A Proton Nuclear Magnetic Resonance Study of Purine and Pyrimidine Nucleoside 5′-Diphosphates. Extent of Macrochelate Formation in Monomeric Metal Ion Complexes and Promotion of Self-Stacking by Metal Ions

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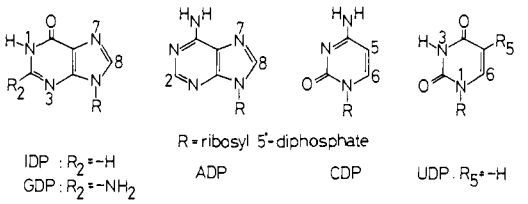
Abstract: The concentration dependence of the chemical shifts for protons H-2, H-8, and H-1′ of ADP^{3-} and IDP^{3-}, H-8 and H-1′ of GDP^{3-}, and H-5, H-6, and H-1′ of CDP^{3-} and UDP^{3-} has been measured in D_2O at 27 °C. The results are consistent with the isodesmic model of indefinite noncooperative stacking; the association constants for the nucleoside 5′-diphosphates (NDP^{3-}) are between 1.8 (ADP^{3-}) and about 0.6 M^{-1} (UDP^{3-}). In agreement with earlier results obtained under the same conditions for nucleosides and nucleoside 5′-triphosphates, the self-stacking tendency of the base moieties of the nucleic acids decreases in the series adenine > guanine ≳ hypoxanthine > cytosine ~ uracil. Due to the repulsion of the negatively charged phosphate moieties, self-association is always less pronounced for NDP^{3-} compared to that of the corresponding nucleoside. In accordance with this observation, the self-stacking tendency of NDP^{3-} systems is promoted by a factor of about 2–3 by coordination of Mg^{2+} to the phosphate moiety, which neutralizes part of the negative charge. However, the self-association tendency of $Zn(ADP)^-$ and $Cd(ADP)^-$ or $Zn(IDP)^-$ and $Cd(IDP)^-$ is much larger than of $Mg(ADP)^-$ or $Mg(IDP)^-$; 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 NDP^{3-} and to N-7 of the purine residue of the other NDP^{3-}. The shifts of H-8 (and H-2) for complete stacking (δ_∞) agree with this interpretation. Comparison 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 nucleoside 5′-diphosphates; 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 the concentration-independent equilibrium between this macrochelate and the open isomer (with phosphate coordination only) is estimated by comparing δ_0 of $M(NDP)^-$ with the shifts of H-8 for complete complex formation of the corresponding metal ion–nucleoside complexes. The NMR study gives no hint for such an N-7 interaction either for the corresponding $Mg(NDP)^-$ complexes or for a base–metal ion interaction in any of the pyrimidine nucleoside 5′-disphosphate complexes. An evaluation of available stability data gives further evidence that macrochelate formation occurs also in the $M(ADP)^-$ complexes of Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}; no evidence for such an interaction is observed for $Mg(ADP)^-$ and $Ni(CDP)^-$. The percentage of the macrochelated isomer is estimated for the above systems and compared with earlier results on monomeric $M(ATP)^{2-}$ complexes; for a given metal ion it appears that the extent of macrochelate formation decreases from $M(ADP)^-$ to $M(ATP)^{2-}$. The ambivalent coordinating properties of nucleotides and their structural versatility are discussed, and upper limits are listed for concentrations that should be employed in studies aiming to evaluate the properties of monomeric nucleoside 5′-diphosphates and their complexes.

The interplay between nucleotides and metal ions is now well established for many enzymic systems.[1] Consequently, metal ion–nucleotide interactions[2] have been studied in the solid state[3] and in solution,[4] and our knowledge on binary metal ion complexes of nucleotide 5′-monophosphates or 5′-triphosphates is considerable: the stability[5-8] of these complexes and their structures[4,9-11] in solution are well characterized, and the tendency for macrochelate formation in monomeric metal ion complexes of nucleoside 5′-triphosphates and the promotion of stacking by metal ions have been quantified.[11] This information, together with the awareness that metal ions accelerate the dephosphorylation of nucleoside 5′-triphosphates,[12,13] has allowed us to proceed to more complicated systems: hence, the stability of mixed-ligand complexes[14-17] was studied, and it was shown that metal ions promote hydrophobic and stacking interactions between the side chains of amino acids and the nucleic bases of nucleoside 5′-triphosphates.[17,18]

Chart I



In view of the above information, and considering that nucleoside 5′-diphosphates are on the metabolic pathway between the corresponding mono- and triphosphates, it is surprising to find that our knowledge on the properties of metal ion–nucleoside 5′-diphosphate systems is very scarce. There is only very limited

(1) (a) Eichhorn, G. L.; Marzilli, L. G., Eds. *Adv. Inorg. Biochem.* **1981**, *3*. (b) Spiro, T. G., Ed. *Met. Ions Biol.* **1980**, *1*.
(2) Sigel, H., Ed. *Met. Ions Biol. Syst.* **1979**, *8*.
(3) Gellert, R. W.; Bau, R. *Met. Ions Biol. Syst.* **1979**, *8*, 1–55. Cf. ref 2.
(4) Martin, R. B.; Mariam, Y. H. *Met. Ions Biol. Syst.* **1979**, *8*, 57–124. Cf. ref 2.
(5) (a) Sillén, L. G.; Martell, A. E. *Spec. Publ.—Chem. Soc.* **1964**, *No. 17*. (b) Sillén, L. G.; Martell, A. E. *Spec. Publ.—Chem. Soc., Suppl. 1* **1971**, *No. 25*.
(6) (a) Högfeldt, E. "Stability Constants of Metal-Ion Complexes"; IUPAC Chemical Data Series; Pergamon Press: Oxford, 1982; Part A. (b) Perrin, D. D. *Ibid.* 1979; Part B.
(7) (a) Smith, R. M.; Martell, A. E. "Critical Stability Constants"; Plenum Press: New York and London, 1975; Vol. 2; (b) *Ibid.* 1976; Vol. 4. (c) Martell, A. E.; Smith, R. M. *Ibid.* 1982; Vol. 5.
(8) Sigel, H. *J. Inorg. Nucl. Chem.* **1977**, *39*, 1903–1911.
(9) Mariam, Y. H.; Martin, R. B. *Inorg. Chim. Acta* **1979**, *35*, 23–28.
(10) Scheller, K. H.; Scheller-Krattiger, V.; Martin, R. B. *J. Am. Chem. Soc.* **1981**, *103*, 6833–6839.
(11) Scheller, K. H.; Hofstetter, F.; Mitchell, P. R.; Prijs, B.; Sigel, H. *J. Am. Chem. Soc.* **1981**, *103*, 247–260.
(12) (a) Sigel, H.; Amsler, P. E. *J. Am. Chem. Soc.* **1976**, *98*, 7390–7400. (b) Sigel, H. *Pure Appl. Chem.* **1983**, *55*, 137–144. (c) Sigel, H.; Hofstetter, F. *Eur. J. Biochem.* **1983**, *132*, 569–577.
(13) Hediger, M.; Milburn, R. M. *J. Inorg. Biochem.* **1982**, *16*, 165–182.
(14) Sigel, H.; Fischer, B. E.; Prijs, B. *J. Am. Chem. Soc.* **1977**, *99*, 4489–4496.
(15) Sigel, H. "Coordination Chemistry—20"; Banerjea, D., Ed.; published by IUPAC through Pergamon Press: Oxford and New York, 1980; pp 27–45.
(16) Saha, N.; Sigel, H. *J. Am. Chem. Soc.* **1982**, *104*, 4100–4105.
(17) (a) Sigel, H.; Naumann, C. F. *J. Am. Chem. Soc.* **1976**, *98*, 730–739. (b) Sigel, H.; Fischer, B. E.; Farkas, E. *Inorg. Chem.* **1983**, *22*, 925–934.
(18) Mitchell, P. R.; Prijs, B.; Sigel, H. *Helv. Chim. Acta* **1979**, *62*, 1723–1735.

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information available on the stability[5-7] of the binary complexes and nothing on ternary complexes. Because self-association of nucleosides and nucleoside 5′-triphosphates via their nucleic bases is well-known,[19] the first information needed is about the corresponding properties of the 5′-diphosphates; only then will it be possible to investigate the monomeric systems, or systems containing several components.

^{1}H NMR shift measurements have proven to be ideal for studying the self-stacking of ATP[20] and several other nucleotides.[11,21-24] Therefore, we have now carried out such a study on self-stacking for purine and pyrimidine nucleoside 5′-diphosphates (see Chart I) in aqueous solution in the neutral pH range. The influence of Mg^{2+}, Zn^{2+}, or Cd^{2+} on the stability of the stacks has also been studied.

There is one further important aspect: extrapolation of the ^{1}H NMR shifts to zero concentration has allowed determination of the chemical shifts for the protons of the monomeric $M(NDP)^-$ complexes. By comparing these chemical shifts with those obtained earlier[11] for the protons in the base moieties of the corresponding nucleoside complexes, the extent of macrochelate formation in several $M(NDP)^-$ complexes could be estimated. The existence of such intramolecular equilibria had previously been shown for $Ni(ADP)^-$ by spectrophotometric measurements.[9]

Experimental Section

Materials. The sodium salts of the nucleotides, ADP, IDP, GDP, CTP, and UDP, were obtained from Sigma Chemical Co. All the other reagents were the same as used earlier.[11]

Apparatus and Measurements. The ^{1}H NMR spectra were recorded with a Bruker WH-90 FT spectrometer (90.025 MHz) at 27 °C by using the center peak of the tetramethylammonium ion triplet as the internal reference. All chemical shifts were converted to a (trimethylsilyl)-propanesulfonate reference by adding 3.188 ppm. The reliability of tetramethylammonium ion as an internal ^{1}H NMR reference in such studies has been discussed previously in detail.[11]

The pD of the solutions was obtained by adding 0.40 to the pH meter reading.[25] The pH was measured with a Metrohm glass electrode EA 125. The experiments were carried out exactly as described[11] by taking into account the acidity constants[5-7] of $H(NDP)^{2-}$ and the stability constants of $M(H{\cdot}ADP)$ and $M(ADP)^-$;[26] the pD was adjusted such that a high degree of formation for $M(NDP)^-$ resulted.[27]

The reactant concentration in the self-stacking experiments varied typically from 0.003 to 0.4 M. $NaNO_3$ was added[28] to increase the ionic strength to 0.1 M, when necessary, although in the more concentrated nucleotide solutions the ionic strength was unavoidably higher. The individual experimental details for several systems are given in Table I and in Figures 1 and 2.

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 and 2) was fitted to eq 3 by using a Newton–Gauss nonlinear least-squares method.

