


Figure 7. Variation of the proportions of ATP present in the monomer (1), dimer (2), trimer (3), ..., and octamer (8) in D_2O solutions in dependence on the total concentratoin of ATP^{4-} ($K = 1.3\ M^{-1}$), $Mg(ATP)^{2-}$ ($K = 4.0\ M^{-1}$), and $Zn(ATP)^{2-}$ ($K_D^* = 20\ M^{-1}$ and $K_{St} = 4\ M^{-1}$; see text in section 4 and footnote 74) at 27 °C, $I = 0.1$ to ~2 ($NaNO_3$) in D_2O.

ionized $M(NTP-H)^{3-}$ complexes even in the physiological pH range;[47,48] therefore the formation of these complex structures might be biologically meaningful.

General Conclusions

The present results show that the self-stacking tendency decreases within the series adenosine > guanosine > inosine >

Table VIII, Comparison of the Available Data on the Percentage of the "Closed" Isomers, $M(NTP)^{2-}_{cl}$, of Purine 5'-Triphosphate Complexes, i.e., on the Percentage of the Macrochelated Form (eq 11)[a]

	ATP^{4-}			ITP^{4-}		GTP^{4-}	
M^{2+}	spect[a]	NMR	stab[a]	NMR	stab[a]	NMR	stab[a]
Mg^{2+}	1-3[b]/0[c]	0[d]	*e*	0[d]	0	0[d]	0
Ca^{2+}	1-3[b]		*e*		0		0
Mn^{2+}	3[b]/10[c]	20[f]	38	11[g]	~7		~0
Co^{2+}	12[b]/35[c]		57		47		
Ni^{2+}	20[b]/30[c]		64[h]/74		74		
Cu^{2+}	80[b]		76		57		50
Zn^{2+}	15[b]	~20[d]	62	48[d]	44	89[d]	36
Cd^{2+}		54[d]		97[d]		~100[d]	

[a] If no reference is given, the data are those of Table VII; $I = 0.1$ ($NaClO_4$), 25 °C. Abbreviations: spect, spectrophotometric measurements; stab, from stability constants of potentiometric titrations (Table VII). [b] Probably at room temperature and natural ionic strength; ref 75. [c] 3 M $NaClO_4$, 23 °C; ref 86 (cf. also ref 4). There is evidence[86] that a third isomer of $Ni(ATP)^{2-}$ (about 30%) with a water molecule between N-7 and Ni^{2+} exists. [d] Cf. Table V; D_2O, $I = 0.1$ ($NaNO_3$), 27 °C. [e] See Table VII and footnote 95. [f] Results of a ^{13}C NMR relaxation study, 27 °C; ref 96. [g] NMR relaxation study, 27 °C; ref 93. Mn^{2+} interacts at two distinct sites on the inosine residue, namely, at N-7 and O-6; cf. also footnote 92. [h] See footnote *f* of Table VII.

cytidine ~ uridine and that this tendency is much less pronounced in the corresponding 5'-triphosphates. The stacking tendency of TTP^{4-} (Chart I) is probably similar to that of CTP^{4-} and UTP^{4-}.

Metal ions promote self-association of nucleoside 5'-triphosphates in two ways: some metal ions (e.g., Mg^{2+}) augment stacking simply by reducing the charge repulsion by coordination at the phosphate residue; others (e.g., Zn^{2+} or Cd^{2+}) enhance the stability of the dimers by forming an *inter*molecular coordinative link between the phosphate residue of one NTP^{4-} and the base residue of the next, in addition to decreasing the electrostatic repulsion.

$Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$ show that this latter kind of promotion is effective, but certain other metal ions, like Cu^{2+}, which have a somewhat increased coordination tendency toward nitrogen *and* a large affinity for phosphate groups, might be even more effective. Comparison of these two examples with the stacking properties of $Zn(ITP)^{2-}$ and $Zn(GTP)^{2-}$ clearly show that this effect depends on the geometry of the stacks:[77] a given metal ion will not promote stacking of all nucleoside 5'-triphosphates with the same efficiency; the coordination sphere of the metal ion will not always be compatible with a good stack. However, ITP^{4-}, GTP^{4-}, UTP^{4-}, and TTP^{4-} may be deprotonated at N-1 or N-3 (see Chart I) at higher pH and this then leads to additional binding sites at the bases.[47] The self-stacking tendency under these conditions has not yet been studied, but one might expect that an *inter*molecular metal ion promotion by cross-linking is possible.

With the association constants listed in Table II it is possible to calculate the variation in the proportions of the various oligomers as the concentration is changed over the range used in the NMR experiments. Examples of such plots are shown for ATP^{4-}, $Mg(ATP)^{2-}$, and $Zn(ATP)^{2-}$ in Figure 7: the promotion of stacking by Mg^{2+} and Zn^{2+} is quite evident. In a system with a lower self-association tendency such as CTP^{4-} ($K = 0.5\ M^{-1}$), in 0.1 M solution 91% is present as the monomer, 8.4% as the dimer, 0.6% as the trimer, and less than 0.04% as larger oligomers; in 0.4 M solution the corresponding numbers are 73, 21, 5, and 1%.