Results and Discussion

By several different methods, among them NMR, it has been shown that AMP^{2-} and other nucleotides,[19] including 5′-triphosphates,[11,23] associate in aqueous solution beyond the dimer stage. It is now generally accepted[11,24] that the self-association of these substances occurs by stacking and that oligomers are formed (vide infra). The situation for these nucleotide systems is best characterized with the isodesmic model for indefinite

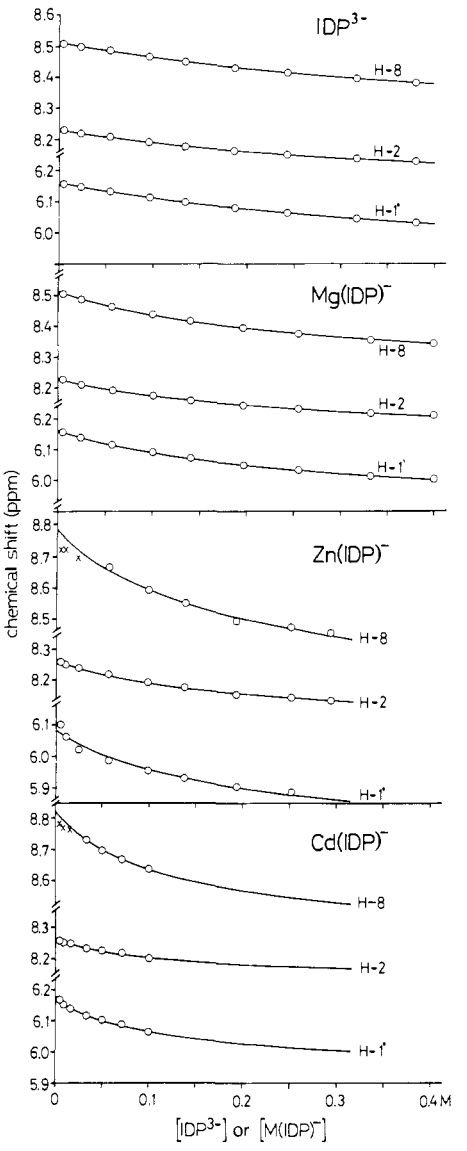


Figure 1. Variation of the chemical shift for H-2, H-8, and H-1′ of IDP with varying concentrations of IDP^{3-} or $M(IDP)^-$. The spectra were measured on a Bruker FT 90 at 90.025 MHz (D_2O; 27 °C; I = 0.1 to ~1.7, $NaNO_3$), relative to internal $(CH_3)_4N^+$, and converted to values relative to sodium (trimethylsilyl)propanesulfonate by adding 3.188 ppm. The curves shown here and in Figure 2 are the computer-calculated best fit of the experimental data (calculated with K_{av} of Table I) by using the indefinite noncooperative stacking model; the resulting shifts are listed in Table II. From top to bottom: IDP^{3-}, pD 8.2; $Mg(IDP)^-$, pD 7.1; $Zn(IDP)^-$, pD 6.6; $Cd(IDP)^-$, pD 7.0. For the latter two complexes only a restricted concentration range could be studied, and the curve shown through the experimental shifts for H-8 was also calculated with K_{av} given in Table I, but for the fit only concentrations greater than 0.033 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.

noncooperative association;[29] the adaptation of this model to ^{1}H NMR shift measurements was recently described in detail.[11,23,30] It is assumed that the equilibrium constants (eq 1) for the equilibria 2 are all equal.

$$K = [N_{n+1}]/([N_n][N]) \quad (1)$$

$$N_n + N \rightleftharpoons N_{n+1} \quad (2)$$

Expression 3 gives the relationship between the observed

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

chemical shift (δ_{obsd}) in a solution of total concentration [N] and

(19) See ref 11 and the references cited therein.
(20) Abbreviations: AMP, ADP, and ATP, adenosine 5′-mono-, 5′-di-, and 5′-triphosphate; CDP, GDP, IDP, UDP, and dTDP, cytidine, guanosine, inosine, uridine, and thymidine 5′-diphosphate; M, general metal ion; N, nucleoside or nucleotide; NMP, NDP, and NTP, nucleoside 5′-mono-, 5′-di-, and 5′-triphosphate; Ns. nucleoside.
(21) Fan, S.; Storer, A. C.; Hammes, G. G. *J. Am. Chem. Soc.* **1977**, *99*, 8293–8298.
(22) Lam, Y.-F.; Kotowycz, G. *Can. J. Chem.* **1977**, *55*, 3620–3630.
(23) Mitchell, P. R.; Sigel, H. *Eur. J. Biochem.* **1978**, *88*, 149–154.
(24) Neurohr, K. J.; Mantsch, H. H. *Can. J. Chem.* **1979**, *57*, 1986–1994.
(25) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188–190.
(26) (a) Khan, M. M. T.; Martell, A. E. *J. Am. Chem. Soc.* **1962**, *84*, 3037–3041. (b) Khan, M. M. T.; Martell, A. E. *J. Am. Chem. Soc.* **1967**, *89*, 5585–5590.
(27) (a) Buisson, D. H.; Sigel, H. *Biochim. Biophys. Acta* **1974**, *343*, 45–63. (b) Sigel, H.; Buisson, D. H.; Prijs, B. *Bioinorg. Chem.* **1975**, *5*, 1–20.
(28) The stability of $Na(NDP)^{2-}$ complexes is low as may be judged from $\log K^{Na}_{Na(ADP)} = 0.65$.[5b]
(29) Heyn, M. P.; Bretz, R. *Biophys. Chem.* **1975**, *3*, 35–45.
(30) Mitchell, P. R. *J. Chem. Soc., Dalton Trans.* **1980**, 1079–1086.

Table I. Equilibrium Constants for Self-Stacking (Equation 1) of Several Nucleoside 5'-Diphosphates and Their Metal Ion Complexes As Determined by ^{1}H NMR Shift Measurements, Together with Some Related Data from the Literature[11,23,24] (D_2O; 27 °C)[a,b,20]

			K, M^{-1}, determined from the shift of				K_{av}, M^{-1}, for the related systems[d]		
system	pD[c]	I	H-2	H-8	H-1'	K_{av}, M^{-1}	Ns	NMP^{2-}	NTP^{4-} or $M(NTP)^{2-}$
ADP^{3-}	8.9	0.1-~1.7	2.19 ± 0.29	1.52 ± 0.37	1.52 ± 0.26	1.8 ± 0.5	15 ± 2	2.1 ± 0.4/*1.9*[e]	1.3 ± 0.2
$Mg(ADP)^-$	7.5	0.1-~1.2	6.75 ± 0.43	4.97 ± 0.71	6.32 ± 0.55	6.4 ± 0.9			4.0 ± 0.5
$Zn(ADP)^-$	7.0	0.1-~0.15	117 ± 53	*f*	(90 000)[g]	~100[h]			~11[h]
$Cd(ADP)^-$	7.0	0.1-~0.15	130 ± 19	*f*	*g*	~100[h]			~17[h]
IDP^{3-}	8.2	0.1-~1.7	1.96 ± 0.30	1.09 ± 0.17	0.97 ± 0.14	1.3 ± 0.6	3.3 ± 0.3	*1.4*[e]	0.4 ± 0.3
$Mg(IDP)^-$	7.1	0.1-~1.2	3.56 ± 0.37	2.10 ± 0.20	2.49 ± 0.21	2.6 ± 0.7			2.0 ± 0.6
$Zn(IDP)^-$	6.6	0.1-~0.9	2.97 ± 0.52	*f*	18.57 ± 7.65	~4[h]			~2.8 ± 1.2[h]
$Cd(IDP)^-$	7.0	0.1-~0.3	3.47 ± 1.80	*f*	11.26 ± 4.77	~7[h]			
GDP^{3-}	8.4	0.1-~1.7		0.63 ± 0.22	1.13 ± 0.24	1.0 ± 0.5	8 ± 3	*1.3*[e]	0.8 ± 0.6
$Zn(GDP)^-$	6.6	0.1				*b*			~1.9 ± 0.6[h]

			K, M^{-1}, determined from the shift of				K_{av}, M^{-1}, for the related systems	
system	pD	I	H-5	H-6	H-1'	K_{av}, M^{-1}	Ns	NTP^{4-} or $M(NTP)^{2-}$
CDP^{3-}	8.9	0.1-~1.7	*i*	0.67 ± 0.06	*i*	0.7	1.4 ± 0.5	0.5 ± 0.2
$Mg(CDP)^-$	7.5	0.1-~1.2	*i*	1.12 ± 0.19	*i*	1.1		*j*
UDP^{3-}	8.4	0.1-~1.7	*i*	0.64 ± 0.34	*i*	0.6	1.2 ± 0.5	~0.4
$Mg(UDP)^-$	7.4	0.1-~1.2	*i*	1.43 ± 0.62	*i*	1.4		*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 of the individual results calculated via log K; the range of error given here is *twice* the standard error. [b] The following systems could not be studied due to precipitation at concentrations ≥0.05 M (pD 6.6-7.2):[c] $[Mg^{2+}] = [GDP]$; $[Zn^{2+}] = [GDP]$, $[CDP]$, or $[UDP]$; $[Cd^{2+}] = [GDP]$, $[CDP]$, or $[UDP]$. [c] pD of the solutions used for the measurements. [d] The values for the self-stacking of adenosine and ATP^{4-} are taken from ref 23, the values printed in italics are from ref 24,[e] and all the others are from ref 11. [e] Taken from the work of Neurohr and Mantsch and measured at 30 °C (ref 24). [f] Due to a Zn^{2+}- or Cd^{2+}-N-7 interaction, the shift for H-8 deviates at low concentrations and can therefore not be used to calculate K for the self-association; see also section 4 and Figure 1. [g] In the Zn^{2+}-ADP and Cd^{2+}-ADP systems the concentrations could only be varied from 0.003 to 0.05 M due to precipitation and gel formation, respectively, and in this concentration range the measured upfield shifts were too small for a reliable curve-fitting procedure. [h] Regarding the validity of this value, see section 4. [i] The upfield shifts were too small (see Figure 2) for a curve-fitting procedure. [j] Only solutions up to $[Mg^{2+}] = [CTP]$ or $[UTP] = 0.1$ M could be studied due to precipitation, and the data for this limited concentration range could be fitted within experimental error with the values of K obtained for CTP^{4-} and UTP^{4-} alone (ref 11). This indicates that the self-association tendency for $Mg(CTP)^{2-}$ and $Mg(UTP)^{2-}$ are only slightly larger than for CTP^{4-} and UTP^{4-}.

the chemical shift for a free molecule (δ_0, the shift at infinite dilution), the chemical shift for an infinitely long stack (δ_∞), and the association constant K (eq 1).[31]

Representative examples of the measurements are shown in Figures 1 and 2: Figure 1 gives the data for the purine system IDP^{3-} with and without metal ions, and Figure 2 contains the corresponding data for the pyrimidine system CDP^{3-}. Further experimental details, especially regarding limitations due to precipitation, are given in Table I.

(1) Comparison of the Self-Stacking Tendency for Nucleosides and Their 5'-Di- and 5'-Triphosphates. The variation of the upfield shifts for H-2, H-8, and H-1' of ADP^{3-} as a function of concentration is similar to the results obtained earlier for ATP^{4-} (see figure 1 in ref 11) and to those for IDP^{3-} shown in Figure 1. Computer-calculated least-squares fits with eq 3 for the variation of the upfield shifts for each of the protons with increasing concentration gave the same stability constant for each of the protons, within experimental error (twice the standard deviation); the values are given in Table I. The association constants for IDP^{3-} (Figure 1), GDP^{3-}, CDP^{3-} (Figure 2), and UDP^{3-} have been obtained in the same way, and the present value for ADP^{3-}, $K = 1.8 \pm 0.5$ M^{-1}, is in agreement with the one published previously:[11] $K = 1.3 \pm 0.4$ M^{-1}.

A comparison of the equilibrium constants given in Table I shows a decreasing tendency for self-stacking within the series adenosine ($K = 15 \pm 2$ M^{-1}) ≫ AMP^{2-} (2.1 ± 0.4 M^{-1}) > ADP^{3-} (1.8 ± 0.5 M^{-1}) > ATP^{4-} (1.3 ± 0.2 M^{-1}); this observation was to be expected due to the repulsion between the negatively charged phosphate groups within the stacks. This result is of a general nature and holds also for all the other corresponding series in Table I: despite the smaller differences between the association constants

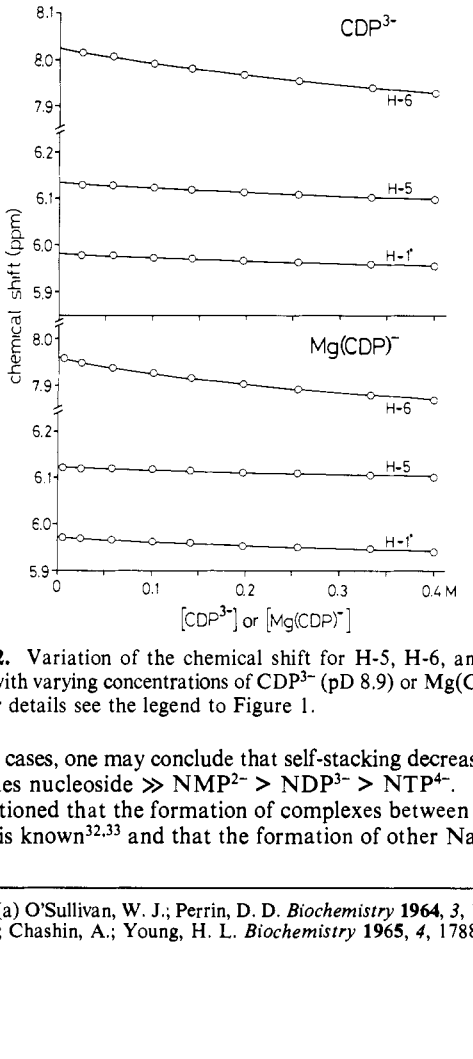


Figure 2. Variation of the chemical shift for H-5, H-6, and H-1' of CDP^{3-} with varying concentrations of CDP^{3-} (pD 8.9) or $Mg(CDP)^-$ (pD 7.5); for details see the legend to Figure 1.

in these cases, one may conclude that self-stacking decreases within the series nucleoside ≫ NMP^{2-} > NDP^{3-} > NTP^{4-}. It should be mentioned that the formation of complexes between Na^+ and ATP^{4-} is known[32,33] and that the formation of other $Na(NTP)^{3-}$

(31) It should be mentioned that if species larger than dimers are ignored, a relationship similar to eq 3 is obtained, but 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.[11,23,30]

(32) (a) O'Sullivan, W. J.; Perrin, D. D. *Biochemistry* **1964**, *3*, 18–26. (b) Botts, J.; Chashin, A.; Young, H. L. *Biochemistry* **1965**, *4*, 1788–1796.

Table II. Chemical Shifts (ppm) for Several Monomeric (δ_0) and Self-Stacked (δ_∞) Nucleoside 5'-Diphosphates and Their Metal Ion Complexes, Together with the Corresponding Upfield Shifts ($\Delta\delta = \delta_0 - \delta_\infty$) and Some Related Data from the Literature[11,23,a-c]

	H-2			H-8			H-1'		
system[c]	δ_0	δ_∞	$\Delta\delta$	δ_0	δ_∞	$\Delta\delta$	δ_0	δ_∞	$\Delta\delta$
adenosine	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
ATP^{4-}	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
ADP^{3-}	8.274 ± 0.015	7.20 ± 0.18	1.07 ± 0.18	8.552 ± 0.009	8.03 ± 0.09	0.52 ± 0.10	6.157 ± 0.007	5.71 ± 0.08	0.45 ± 0.08
$Mg(ADP)^-$	8.273 ± 0.012	7.44 ± 0.05	0.83 ± 0.05	8.535 ± 0.010	8.10 ± 0.03	0.44 ± 0.04	6.155 ± 0.007	5.73 ± 0.03	0.43 ± 0.03
$Zn(ADP)^-$ [d]	8.178 ± 0.009	7.89 ± 0.01	0.29[e]	[f]	8.57 ± 0.02[g]		6.006 ± 0.013	5.83 ± 0.02	0.18[e]
$Cd(ADP)^-$ [d]	8.240 ± 0.005	7.84 ± 0.01	0.40[e]	[f]	8.74 ± 0.04[g]		5.999 ± 0.015	5.99 ± 0.02	0.01[e]
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
IDP^{3-}	8.229 ± 0.006	7.84 ± 0.13	0.39 ± 0.13	8.513 ± 0.007	8.01 ± 0.15	0.50 ± 0.15	6.160 ± 0.007	5.67 ± 0.15	0.49 ± 0.15
$Mg(IDP)^-$	8.228 ± 0.006	7.92 ± 0.05	0.31 ± 0.05	8.512 ± 0.008	8.08 ± 0.07	0.43 ± 0.07	6.161 ± 0.007	5.75 ± 0.06	0.41 ± 0.06
$Zn(IDP)^-$ [d]	8.265 ± 0.003	7.95 ± 0.01	0.32[e]	[f]	7.95 ± 0.06[h]		6.088 ± 0.020	5.53 ± 0.07	0.55[e]
$Cd(IDP)^-$ [d]	8.263 ± 0.003	8.08 ± 0.01	0.18[e]	[f]	8.24 ± 0.03[h]		6.172 ± 0.006	5.84 ± 0.02	0.33[e]
guanosine				8.006 ± 0.003			5.921 ± 0.003		
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
GDP^{3-}				8.139 ± 0.006	7.74 ± 0.16	0.40 ± 0.16	5.937 ± 0.006	5.51 ± 0.16	0.43 ± 0.16

	H-5			H-6			H-1'		
system[c]	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
CDP^{3-} [b]	6.133 ± 0.003	5.93 ± 0.04	0.20 ± 0.05	8.023 ± 0.002	7.47 ± 0.08	0.55 ± 0.08	5.981 ± 0.002	5.84 ± 0.03	0.14 ± 0.03
$Mg(CDP)^-$ [b]	6.123 ± 0.003	6.04 ± 0.03	0.08 ± 0.03	7.959 ± 0.004	7.60 ± 0.09	0.36 ± 0.09	5.972 ± 0.002	5.85 ± 0.03	0.12 ± 0.04
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			7.993 ± 0.004			5.996 ± 0.002		
UDP^{3-} [d]	5.971 ± 0.001	5.85 ± 0.01	0.12	8.010 ± 0.002	7.74 ± 0.02	0.27	5.967 ± 0.001	5.84 ± 0.01	0.13
$Mg(UDP)^-$ [d]	5.965 ± 0.002	5.96 ± 0.01	0.01	7.964 ± 0.002	7.80 ± 0.01	0.16	5.963 ± 0.005	5.91 ± 0.12[i]	0.05[i]

[a] The experimental conditions are the same as given in Table I. 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. [b] The shifts were calculated by using from Table I the values of K_{av} together with their corresponding errors; except for the CDP^{3-} and $Mg(CDP)^-$ systems the values resulting from H-6 were used. The range of error given with the calculated shifts is *twice* the standard deviation. [c] The values for adenosine and ATP^{4-} are taken from ref 23, and those for AMP^{2-}, the nucleoside, and NTP^{4-} systems from ref 11; all the data referring to NDP systems are from the present work. [d] The listed values result if the calculation is done with K_{av} of Table I; the given error limits reflect *only* the fit between the experimental data and the calculated curve, but they contain *no* contribution from the uncertainty in K_{av} (as is the case with the other systems).[b] [e] There is some uncertainty in these values as the result depends somewhat on K_{av}; see also footnotes *f* and *g* of Table I and section 4. [f] See Figure 1 (for the systems with IDP), Table V, and section 7. [g] The curve through the experimental shifts for H-8 was also calculated with K_{av} given in Table I, but for the fit only concentrations greater than 8 mM were used. The deviations 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. [h] See the legend to Figure 1. [i] The signals for H-1' were not well resolved; only three experiments at concentrations ≤0.06 M could be evaluated.

complexes must also be anticipated; hence, the association constants given in Table I for the NTP^{4-} systems should be considered rather as an upper limit.[11] The extent of complex formation between Na^+ and NDP^{3-} is considerably smaller[28] as one would expect.

A detailed comparison of the stacking tendency for the various nucleoside 5'-diphosphates (and for the NTPs) is hindered by their low association constants, which is a result of the high charge of these nucleotides. However, the trend observed for these 5'-phosphates becomes unequivocal with the nucleosides (Table 1): the association tendency for the adenine moiety ($K = 15 \pm 2\ M^{-1}$) is more pronounced than that for the guanine ($K = 8 \pm 3\ M^{-1}$) or the hypoxanthine moiety ($K = 3.3 \pm 0.3\ M^{-1}$); the lower stacking ability of the pyrimidine moieties compared with purine residues is also evident. One may conclude that the association tendency for the base moieties of the nucleic acids decreases in the series adenine > guanine ≳ hypoxanthine > cytosine ~ uracil.

(2) Influence of Stacking on the Position of the Proton Resonances of the Nucleic Base Moieties. The chemical shifts for the protons in monomeric NDP^{3-} and in completely stacked NDP^{3-} are given in Table II, together with the corresponding data for some related systems. The upfield shifts, $\Delta\delta = \delta_0 - \delta_\infty$, especially for H-2 of AMP^{2-}, ADP^{3-}, and ATP^{4-}, are much higher than would be expected for the shift due to a single adjacent molecule, as in the dimer. This observation confirms that stacking proceeds beyond the dimer stage. In this connection it should be mentioned that for the inosine and guanosine derivatives and especially for the pyrimidines smaller upfield shifts are expected because their ring currents are smaller.[34]

A comparison of the upfield shifts $\Delta\delta$ 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-} ≲ ADP^{3-} ≲ ATP^{4-} (Table II). The same trend is indicated for the hypoxanthine systems despite the less accurate values: inosine < IDP^{3-} ≲ ITP^{4-}. For the guanosine systems no conclusions can be drawn as not enough data are available. Though the upfield shifts of H-5, H-6, and H-1' for the pyrimidine derivatives are smaller, it still seems that $\Delta\delta$ increases somewhat also here with increasing charge, i.e., Ns ≲ NDP^{3-} ≲ NTP^{4-}.

The δ_0 shifts for H-5, H-6, and H-1' of the pyrimidines or for H-8 and H-1' of the purines differ somewhat for the nucleosides and nucleotides, whereas the δ_0 shifts for H-2 of the adenine and hypoxanthine substrates are identical within a series. This observation is not surprising, because in solution these nucleotides assume the anti conformation with respect to the glycosyl bond,[4] and therefore the negatively charged phosphate groups are close to H-5 and H-6 of the pyrimidines or to H-8 of the purines but not to H-2 of the hypoxanthine or adenine derivatives. However, δ_∞ decreases for these systems with increasing negative charge (Table II). These differences in δ_∞ for H-2 are probably due to variations in the relative orientation of the molecules in the stack,[24,30,35] which result from changes in the repulsion between

(33) Na^+–ATP^{4-}: $\log K^{Na}_{Na(ATP)} = 1.2$. 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%).