With these results in mind we may conclude two things. (i) Experiments which are aimed at learning properties of the monomeric nucleotides should be carried out at low concentrations; e.g., at 10^{-3} M > 96% of $Zn(ATP)^{2-}$ is in the monomeric form. (ii) It must be assumed that in living systems self-association of nucleotides may be considerable under certain conditions; if [ATP] = 0.1 M in the adrenal chromaffin granules,[12-15] as has been claimed, then considerable self-stacking must occur.

Another important point is the formation of *intra*molecular macrochelates in monomeric $M(NTP)^{2-}$ complexes (eq 11). The extent of base–metal ion interaction varies from nucleotide to nucleotide and from metal ion to metal ion, thus ranging from insignificant traces to nearly 100% of the macrochelated isomer (Tables VIII and IX). The number of complex species simultaneously present in equilibrium is increased further in those cases where the base moiety may be ionized (ITP, GTP, UTP, and

Table IX, Extent of the Intramolecular Macrochelate Formation in Metal Ion Complexes of Purine and Pyrimidine 5'-Triphosphates with Ionized N-1 and N-3, Respectively: Estimations of the Intramolecular Dimensionless Equilibrium Constant K_I and of the Percentage of the "Closed" Isomer $M(NTP\text{-}H)^{3-}_{cl}$ ($I = 0.1$, $NaClO_4$, 25 °C)[a]

no.	complex	$pK^H_{NTP} - pK^H_{M(NTP)}$[a]	log Δ (analogous to eq 17)	K_I[b] (analogous to eq 12)	% $M(NTP\text{-}H)^{3-}_{cl}$ (analogous to eq 11)
1	$Mg(ITP\text{-}H)^{3-}$	0.2			
2	$Mg(GTP\text{-}H)^{3-}$	0.2 } 0.2[c]			
3	$Mg(UTP\text{-}H)^{3-}$	0.2			
4	$Mn(ITP\text{-}H)^{3-}$	0.33	0.13	0.35	26
5	$Mn(GTP\text{-}H)^{3-}$	0.43	0.23	0.70	41
6	$Mn(UTP\text{-}H)^{3-}$	0.25	~0	~0	≲20[a]
7	$Mn(TTP\text{-}H)^{3-}$	~0.22[d]	~0	~0	≲20[a]
8	$Ni(ITP\text{-}H)^{3-}$	0.87	0.67	3.7	79
9	$Ni(GTP\text{-}H)^{3-}$	1.15	0.95	7.9	89
10	$Ni(UTP\text{-}H)^{3-}$	0.60	0.40	1.5	60
11	$Ni(TTP\text{-}H)^{3-}$	~0.81[d]	~0.61	~3.1	~76
12	$Cu(ITP\text{-}H)^{3-}$	1.7	1.5	31	97
13	$Cu(GTP\text{-}H)^{3-}$	1.9	1.7	49	98
14	$Cu(UTP\text{-}H)^{3-}$	1.7	1.5	31	97
15	$Cu(TTP\text{-}H)^{3-}$	~2.1[d]	~1.9	~78	~99
16	$Zn(ITP\text{-}H)^{3-}$	0.95	0.75	4.6	82
17	$Zn(GTP\text{-}H)^{3-}$	1.40	1.20	15	94
18	$Zn(UTP\text{-}H)^{3-}$	0.99	0.79	5.2	84
19	$Zn(TTP\text{-}H)^{3-}$	~1.54[d]	~1.34	~21	~95

[a] These data are from Table IV of ref 47; log Δ = 0.1 corresponds to about 20% of the "closed" isomer. [b] Calculated with $K_I = (K^H_{M(NTP)}/K^H_{M(NTP)_{op}}) - 1$ which is analogous to eq 16 (see also footnote *c*). [c] This value is used as a basis for the evaluation because the change in the pK_A values is here due only to charge neutralization and not to a Mg^{2+}/base interaction (cf. ref 47); hence this value represents the "stability" of the "open" isomer, i.e., of $M(NTP\text{-}H)^{3-}_{op}$. [d] These values resulted only from a single titration due to the scarcity of TTP; cf. ref 47.

TTP): the $M(NTP-H)^{3-}$ species also occur in "open" and "closed" forms. In several M^{2+}/NTP systems[47,48] all these different complexes must exist simultaneously in the physiological pH range.