(34) (a) Giessner-Prettre, C.; Pullman, B. *J. Theor. Biol.* **1970**, *27*, 87–95. (b) Ribas Prado, F.; Giessner-Prettre, C. *J. Mol. Struct.* **1981**, *76*, 81–92.

(35) (a) Schweizer, M. P.; Broom, A. D.; Ts'o, P. O. P.; Hollis, D. P. *J. Am. Chem. Soc.* **1968**, *90*, 1042–1055. (b) Son, T.-D.; Chachaty, C. *Biochim. Biophys. Acta* **1973**, *335*, 1–13.

Table III. Dependence of the Coupling Constant, $J_{1'-2'}$ (Hz), between H-1' and H-2' of the Ribosyl Moiety of Several Nucleoside 5'-Diphosphates and Their Mg^{2+} Complexes on the Total Substrate Concentration (D_2O; 27 °C)[a-c]

system[a]	$J_{1'-2'}$, Hz,[b] at concentrations of ~0.005 M	~0.4 M
ADP^{3-}	4.84 (98)[c]	4.10 (45)[c]
$Mg(ADP)^-$	5.28 (94)	~3.2 (21)
IDP^{3-}	4.84 (99)	3.81 (53)
$Mg(IDP)^-$	4.98 (98)	3.96 (37)
GDP^{3-}	5.35 (99)	3.44 (59)
CDP^{3-}	3.4 (99)	3.1 (66)

[a] The experimental conditions are the same as given in Table I. The NDP systems not listed above were either not sufficiently soluble at concentrations of 0.4 M or the resonances for H-1' were not satisfactorily resolved at the high concentrations. [b] The estimated errors are ±0.2 Hz. [c] Percentage of the total substrate concentration present in the monomeric form in 0.005 or 0.4 M solutions.

the differently charged phosphate residues; in adenosine and inosine stacks there is of course no such repulsion.

That stacking influences the orientation of the different parts of the molecules to each other is evident from the coupling constants between H-1′ and H-2′, which are dependent on the total concentrations of the substrates. The results listed in Table III for several NDP systems show that, at least for the purine derivatives, the self-association is connected with an alteration of the conformation of the ribosyl moiety. Similar observations regarding the H-1′–H-2′ coupling constant have previously been made for free AMP^{2-} and GMP^{2-} and their tryptamine stacking adducts.[36]

(3) Influence of Mg^{2+} and Related Metal Ions on the Self-Association Tendency of Nucleoside 5′-Diphosphates. Comparison of the association constant for $Mg(ADP)^-$ with the corresponding value for uncomplexed ADP^{3-} shows that the addition of Mg^{2+} to solutions of ADP^{3-} favors self-stacking; K is increased by a factor of about 3, a result that corresponds to the observations made with ATP^{4-} and $Mg(ATP)^{2-}$.[11] This promotion of the self-stacking tendency is undoubtedly due to partial neutralization of the negative charge at the phosphate moiety by formation of a Mg^{2+} complex. Indeed, the value for the single negatively charged $Mg(ADP)^-$ ($K = 6.4 \pm 0.9\ M^{-1}$) is between the values for the neutral adenosine ($K = 15 \pm 2\ M^{-1}$) and the 2-fold negative species AMP^{2-} ($K = 2.1 \pm 0.4\ M^{-1}$) and $Mg(ATP)^{2-}$ ($K = 4.0 \pm 0.5\ M^{-1}$).

The addition of Mg^{2+} to IDP^{3-} also favors self-stacking as is already obvious from the experimental data shown in Figure 1: the curvature of the lines is more pronounced for the $Mg(IDP)^-$ system than for the IDP^{3-} system. Indeed, comparison of the association constants (Table I) shows that the self-stacking tendency is promoted by a factor of about 2; this is the same factor observed also for the pyrimidine derivatives, i.e., from CDP^{3-} to $Mg(CDP)^-$ and from UDP^{3-} to $Mg(UDP)^-$. For these pyrimidine systems, which show a relatively weak self-association tendency, the association constants for $Mg(NDP)^-$ and cytidine or uridine are identical within experimental error (Table I). The decreasing order of the association constants for the complexes $Mg(ADP)^- > Mg(IDP)^- > Mg(CDP)^- \sim Mg(UDP)^-$ confirms the decreasing stacking tendency of the nucleic base moieties within this series, as discussed in section 1.

It is clear that the promotion of the stacking by the coordination of Mg^{2+} to NDP^{3-} is due to charge neutralization; an effect that also stabilizes stacked poly(adenylic acid).[37] It is also evident that any other metal ion that coordinates to the phosphate moiety, but not to the nucleic base (see sections 4–6), e.g., Ca^{2+} and the trivalent lanthanide ions, will facilitate the self-association in the same way.

(36) Wagner, K. G.; Lawaczeck, R. *J. Magn. Reson.* **1972**, *8*, 164–174.
(37) Dewey, T. G.; Turner, D. H. *Biochemistry* **1979**, *18*, 5757–5762.

Since the size of an upfield shift is dependent on the geometric arrangement of the stacks,[30] among several other factors, it is interesting to compare the upfield shifts $\Delta\delta$ of H-2, H-8, and H-1′ for the various ADP and IDP stacks listed in Table II. The values within each class of NDP^{3-} and $Mg(NDP)^-$ are similar to each other yet are somewhat smaller for $Mg(NDP)^-$ due to the decreased charge (section 2). The influence of charge is also indicated for the pyrimidines: $\Delta\delta$ is smaller for $Mg(NDP)^-$ compared with that for CDP^{3-} and UDP^{3-} (Table II). Finally for $Mg(ADP)^-$ $\Delta\delta$ for H-2 is rather large, thus again confirming that stacking proceeds beyond dimers (see also section 2).

(4) Promotion of the Self-Stacking Tendency for ADP^{3-} and IDP^{3-} by Zn^{2+} and Cd^{2+}. Zn^{2+} and Cd^{2+} also clearly promote the self-association of ADP^{3-} and IDP^{3-}, but the situation is more complicated. In the case of $Zn(ADP)^-$, $Cd(ADP)^-$, $Zn(IDP)^-$ and $Cd(IDP)^-$, only the shifts for H-2 and H-1′ seem to behave "normally" (Figure 1), while the shifts for H-8 deviate downfield at low concentrations (<0.03 M). However, evaluation of the experimental data for H-2 and H-1′ with eq 3 leads to two *different* values for the association constant of these $M(NDP)^-$ complexes. Despite the resulting uncertainty in calculating K_{av}, it is evident that these values (Table I) are considerably larger than the corresponding association constants for the $Mg(NDP)^-$ complexes; this indicates that in the Zn^{2+} and Cd^{2+} complexes an additional effect, besides charge neutralization, must be operating. This observation, together with the relatively poor fit of the experimental data by the calculations using the values of K_{av} [see, e.g., H-1′ for $Zn(IDP)^-$ in Figure 1], and the deviations of the shifts for H-8 at low concentrations show that the indefinite noncooperative model (eq 1–3) does not adequately describe the results and that an additional equilibrium must be considered.

The interaction between N-7 of purine derivatives with metal ions such as Zn^{2+} or Cd^{2+} is well documented[3,4] and has already been shown to occur in the monomeric $M(NTP)^{2-}$ complexes of Zn^{2+} and Cd^{2+} with ATP^{4-}, ITP^{4-}, and GTP^{4-}.[11] 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 will coordinate to the phosphate moiety of one ADP^{3-} or IDP^{3-} and to N-7 of the purine moiety of the next.[38]

Such an *inter*molecular metal ion bridge should enhance the stability of dimeric species and should also alter the shifts of the resonances, especially of H-8, which is next to N-7. This reasoning has led to the following model:[11]

$$2M(NDP)^- \rightleftharpoons M_2(NDP)_2^{2-} \quad (4)$$

$$K_D^* = [M_2(NDP)_2^{2-}]/[M(NDP)^-]^2 \quad (5)$$

These *inter*molecular metal ion bridged dimers of eq 4 may stack with each other, as well as with monomeric $M(NDP)^-$; the association constants for such nonbridged stacks are again expected to be equal. This may be expressed in a general form by eq 6 and 7. Due to the increased number of variable parameters (K_D^*,

$$M_2(NDP)_2^{2-} + M_n(NDP)_n^{n-} \rightleftharpoons M_{2+n}(NDP)_{2+n}^{(2+n)-} \quad (6)$$

$$K_{st} = [M_{2+n}(NDP)_{2+n}^{(2+n)-}]/[[M_2(NDP)_2^{2-}][M_n(NDP)_n^{n-}]] \quad (7)$$

K_{st}, δ_0, δ_0^*, and δ_∞), the scatter of the experimental results, and the limited accessible concentration range (Figure 1 and footnote *g* in Table I), 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 shift of H-8, which is initially rather *down*field, in the systems of Zn^{2+} and Cd^{2+} with ADP^{3-} or IDP^{3-} (cf. Figure 1). Several least-squares calculations indicated that the equilibrium constants for stacking, K_{st}, are of

(38) The corresponding conclusion has also been reached for the promotion of self-association for the purine nucleoside 5′-triphosphates by Zn^{2+} and Cd^{2+}.[11] Similarly, the enhanced self-association of ATP at pH 2.8, i.e., under conditions where the adenine moiety is protonated at N-1, has been explained by an electrostatic interaction between the phosphate tail of one ATP and the adenine ring of the other; see Gilligan, T. J., III; Schwarz, G. *Biophys. Chem.* **1976**, *4*, 55–63.

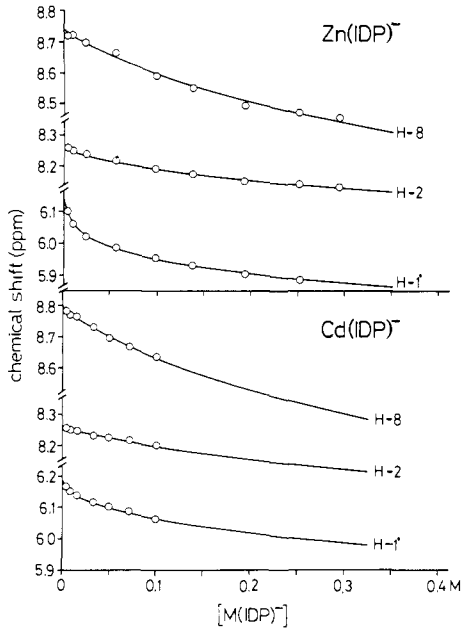


Figure 3. Variation of the chemical shift for H-2, H-8, and H-1′ of $Zn(IDP)^-$ (upper part) and of $Cd(IDP)^-$ (lower part) with varying concentrations of $M(IDP)^-$. The experimental data are those of Figure 1, where the experimental conditions are also given. The curves shown are the computer-calculated best fit of the experimental data by using the model given by eq 4–7 and the constants K_{st} = 2.6 M^{-1} (see ref 39) and K_D^* = 50 M^{-1} for both the $Zn(IDP)^-$ and $Cd(IDP)^-$ 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 the text in section 4).

the order of K_{av} obtained with the noncooperative stacking model for $Mg(ADP)^-$ and $Mg(IDP)^-$, respectively,[39] and that the dimerization constant K_D^* is within the range 25–500 M^{-1} for $Zn(ADP)^-$ and $Cd(ADP)^-$, and 25–250 M^{-1} for $Zn(IDP)^-$ and $Cd(IDP)^-$. The curves plotted in Figure 3 are a least-squares fit of the experimental data for $Zn(IDP)^-$ and $Cd(IDP)^-$ from Figure 1, calculated with K_{st} and K_D^* fixed at 2.6 M^{-1} and 50 M^{-1}, respectively, for both systems.[40] It is evident that the model according to eq 4–7 is able to explain the experimental data.

(5) Further Evidence from Chemical Shifts for the Formation of Intermolecular Bridges by Zn^{2+} or Cd^{2+} in Purine Nucleotide Stacks. It is well-known that protonation or coordination of a diamagnetic metal ion to a binding site deshields neighboring protons and therefore the resonance signals for such protons shift *down*field. Although in aromatic systems no simple relation between the distance of the site of protonation or metalation and the size of the downfield shifts of the observed protons exists,[10,41] we have compiled in Table IV the values of δ_∞ for H-8 on different ADP and IDP stacks, together with some related data, including those[11] for ATP and ITP stacks. If Zn^{2+} and Cd^{2+} coordinate in the dimeric stacks to N-7 forming the *inter*molecular metal ion bridge discussed in section 4, this should also affect the values of δ_∞, even though the orientation in the stacks might be somewhat different and the δ_∞ values were calculated with the noncooperative stacking model (Table II). Hence, at least δ_∞ for H-8 of $Zn(ADP)^-$, $Cd(ADP)^-$, $Zn(IDP)^-$, and $Cd(IDP)^-$ should be shifted downfield compared to the δ_∞ shifts for H-8 of $Mg(ADP)^-$ and $Mg(IDP)^-$, because in these Mg^{2+}-containing stacks the effect of charge neutralization operates but practically no N-7 interaction[4,9,11] should occur.

Table IV. Importance of the M^{2+}–N-7 Interaction for the Self-Association of $M(NDP)^-$ and $M(NTP)^{2-}$ Complexes, As Judged from a Comparison of the Chemical Shifts (ppm) of H-8 in Several Stacks (δ_∞)[a] and from the Downfield Shifts, $\Delta\delta_\infty$, between Stacks Containing Zn^{2+} or Cd^{2+} and Those with Mg^{2+} (D_2O; 27 °C)[b]

	shifts for H-8		downfield shifts, $\Delta\delta_\infty$,[d] for	
systems[a,b]	δ_∞	$\Delta\delta_\infty$ (downfield)[c]	H-2	H-1′
adenosine	8.07 ± 0.04			
ADP^{3-}	8.03 ± 0.09			
$Mg(ADP)^-$	8.10 ± 0.03			
$Zn(ADP)^-$	8.57 ± 0.02	0.47 ± 0.05	0.45 ± 0.06	0.10 ± 0.05
$Cd(ADP)^-$	8.74 ± 0.04	0.64 ± 0.07	0.40 ± 0.06	0.26 ± 0.05
inosine	8.06 ± 0.03			
IDP^{3-}	8.01 ± 0.15			
$Mg(IDP)^-$	8.08 ± 0.07			
$Zn(IDP)^-$	7.95 ± 0.06	−0.13 ± 0.13	0.03 ± 0.06	−0.22 ± 0.13
$Cd(IDP)^-$	8.24 ± 0.03	0.16 ± 0.10	0.16 ± 0.06	0.09 ± 0.08
ATP^{4-}	7.92 ± 0.06			
$Mg(ATP)^{2-}$	8.02 ± 0.04			
$Zn(ATP)^{2-}$	8.36 ± 0.06	0.34 ± 0.10	0.40 ± 0.13	0.18 ± 0.08
$Cd(ATP)^{2-}$	8.49 ± 0.08	0.47 ± 0.12	0.42 ± 0.21	0.25 ± 0.14
ITP^{4-}	7.59 ± 0.60			
$Mg(ITP)^{2-}$	8.05 ± 0.08			
$Zn(ITP)^{2-}$	8.08 ± 0.14	0.03 ± 0.22	0.02 ± 0.14	0.00 ± 0.16

[a] The corresponding shifts for the nucleosides are given for comparison (Table II).[11] [b] The values for the diphosphates are from Table II and those for the triphosphates are from Table III of ref 11. [c] Shift difference $\Delta\delta_\infty$ for H-8 between the shifts of $Zn(NDP \text{ or } NTP)^{-/2-}$ or $Cd(NDP \text{ or } NTP)^{-/2-}$ and $Mg(NDP \text{ or } NTP)^{-/2-}$. The errors given are the sum of the errors (see footnotes *b* and *d* in Table II) of the values used for the calculation of $\Delta\delta_\infty$. [d] Differences for H-2 and H-1′ as defined for H-8 in footnote *c*; calculated from the values of δ_∞ listed in Table II and in Table III of ref 11.

Indeed, the values in Table IV of δ_∞ for H-8 of ADP^{3-} and $Mg(ADP)^-$ are the same within experimental error, while these shifts for $Zn(ADP)^-$ and $Cd(ADP)^-$ are significantly further downfield. The same observation is made for the corresponding systems with IDP, ATP, and ITP, and all these downfield shifts are of a reasonable order as is indicated by a comparison with the shifts obtained for the monomeric systems (see section 7 and Table V).

To facilitate further the comparison of the properties between the complexes of Zn^{2+} or Cd^{2+} and those of Mg^{2+} the shift differences, $\Delta H_\infty = \delta_{\infty(Zn,Cd\text{-}NDP,NTP)} - \delta_{\infty(Mg\text{-}NDP,NTP)}$, were calculated for H-8, H-2, and H-1′; these results are also listed in Table IV.

It is evident that the downfield shift difference $\Delta\delta_\infty$ for a given system is about the same size for the aromatic protons H-8 and H-2, while it is considerably smaller for the ribosyl proton H-1′ (Table IV). The latter result is expected because H-1′ is relatively far from the metal binding site at the nucleic base moiety. However, the comparable size of $\Delta\delta_\infty$ for H-8 and H-2 should not lead to the conclusion that both N-7 *and* N-1 are involved in bridging of the adenine nucleotide stacks, because a similar result is observed also for the hypoxanthine derivatives where N-1 carries a proton and therefore has no metal ion coordinating properties.[42] This result is rather a further manifestation of the observation[10,41] that for aromatic ring systems no simple relation exists between the distance of the binding site and the protons at the aromatic system whose downfield shifts are observed.

Taken together, the results of Table IV provide further support for the formation of *inter*molecular bridges by Zn^{2+} and Cd^{2+} in stacks of purine nucleotides. In addition, the downfield shifts $\Delta\delta_\infty$ are larger and the stacks more stable for the adenine derivatives than for the hypoxanthine derivatives (Table I; sections 1 and 3). Moreover, the Zn^{2+}-bridged stacks are apparently less stable, as is suggested by the "crude" values of K_{av} in Table I and also by

(39) $K_{st} \simeq 6.4$ M^{-1} for $Zn(ADP)^-$ or $Cd(ADP)^-$ and $K_{st} \simeq 2.6$ M^{-1} for $Zn(IDP)^-$ or $Cd(IDP)^-$ are reasonable, because in the corresponding equilibria (eq 6), like in those for $Mg(ADP)^-$ or $Mg(IDP)^-$ (eq 2), only charge effects are operating.

(40) The experimental data for the $Zn(ADP)^-$ and $Cd(ADP)^-$ systems could equally well be fitted with K_{st} = 6.4 M^{-1} (see ref 39) and K_D^* = 50 M^{-1}.

(41) Orbell, J. D.; Solorzano, C.; Marzilli, L. G.; Kistenmacher, T. J. *Inorg. Chem.* **1982**, *21*, 2630–2636.

(42) Only after deprotonation does N-1 of ITP^{4-} (or IDP^{3-}) show coordinating properties.[11,43]

(43) Sigel, H. *J. Am. Chem. Soc.* **1975**, *97*, 3209–3214.

the values of $\Delta\delta_\infty$ in Table IV: the downfield shifts are more pronounced in the stacks containing Cd^{2+}, suggesting a larger degree of bridging with this metal ion than with Zn^{2+}. In fact, it appears from these data that the *inter*molecular interaction in the stacks of $Zn(IDP)^-$ and $Zn(ITP)^{2-}$ is not very pronounced, a conclusion that is further supported by the stability data of Table I: comparison of the association constants for $Mg(IDP)^-$ and $Mg(ITP)^{2-}$ with the corresponding apparent constants for $Zn(IDP)^-$ and $Zn(ITP)^{2-}$ indicates that the stability of the stacks for the two latter complexes may only be slightly larger.

(6) Some General Considerations on the Influence of Metal Ions on the Self-Stacking Tendency of Nucleoside 5′-Diphosphates. 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 ions, only a limited number of complexes with nucleoside 5′-diphosphates could be studied (see footnote *b* in Table I). Nevertheless, several extrapolations can be made from the results obtained by taking into account some general principles of coordination chemistry.

No $M(GDP)^-$ complexes could be studied, but it is to be expected that metal ions like Mg^{2+} or Ca^{2+} promote the self-association also simply via charge neutralization (see section 3), while Zn^{2+} and Cd^{2+} may also form *inter*molecular bridges within the stacks (sections 4 and 5); to a first approximation properties similar to those of the M^{2+}–IDP^{3-} systems may be anticipated.

Regarding the properties of purine-NDP systems in the presence of metal ions other than Zn^{2+} or Cd^{2+}, one may conclude that all metal ions that have a reasonable coordination tendency toward both oxygen *and* nitrogen binding sites will facilitate self-association mainly via the formation of *inter*molecular metal ion bridged dimers, $[M(NDP)]_2^{2-}$ (sections 4 and 5). Such ions include Ni^{2+} and Cu^{2+} and to some extent also Mn^{2+}. All metal ions with a strong preference for O binding sites will act only via charge neutralization (section 3).

No data are available on the influence of Zn^{2+} or Cd^{2+} on the self-association of pyrimidine-NDP systems. However, it appears that a promotion via an *inter*molecular interaction is not likely for UDP^{3-} (and $dTDP^{3-}$) because the base moiety has only carbonyl-oxygen binding sites if N-3 is not deprotonated (see Chart I). An *inter*molecular interaction in M^{n+}–CDP^{3-} systems seems also not very likely, as the cytosine ring has little aromaticity[34,44] and therefore its "tendency to form mixed phosphate complexes (cf. ref 14–16) may be expected to be weaker than of the more aromatic purines".[4] Indeed, as will be shown in section 7, there is no metal ion–N-3 interaction in the monomeric $Zn(CDP)^-$ and $Cd(CDP)^-$ complexes. Hence, one may predict that promotion of the self-association of pyrimidine nucleoside 5′-diphosphates occurs only via charge neutralization, as it has been observed for $Mg(CDP)^-$ and $Mg(UDP)^-$ (section 3).