To conclude, the versatility of the interactions of nucleotides with surrounding ions and molecules is indeed impressive; they form hydrogen bonds and stack with each other as well as with other species; their coordinating properties are truly ambivalent: they offer not only different binding sites for different metal ions but also change their ligating qualities toward the same metal ion as the concentrations change. At low concentrations isomeric equilibria exist involving macrochelates, while at higher concentrations metal-ion-bridged stacks of dimers may be formed. Moreover, it appears that all these properties may be combined in several ways to create additional properties—here exists obviously a huge reservoir to achieve selectivity, and indeed nucleotides are involved in some of the most selective processes occurring in nature.

Acknowledgment. We thank Mr. K. Aegerter for recording the 90-MHz NMR spectra, the CIBA-Stiftung Basel for support toward the costs of these spectra, and the Swiss National Science Foundation for a research grant.

Nonpotential Energy (NPE) Effects in Organic Chemical Reactions: Development of a Suitable Force Field

Christine D. Chalk, Barrie G. Hutley, James McKenna,* Leslie B. Sims, and Ian H. Williams

Contribution from the Department of Chemistry, The University, Sheffield S3 7HF, England.
Received December 6, 1979

Abstract: A preliminary analysis is given of the role of NPE effects in organic chemistry. Calculation of these effects centers around a vibrational analysis program based on a suitable force field. A simple valence force field (SVFF) is chosen for the purpose, initially on the basis of a limited range of molecular types (alkanes and alkyl halides) but capable in principle of extension as required. Special weighting is given to accurate reproduction of the entropy-rich lower frequencies, and particular attention is paid to analysis of torsional force constants and torsional frequencies.

Introduction

Most interpretations of equilibria or reactivities in organic chemistry have traditionally been made on the basis of potential energy changes (bond energies, resonance energies, aromaticity or antiaromaticity, strain energies, coulombic aspects of solvation, etc.) with little or no attention being paid to contributions from other (nonpotential energy (NPE)) effects, apart from occasional usually rather qualitative interpretations of entropy changes where these are experimentally available and relatively large, and treatment of kinetic isotope effects. A more general analysis of NPE effects seems desirable, particularly since such contributions seem likely to be substantial for many reactions in solution, including enzyme-catalyzed processes.[1] In this paper we describe the development of a suitable force field for such analysis, and in accompanying papers we deal with calculations of NPE effects and related kinetic isotope effects for some nucleophilic substitutions and 1,2-eliminations. These calculations apply strictly to ideal gas conditions; in the future we shall report on solvation effects and simple enzyme models where the calculated NPE effects are, as expected, very much larger.

The expression "nonpotential energy" which we abbreviate to "NPE" suffers from an obvious negative character, but it is nevertheless both comprehensive and unambiguous, and it seems preferable to various other designations which are inadequate, incomplete, or otherwise objectionable for one reason or another: for example, mass, "ponderal",[2] or partition function (effects). Nonpotential energy effects, as defined, do of course depend on the topography of the relevant potential energy surfaces.

The drawbacks of overemphasis of potential energy interpretations in organic chemistry were recognized by Hammett, who in his classical text (1940) on physical organic chemistry wrote[3] that "any approach to the effect of structure upon equilibrium or heat of reaction that treats a molecule as a rigid lifeless structure can be no more than the crudest approximation". He might equally well have commented on similar interpretations of rate constants. Conventional discussions of equilibria or reactivity dealing only, and usually implicitly, with potential energy changes refer just to the behavior of molecules at absolute zero which have lost not only their thermal energies but also, more mysteriously, their zero-point energies, perhaps by the intervention of a Maxwell demon!

A very considerable improvement in interpretation is of course effected when free energies of reaction or of activation are determined and separated into enthalpy and temperature-entropy effects (eq 1). While such analyses are conveniently based on

$$\Delta G = \Delta H - T\Delta S \quad (1)$$

experimental measurement of the temperature coefficients of equilibrium or rate constants, there are nevertheless some drawbacks. First, the potential energy change, which chemists are so interested in interpreting, if usually implicitly rather than explicitly, is not specifically isolated. This term is not of course equivalent to the enthalpy change, although it is often by implication assumed to be so. Second, the enthalpy and entropy changes are both dependent on partition-function changes in a way which causes a degree of opposition between the two. Third, there is no isolation of zero-point energy changes, which correspond to perhaps the most unknown territory of all in the whole region of analysis of reactivities and equilibria.

A free energy change ΔG may be expressed[1] by eq 2 in which $\Delta\epsilon$, ΔE_{zp}, ΔE_{th}, and $P\Delta V$ represent changes in potential energy, zero-point energy, thermal energy, and pressure–volume terms, respectively; these terms together constitute an enthalpy change

(1) D. B. Cook and J. McKenna, *J. Chem. Soc., Perkin Trans 2*, 1223 (1974).
(2) C. K. Ingold, *Q. Rev., Chem. Soc.*, **11**, 1 (1957).
(3) L. P. Hammett, "Physical Organic Chemistry"; McGraw-Hill, New York, 1940, p 76.

0002-7863/81/1503-260$01.00/0