The general trends indicated in this section for the self-association of NDP^{3-} complexes are also in accordance with present knowledge on NTP^{4-} complexes.[11]

(7) Intramolecular Metal Ion–N-7 Interaction in Monomeric Nucleoside 5′-Diphosphate Complexes of Zn^{2+} and Cd^{2+}. The possibility that for certain metal ion–nucleotide combinations an *intra*molecular chelate formation between the phosphate residue and the base moiety may occur has fascinated chemists for many years.[45,46] The formation of such macrochelates is now well established for several purine-NTP complexes,[4,11] but for nucleoside 5′-diphosphates only a single example is known in detail, $Ni(ADP)^-$,[9,47] although for another, $Cu(ADP)^-$,[48] some early hints exist.

Table V. Evidence for a Zn^{2+}- or Cd^{2+}–N-7 Interaction in $M(NDP)^-$ Complexes, from a Comparison of the Chemical Shifts (δ_0; ppm) of H-8 for Monomeric Purine Nucleoside 5′-Diphosphates and Their Monomeric Metal Ion Complexes (D_2O; 27 °C; I = 0.1, $NaNO_3$)[a]

system	nucleotide: δ_0 of H-8 (or H-5)[a]	downfield shift[b] $\Delta\delta_0$	downfield shift $\Delta\delta'$ for the corresponding nucleoside system[c]	estimated % $M(NDP)_{cl}^-$ (eq 8, 9)
ADP^{3-}	8.552 ± 0.009[d]			
$Mg(ADP)^-$	8.535 ± 0.010[d]			0
$Zn(ADP)^-$	8.62[e]	0.08	~0.4[f]	~20
$Cd(ADP)^-$	8.70[e]	0.16	0.39	41
IDP^{3-}	8.513 ± 0.007[d]			
$Mg(IDP)^-$	8.512 ± 0.008[d]			0
$Zn(IDP)^-$	8.72[e,g]	0.21	0.44	48
$Cd(IDP)^-$	8.79[e,g]	0.28	0.29	97
GDP^{3-}	8.139 ± 0.006[d]			
$Mg(GDP)^-$	8.16[e,h]			0
$Zn(GDP)^-$	8.43[e,h]	0.27	0.36	75
$Cd(GDP)^-$	8.48[e,h]	0.32	0.27	~100
CDP^{3-}	6.133 ± 0.003[d,i]			
$Mg(CDP)^-$	6.123 ± 0.003[d,i]			0
$Zn(CDP)^-$	6.09[e,h,i]	0		0[j]
$Cd(CDP)^-$	6.09[e,h,i]	0		0[j]
UDP^{3-}	5.971 ± 0.004[d,i]			
$Mg(UDP)^-$	5.965 ± 0.002[d,i]			0
$Zn(UDP)^-$	5.98[e,h,i]	0		0
$Cd(UDP)^-$	5.97[e,h,i]	0		0

[a] The corresponding data (δ_0 of H-5) for the pyrimidine-NDP systems are given for comparison. The percentage of the macrochelated isomers, $M(NDP)_{cl}^-$, is estimated from the downfield shifts $\Delta\delta'$ observed for H-8 in the corresponding nucleoside–metal ion systems.[c] [b] Shift difference $\Delta\delta_0$ for H-8 (or H-5) between $Zn(NDP)^-$ or $Cd(NDP)^-$ and $Mg(NDP)^-$. [c] Downfield shifts obtained upon complexation between the nucleoside and Zn^{2+} or Cd^{2+}; the values are taken from Tables V and VI of ref 11. With these $\Delta\delta'$ values and $\Delta\delta_0$, the percentage of $M(NDP)_{cl}^-$ is calculated [% closed isomer = $(\Delta\delta_0/\Delta\delta') \times 100$]; this then allows calculation of the intramolecular equilibrium constant K_I (eq 9). [d] Value from Table II. [e] Graphically extrapolated; the estimated error is ±0.01 ppm. [f] Estimation based on the Cd^{2+}–adenosine system (see ref 11). [g] See Figure 1. [h] Value determined from dilute solutions (~0.002–0.005 M); see also footnote *b* of Table I. [i] δ_0 of H-5. [j] Even though there is no indication for a metal ion interaction with the cytosine moiety in $M(CDP)^-$ [or $M(CTP)^{2-}$], $M(cytidine)^{2+}$ complexes exist.[19]

We have seen in section 3 that the $Mg(NDP)^-$ complexes behave "normally", i.e., the shifts for H-2, H-8, and H-1′ (Figure 1) or for H-5, H-6, and H-1′ (Figure 2) vary continuously with increasing concentration and, for each complex, curve fitting of all three protons (where possible) results in the same value of the association constants (Table I). The Zn^{2+} and Cd^{2+} complexes of ADP^{3-} and IDP^{3-} behave differently (section 4): the shifts for H-8 deviate at low complex concentration from the curve expected on the basis of the shifts for H-2 and H-1′, indicating a metal ion–base interaction (Figure 1). Therefore, to learn details about the extent of *intra*molecular chelate formation in these Zn^{2+} and Cd^{2+} purine-NDP complexes, we have extrapolated the shifts for H-8 to infinite dilution (δ_0) and have thus obtained the shifts for H-8 in the monomeric $M(NDP)^-$ complexes.[49a] This procedure was also used for the shifts for H-5 of the pyrimidine systems.[49b] These values are listed in Table V together with the corresponding values of δ_0 for $Mg(NDP)^-$ and uncomplexed NDP^{3-}. The δ_0

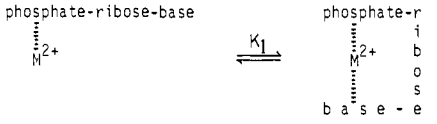
(44) Schweizer, M. P.; Chan, S. I.; Ts'o, P. O. P. *J. Am. Chem. Soc.* **1965**, *87*, 5241–5247.
(45) Szent-Györgyi, A. In "Enzymes: Units of Biological Structure and Function", Gaebler, O. H., Ed.; Academic Press: New York, 1956; p 393.
(46) Schneider, P. W.; Brintzinger, H.; Erlenmeyer, H. *Helv. Chim. Acta* **1964**, *47*, 992–1002.
(47) Miller Frey, C.; Stuehr, J. E. *J. Am. Chem. Soc.* **1978**, *100*, 139–145.
(48) (a) Cohn, M.; Hughes, T. R. *J. Biol. Chem.* **1962**, *237*, 176–181. (b) Schneider, P. W.; Brintzinger, H. *Helv. Chim. Acta* **1964**, *47*, 1717–1733. (c) Sigel, H. *Experientia* **1966**, *22*, 497–499.

(49) (a) The shifts for H-2 (where available, and partly also of H-1′) could also have been used for this evaluation, but their shift differences are smaller than those for H-8; this is expected as the site of coordination, i.e., N-7,[4,9,11] is next to H-8 (see also the discussion in section 5). (b) For the pyrimidine derivatives the largest shift difference is expected for H-5; see footnote 87 in ref 11.

values of those systems that formed precipitates at higher concentrations (Table I, footnote *b*) were obtained from measurements in diluted solutions and are also included in Table V.

It is clear from the data in Table V that the shifts for NDP^{3-} and $Mg(NDP)^-$ are similar and no downfield shift can be observed for any of the Mg^{2+} complexes. This is in accord with the low coordination tendency of Mg^{2+} toward N-7 of the purine residue and toward N-3 of the cytosine moiety,[4,9,50,51] and it also agrees with the results obtained for $Mg(NTP)^{2-}$ complexes.[11] Moreover, an *intra*molecular macrochelate formation in $Zn(CDP)^-$, $Cd(CDP)^-$, $Zn(UDP)^-$, and $Cd(UDP)^-$ also does not occur, a result that again agrees with earlier observations for complexes of CTP^{4-} and UTP^{4-}.[4,9,11,50]

However, for all purine-NDP complexes with Zn^{2+} or Cd^{2+}, i.e. $Zn(ADP)^-$, $Cd(ADP)^-$, $Zn(IDP)^-$, $Cd(IDP)^-$, $Zn(GDP)^-$, and $Cd(GDP)^-$, there is clearly a downfield shift for 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, $M(NDP)_{op}^-$, and a "closed" species, $M(NDP)_{cl}^-$, exists:



(8)

$$K_1 = [M(NDP)_{cl}^-]/[M(NDP)_{op}^-] \quad (9)$$

The position of this intramolecular equilibrium 8 and hence a value for the dimensionless equilibrium constant K_1 (eq 9) can be estimated from the downfield shifts $\Delta\delta_0$ observed for H-8 of the monomeric purine-NDP complexes of Zn^{2+} or Cd^{2+} (Table V, column 3) and the values $\Delta\delta'$ for complete complexation at N-7 (column 4), which were obtained previously[11] from experiments with the corresponding nucleosides, adenosine, and inosine, and guanosine.[52] The resulting estimates for the percentages of the "closed" isomers $M(NDP)_{cl}^-$, are given at the right 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 $Cd(NDP)^-$ complexes, which is in agreement with the slightly larger stability observed[11] for the $Cd(nucleoside)^{2+}$ complexes and the apparently somewhat more effective promotion of purine-NDP stacking by Cd^{2+} (section 5). We assume that in the macrochelated complexes of IDP^- and CDP^- the metal ion coordinates only to a single position at the base, namely, mainly to N-7, just as in $Zn(ADP)^-$ and $Cd(ADP)^-$; 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".

(8) Extent of Macrochelate Formation in Monomeric Adenosine 5′-Diphosphate Complexes. Even though the position of the intramolecular equilibrium 8 could be estimated only for the Zn^{2+} and Cd^{2+} complexes of the purine nucleoside 5′-diphosphates, the results described in section 7 prove that both the open and closed isomers exist in aqueous solution. This should provide a solid basis for a general estimation of the position of equilibrium 8 for $M(NDP)^-$ complexes from stability data; unfortunately, only a very limited number of such data is presently available.[5-7] Stability constants determined by potentiometric pH titrations exist only for some $M(ADP)^-$ and $M(CDP)^-$ complexes;[26,53] fortunately, the corresponding experiments have been carried out at relatively low concentrations in which only little self-association occurs.

Mariam and Martin[9] have recently shown that the intramolecular equilibrium constant K_1 (eq 8 and 9) can be deduced (see also ref 47 and 54–56) from the experimentally accessible overall stability constant, $K^M_{M(NDP)}$ (eq 10), using eq 11:

$$K^M_{M(NDP)} = [M(NDP)^-]/([M^{2+}][NDP^{3-}]) \quad (10)$$

$$K_1 = \left(K^M_{M(NDP)}/K^M_{M(NDP)_{op}}\right) - 1 \quad (11)$$

$K^M_{M(NDP)_{op}}$ is the stability constant of the open isomer $M(NDP)_{op}^-$ (eq 8 and 9). The accuracy of the logarithm of the ratio from eq 11

$$\log \Delta = \log K^M_{M(NDP)} - \log K^M_{M(NDP)_{op}} \quad (12)$$

depends very much on the experimental error in the constants, and this error becomes more important the more similar the two constants are. Moreover, $K^M_{M(NDP)_{op}}$ is usually not directly accessible by experimental determinations; in the present case the stability constants determined for $M(H{\cdot}P_2O_7)^-$ complexes,[5b,7b,57] which cannot exist in a closed form, provide estimates for log $K^M_{M(NDP)_{op}}$ (see footnotes *b* and *c* in Table VI). Fortunately, the differences log Δ (eq 12) are relatively large in the present cases; hence, reasonable estimates may be expected for the intramolecular equilibrium constants K_1 (eq 8 and 9) and the percentage of the closed isomer $M(NDP)_{cl}^-$ (see footnotes *c* and *e* of Table VI). The results of these calculations, together with the data on which they are based, are given in Table VI.

Entries no. 1–6 (I = 0.1, KNO_3; 15 °C) of Table VI, which are based on a single and very careful study,[53] support several conclusions from section 7: (i) there is no metal ion–base interaction in $Mg(CDP)^-$ or $Mg(ADP)^-$; (ii) such an interaction is also not expected (based on section 7) for $Ni(CDP)^-$ and is indeed not observed, a result also in agreement with spectrophotometric measurements;[9] (iii) such an interaction is expected for $Ni(ADP)^-$, and in this case a considerable percentage of $Ni(ADP)^-$ in fact exists as the macrochelated isomer, a result again in accord with spectrophotometric measurements.[9] Overall, the data of Table VI clearly confirm the conclusions of section 7 and extent our knowledge about the intramolecular equilibrium 8 in $M(NDP)^-$ complexes to additional examples.

In the present context it should be mentioned that Mariam and Martin[9] have recently suggested the existence of a third isomer in which the metal ion is coordinated inner sphere to the phosphate residue but only outer sphere, via a water molecule, to the adenine moiety. Mariam and Martin estimate that for $Ni(ADP)^-$ about 65% exists as the macrochelated inner-sphere isomer, about 15% as the water-bridged outer-sphere isomer, and the remaining 20% in the "open" (i.e., phosphate-bound) form. The existence of such a third outer-sphere isomer would also explain why the NMR measurements indicated only about 20% of $Zn(ADP)_{cl}^-$ (Table V), while evaluation of the potentiometrically determined stability data leads to an estimate of about 70% (no. 21 in Table VI). Similar "discrepancies" have also been observed between the percentages obtained by different methods for the closed isomers of $M(ATP)^{2-}$ complexes.[11] Perhaps this is a further indication that such a third isomer actually exists in adenine nucleotide complexes.

It is gratifying to note that the two evaluations of independent stability data for $Ni(ADP)^-$ both give about 80% for the closed isomer (no. 6 and 16 in Table VI). Moreover, the percentage of the macrochelated isomer varies within the series $Mn(ADP)_{cl}^- < Co(ADP)_{cl}^- < Ni(ADP)_{cl}^- < Cu(ADP)_{cl}^- > Zn(ADP)_{cl}^-$. This

(50) Miller Frey, C.; Stuehr, J. E. *Met. Ions Biol. Syst.* **1974**, *1*, 51–116. Cf. Vol. 1 of ref 2.

(51) Banyasz, J. L.; Stuehr, J. E. *J. Am. Chem. Soc.* **1973**, *95*, 7226–7231.

(52) (a) An evaluation by curve fitting (see ref 11) of recently published ^1H NMR shift data (see figure 2 in ref 52b) obtained in D_2O for the Zn^{2+}–9-(β-D-ribofuranosyl)purine system gives $\Delta\delta'_{H\text{-}8} = 0.28$ ppm, $\Delta\delta'_{H\text{-}6} = 0.40$ ppm, $\Delta\delta'_{H\text{-}2} = 0.27$ ppm, and log $K^{Zn}_{Zn(L)} = 0.7 \pm 0.1$. These results agree well with those obtained previously for other purine nucleoside systems.[11] (b) Lönnberg, H.; Arpalahti, J. *Inorg. Chim. Acta* **1981**, *55*, 39–42.

(53) Miller Frey, C.; Stuehr, J. E. *J. Am. Chem. Soc.* **1972**, *94*, 8898–8904.

(54) Schwarzenbach, G.; Anderegg, G.; Schneider, W.; Senn, H. *Helv. Chim. Acta* **1955**, *38*, 1147–1170.

(55) Taylor, R. S.; Diebler, H. *Bioinorg. Chem.* **1976**, *6*, 247–264.

(56) (a) Sigel, H.; Scheller, K. H.; Rheinberger, V. M.; Fischer, B. E. *J. Chem. Soc., Dalton Trans.* **1980**, 1022–1028. (b) Sigel, H. *Angew. Chem.* **1982**, *94*, 421–432; *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 389–400.

(57) Sigel, H.; McCormick, D. B. *Acc. Chem. Res.* **1970**, *3*, 201–208.

Table VI. Evidence for Intramolecular Macrochelate Formation in Metal Ion Complexes of Adenosine 5′-Diphosphate: Estimations of the Intramolecular Dimensionless Equilibrium Constant K_I and of the Percentage of the Closed Isomer $M(ADP)_{cl}^-$ ($I \simeq 0.1$; 15 or 25 °C); the Percentage of $M(ATP)_{cl}^{2-}$ Is Given for Comparison

no.	complex	log $K^M_{M(H\cdot P_2O_7)}$ or log $K^M_{M(NDP)}$ (eq 10)[a]	log $K^M_{M(NDP)_{op}}$ (cf. *b*)	log Δ (eq 12)	K_I (eq 9, 11)	% $M(NDP)_{cl}^-$ (eq 8)	% $M(NTP)_{cl}^{2-}$ (ref 11)
1	$Mg(H\cdot P_2O_7)^-$	3.18 } *b*	3.20[c]				
2	$Mg(CDP)^-$	3.22 }					
3	$Mg(ADP)^-$	3.21	3.20	0	0	0	
4	$Ni(H\cdot P_2O_7)^-$	3.50 } *b*	3.49[c]				
5	$Ni(CDP)^-$	3.48 }					
6	$Ni(ADP)^-$	4.18	3.49	0.69	3.9	80	
7	$Mn(ADP)^-$	4.16	3.8[d]	0.3 (5)	1.2	55 (71)[e]	38
8	$Co(H\cdot P_2O_7)^-$	3.4					
9		*4.07* }	3.76[c,e]				
10		3.5					
11		*4.05* }					
12	$Co(ADP)^-$	4.20	3.8	0.4	1.5	60 (80)[e]	57
13	$Ni(H\cdot P_2O_7)^-$	3.71					
14		*3.81* }	3.78[c,e]				
15		*3.83* }					
16	$Ni(ADP)^-$	4.50	3.8	0.7	4	80 (84)[e]	74
17	$Cu(H\cdot P_2O_7)^-$	4.45					
18		*5.37* }	4.74[c,e]				
19		4.4					
20	$Cu(ADP)^-$	5.90	4.7	1.2	15	94 (97)[e]	76
21	$Zn(ADP)^-$	4.28	3.8[d]	0.5	2	67 (80)[e]	62

[a] These constants were determined by potentiometric pH titrations. Entries no. 1–6 are from ref 53 ($I = 0.1$, KNO_3; 15 °C), no. 7, 12, 16, 20, and 21 from ref 26 ($I = 0.1$, KNO_3; 25 °C; given error limits, ±0.02 log unit), no. 8, 9, 13, 14, 17, and 18 ($I = 0.1$–1.0; 25 °C) from ref 7b, and no. 10, 11, 15, and 19 ($I = 0.1$, $NaNO_3$ or $(CH_3)_4N^+Cl^-$; 25 °C) from ref 5b. There were no other usable constants available in the literature (ref 5–7). [b] The acidity constants (at $I = 0.1$, KNO_3; 15 °C)[53] of $H(CDP)^{2-}$ ($pK^H_{H(CDP)} = 6.38$) and of $H(ADP)^{2-}$ ($pK^H_{H(ADP)} = 6.41$) are very similar, while the value for $H_2(P_2O_7)^{2-}$ ($pK^H_{H(H\cdot P_2O_7)} = 6.02$ is somewhat smaller, i.e. $H(P_2O_7)^{3-}$ is somewhat less basic than CDP^{3-} and ADP^{3-}. Despite these differences in basicity the stability constants of $Mg(H\cdot P_2O_7)^-$, $Mg(CDP)^-$, and $Mg(ADP)^-$ are identical within experimental error (no. 1–3); this is also true for $Ni(H\cdot P_2O_7)^-$ and $Ni(CDP)^-$ (no. 4 and 5). This means it is possible to use the constants obtained for $M(CDP)^-$ complexes directly as log $K^M_{M(NDP)_{op}}$, because in monomeric $M(CDP)^-$ complexes no metal ion–base interaction occurs (see section 7) and because the acidity constants of $H(CDP)^-$ and $H(ADP)^-$ are the same; furthermore, the constants obtained for $M(H\cdot P_2O_7)^-$ complexes can also be used as log $K^M_{M(NDP)_{op}}$ because evidently the lower basicity of $H(P_2O_7)^{3-}$ (compared with that of CDP^{3-} or ADP^{3-}) is compensated by the smaller steric hindrance of its hydrogen compared with that of the cytidine moiety in CDP^{3-} (compare no. 1 with 2 and 4 with 5). It should be emphasized that all the constants discussed in this footnote have been determined under the same conditions in the same laboratory.[53] [c] This value is the average of the constants connected by the brace. Stability constants of $M(H\cdot P_2O_7)^-$ may be used as reasonable estimates for log $K^M_{M(NDP)_{op}}$ as outlined in footnote *b*. The apparently large differences between the listed values arise from the different background electrolytes used in the experiments: the values printed in italics were determined in the presence of $(CH_3)_4N^+$ salts while all the other values were measured in the presence of Na^+ or K^+ salts. We have deliberately included the values determined in the presence of $(CH_3)_4N^+$ salts because this brings us certainly to the "safe side", as in this way log $K^M_{M(NDP)_{op}}$ is rather too large and therefore % $M(NDP)_{cl}^-$ rather too small; hence, all our conclusions regarding the intramolecular equilibrium 8 are definitely correct (see also footnote *e*). [d] The stability constants of $Mn(H\cdot P_2O_7)^-$ and $Zn(H\cdot P_2O_7)^-$ are not known.[5,6a,7b,7c] However, the stability constants of the complexes formed between Mn^{2+}, Co^{2+}, Ni^{2+} or Zn^{2+}, and HPO_4^{2-} or hydrogen triphosphate ($H\cdot P_3O_{10}^{4-}$) are very similar within a phosphate series;[57] this is also confirmed by the present data for $Co(H\cdot P_2O_7)^-$ (no. 8–11; average, 3.76) and $Ni(H\cdot P_2O_7)^-$ (no. 13–15; average, 3.78). Therefore the corresponding average, i.e., log $K^M_{M(H\cdot P_2O_7)}$ = log $K^M_{M(NDP)_{op}}$ = 3.8, was also used as a basis for the evaluation of the stability of the $Mn(ADP)^-$ and $Zn(ADP)^-$ complexes. [e] To show the influence of log $K^M_{M(NDP)_{op}}$ on the resulting calculations, these were also carried out by using the average obtained when neglecting the values printed in italics (see footnote *c*); the corresponding results are given in parentheses in the column of % $M(NDP)_{cl}^-$.

variation reflects very well the different affinities of these metal ions toward ligands with N donors (see, e.g., ref 16). Finally, it should be pointed out that for a given metal ion the percentage of the closed isomer is larger for $M(ADP)^-$ than for $M(ATP)^{2-}$ (Table VI); because the macrochelate is smaller in $M(ADP)^-$ this observation seems also reasonable.

General Conclusions

The results presented show that metal ions promote the self-association of nucleoside 5′-diphosphates in two ways: some metal ions, like Mg^{2+}, augment stacking simply by reducing charge repulsion by coordination to the phosphate residue (section 3); others, like Zn^{2+} or Cd^{2+}, enhance the stability of the dimers by forming an *inter*molecular coordinative link between the phosphate residue of one NDP^{3-} and the base residue of the next, in addition to decreasing the electrostatic repulsion (section 4). This second way is observed only with purine nucleoside 5′-diphosphates and metal ions that have an affinity for phosphate groups *together* with a significant coordination tendency toward nitrogen (like Zn^{2+} or Cd^{2+}). The first way operates in all those cases where the nucleic base of the NDP^{3-} offers no (accessible) binding site (like UDP^{3-} or CDP^{3-}) or where the metal ion has a coordination tendency only toward the phosphate residue (like Mg^{2+} or Ca^{2+}). These observations parallel those made with nucleoside 5′-*tri*phosphates.[11]

That stacking is important also in metal ion–nucleoside 5′-monophosphate systems is indicated by observations made in a thorough study[53] on Ni^{2+} complexes: in Ni^{2+}–5′-AMP^{2-} and Ni^{2+}–2′-AMP^{2-} systems $Ni(AMP)_2^{2-}$ complexes of relatively high stability are formed, while no such 1:2 complexes could be detected in the Ni^{2+} systems with HPO_4^{2-}, ribose 5′-monophosphate (RP^{2-}), or 5′-CMP^{2-}. The simplest explanation focuses on the described stacking properties of the nucleic bases: HPO_4^{2-} and RP^{2-} cannot stack and the stacking tendency of the cytidine moiety in CMP^{2-} is certainly very small, while that for the adenosine moiety in AMP^{2-} is significant (Table I); therefore the formation of $M(AMP)_2^{2-}$ complexes should be augmented by an *intra*molecular stacking interaction between the two AMP^{2-} ligands coordinated

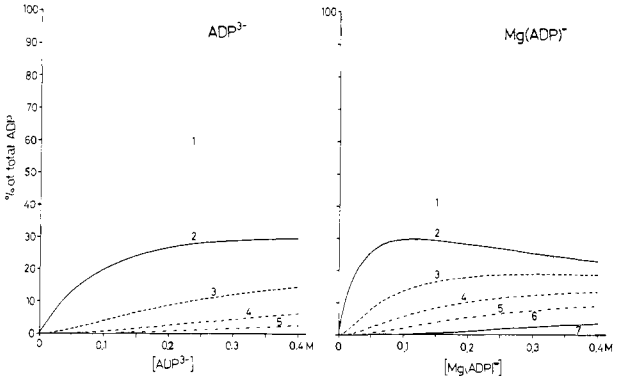


Figure 4. Variation of the proportions of ADP present in the monomer (1), dimer (2), trimer (3), ..., and heptamer (7) in D_2O solutions as a function of the total concentration of ADP^{3-} (K = 1.8 M^{-1}; I = 0.1–1.7) and $Mg(ADP)^-$ (K = 6.4 M^{-1}; I = 0.1–1.2) at 27 °C.

to the same metal ion.[58]

To provide a realistic feeling of how the proportions of the various oligomers vary as the concentration is changed, calculations with the association constants listed in Table I were carried out; two examples are shown in Figure 4. It is evident that the percentage of ADP^{3-} present in the monomeric form decreases rapidly with increasing total concentration of ADP^{3-}; the promotion of stacking by Mg^{2+} is also impressively seen.

One of the main aims of this work was the search for the conditions that must be kept in studies aiming to obtain properties of monomeric nucleoside 5′-diphosphates and their metal ion complexes. To make this information easily accessible Table VII was prepared. This table lists the conditions that must be kept if a certain substrate is to be present to 90, 95, or 97% in the monomeric form. For example, 95% of the substrate is present in the monomeric form in solutions that are 0.044 M in UDP^{3-}, 0.02 M in IDP^{3-}, 0.01 M in $Mg(IDP)^-$, or about 10^{-3} M in $Cd(IDP)^-$ (see footnote *c* in Table VII).

Another important aspect of this work is its proof for the formation of *intra*molecular macrochelates in monomeric $M(NDP)^-$ complexes (eq 8). The extent of nucleic base–metal ion interaction varies from nucleotide to nucleotide and from metal ion to metal ion (Tables V and VI) and may range in practice from insignificant traces to nearly 100% of the macrochelated isomer. The formation of such macrochelates occurs also in $M(NTP)^{2-}$ complexes,[11] and it appears now that for a given metal ion the extent of macrochelate formation decreases from $M(ADP)^-$ to $M(ATP)^{2-}$. There is not yet much known in this respect about M^{2+}–NMP^{2-} systems, but the formation of a macrochelate has been suggested[53] to occur in Ni(AMP), and based on spectrophotometric measurements it was concluded[9] that about 80% of Ni(AMP) exists in a form with Ni^{2+} inner sphere coordinated to the adenine base with possibly a water molecule bridging Ni^{2+} and the (α-)phosphate group, the remaining 20% being phosphate coordinated. Some information exists also about Co(AMP) and Ni(IMP).[59]

It must be assumed that in living systems the self-association of some nucleotides may be considerable under certain conditions, especially in the presence of metal ions suitable for bridging. Moreover, in very dilute solutions where only monomeric species exist, metal ions will enforce different structures through complexation: e.g., Mn^{2+} and Zn^{2+} will form macrochelates with purine nucleotides to some exent, while Mg^{2+} and Ca^{2+} will not. It is expected that such structural differences are reflected in the course of enzymic reactions. Indeed, there is a related example:

(58) (a) Such intramolecular ligand–ligand interactions are well known for mixed-ligand complexes.[15,17,56b,58b] (b) Fischer, B. E.; Sigel, H. *J. Am. Chem. Soc.* **1980**, *102*, 2998–3008.

(59) (a) Peguy, A.; Diebler, H. *J. Phys. Chem.* **1977**, *81*, 1355–1358. (b) Nagasawa, A.; Diebler, H. *J. Phys. Chem.* **1981**, *85*, 3523–3528.

Table VII. Concentrations of D_2O Solutions for Several NDP Substrates in Which 90%, 95%, or 97% of the Total Concentrations Are Present in Monomeric Form (27 °C)[a]

system	K,[b] M^{-1}	at the concentrations given below, in mM, are present in the monomeric form 90%	95%	97%
ADP^{3-}	1.8	32	15	9
$Mg(ADP)^-$	6.4	9	4	2.5
$Zn(ADP)^-$/$Cd(ADP)^-$	100[c]	*0.57*	*0.27*	*0.16*
	(6.4/50)[c]	*1.2*	*0.55*	*0.32*
IDP^{3-}	1.3	44	20	12
$Mg(IDP)^-$	2.6	22	10	6
$Zn(IDP)^-$	4[c]	*14*	*6.7*	*3.9*
	(2.6/50)[c]	*1.2*	*0.55*	*0.32*
$Cd(IDP)^-$	7[c]	*8.1*	*3.8*	*2.2*
	(2.6/50)[c]	*1.2*	*0.55*	*0.32*
GDP^{3-}	1.0	57	27	16
CDP^{3-}	0.7	81	38	22
$Mg(CDP)^-$	1.1	52	24	14
UDP^{3-}	0.6	95	44	26
$Mg(UDP)^-$	1.4	41	19	11

[a] The detailed experimental conditions are given in Table I. [b] The constants used for the calculations are the values given under K_{av} in Table I, except those values in parentheses that are from section 4 (see also Figure 3); the first value in parentheses is always due to K_{st} and the second to K_D*.[c] [c] It is evident that the calculations for $Zn(ADP)^-$, $Cd(ADP)^-$, $Zn(IDP)^-$, and $Cd(IDP)^-$ with K_{av} (Table I) and K_{st}/K_D* (section 4) give different results. We have deliberately *not* adjusted the several K values in such a way that the same concentrations of monomeric species result (which could easily have been done); due to the experimental difficulties we cannot obtain association constants for these complexes of a higher accuracy (but the given values are of course in accordance with the experiments; see Figures 1 and 3 and section 4). To indicate this uncertainty the concentrations due to these four complexes are printed in italics. It is our recommendation to work with 10^{-3} M (or more diluted) solutions in any study that aims for properties of the monomeric complexes.

RNA polymerase of *Escherichia coli* contains 2 mol of Zn^{2+}/mol of enzyme, and for the Co^{2+}/Zn^{2+} derivative (one Zn^{2+} exchanged for one Co^{2+}) it has been suggested that the cobalt ion is located at the initiation site, that ATP is in direct coordination with this intrinsic metal ion, probably via N-7, and that the metal ion plays a role in discriminating the initiating nucleotide and in orientating it in a stereospecific position suitable for catalysis.[60]

To conclude, it is evident that nucleotides are very versatile in their interactions with metal ions already in binary systems and certainly even more so in mixed-ligand systems[15,17] where ternary complexes may be formed.

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Registry No. ADP^{3-}, 52322-03-9; Mg(ADP), 7384-99-8; Zn(ADP), 12295-00-0; Cd(ADP), 79189-47-2; IDP^{3-}, 86527-68-6; Mg(IDP), 7219-40-1; Zn(IDP), 86527-65-3; Cd(IDP), 75898-68-9; GDP^{3-}, 86527-69-7; Zn(GDP), 86527-67-5; CDP^{3-}, 86542-47-4; CDP, 63-38-7; UDP^{3-}, 86527-70-0; UDP, 58-98-0; AMP^{2-}, 6042-43-9; ATP^{4-}, 13265-06-0; ITP^{4-}, 86527-71-1; GTP^{4-}, 86527-72-2; CTP^{4-}, 86527-73-3; UTP^{4-}, 86527-74-4; Mg(ATP), 1476-84-2; Zn(AtP), 6602-83-1; Cd(ATP), 72052-13-2; Mg(ITP), 7219-40-1; Zn(ITP), 75898-66-7; Mg(GDP), 7277-99-8; Cd(GDP), 86527-66-4; Zn, 7440-66-6; Cd, 7440-43-9; Mg, 7439-95-4; Ni, 7440-02-0; Ni(ADP), 83862-74-2; Mn(ADP), 69828-68-8; Co(ADP), 78969-59-2; Cu(ADP), 69828-70-2; adenosine, 58-61-7; inosine, 58-63-9; guanosine, 118-00-3; cytidine, 65-46-3; uridine, 58-96-8.

(60) (a) Chatterji, D.; Wu, F. Y.-H. *Biochemistry* **1982**, *21*, 4657–4664. (b) Wu, F. Y.-H.; Wu C.-W. *Met. Ions Biol. Syst.* **1983**, *15*, 157–192. Cf. vol. 15 of ref 2.