Nuclear Magnetic Resonance Investigations of Adenosine Phosphates and their Mercury and Cadmium Salt Adducts

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Chemical shift and relaxation time measurements (^1H, ^{13}C, and ^{31}P) show that the adenosine di- and triphosphates (ADP and ATP), and to a lesser extent the monophosphate (AMP), are predominantly in the *syn*-conformation at low pH. Furthermore, all three phosphates form well defined complexes with Cd^{II} salts at pH 8, whereas only for ADP and ATP are well defined Hg^{II} and Cd^{II} salt complexes at pH 3 observed. Both metal ions affect the tendency towards stack formation. The conclusions based on relaxation and chemical shift measurements are supported by ^1H nuclear Overhauser effect (n.O.e.) measurements, as far as possible. The different origins of the dipolar relaxation of the base protons are discussed. It is shown that the ribose protons play a major role in the relaxation of H-8 whereas the protons at position 2 are subject to a strong relaxation influence from the phosphate groups. Internucleotide dipolar interactions, however, are of some importance in the case of AMP, whereas in ATP and ADP for H-8, and in ATP also for H-2, their importance is reduced.

Mercury(II) salts are known to bind to and destabilise double-stranded deoxyribonucleic acid (DNA).[1–3] We have decided to investigate the properties of adenosine phosphates in the presence of Hg^{II} to determine whether specific binding sites exist. We have also investigated the effect of Cd^{II} ions. Because of the low solubility of Hg^{II} salts at neutral pH, we were compelled to work at about pH 3. We report here an investigation of the dependence of chemical shifts and relaxation times on the concentration of nucleotides in the absence and in the presence of the metal ions.

Results

Our approach involved the determination of the equilibrium constants for stacking using the concentration dependence of the chemical shifts, a study of the relaxation properties of the stacked and monomeric states, and investigations of the uncomplexed states. Upfield shifts are well known to be induced by stacking processes,[4,5] whereas downfield shifts are representative of complexation processes.[6,7]

In Table 1 the chemical shift values of the different adenosine phosphates in the absence and in the presence of Cd^{II} and Hg^{II} are shown. Neither H-2 nor H-8 of AMP at pH 3 is altered by adding Cd^{II} or Hg^{II}, whereas at pH 8 a downfield shift for H-8 and the opposite for H-2 can be observed. This means that the predominant effect of complexation with Cd occurs at H-8. However, H-2 is affected by the salt-induced increase in stacking tendency. The same is true for ATP (both pH values and both ions) and for the reaction of ADP with Cd^{II}. ADP, however, interacts with Hg^{II} in a very complex manner (see Figure 1). At small Hg^{II} concentrations the stacking is increased, whereas larger salt concentrations force complexation. This complexation affects H-2 more, in contrast to all other cases reported herein. To explain this finding one has to assume that Hg is bound to the ADP base in the neighbourhood of H-2 (probably N-1), whereas in all other cases N-7 is complexed.

With the two alternative processes (complexation and stack formation) in mind, one may try to estimate the equilibrium constants for stacking. By using the isodesmic formulation[8,9] [see equations (1) and (2)] a calculation is possible, at least for those protons which are unaffected by complexation. Figure 2 shows a graph of the concentration dependence for the two base protons in two representative cases. The results of all analyses are given in Table 2.

$$\Delta\delta(k) = \delta_1(k) + H_r(k)[\delta_\infty(k) - \delta_1(k)] \quad (1)$$

$$H_r(k) = F(2)/\gamma + F(3) \quad (2)$$

$$F(2) = 2/a_0 \sum_i a_i$$

$$F(3) = 1/a_0 \sum_i (i-2)a_i$$

$$\gamma = (\delta_3 - \delta_1)/(\delta_2 - \delta_1)$$

where δ_∞ = stack shift, δ_1 = monomer shift, δ_2 = dimer shift, δ_3 = trimer shift (neighbour molecule on both sides), a_0 = total concentration, a_i = concentration of an individual stack, $\Delta\delta$ = calculated shift

Table 2 shows that a striking increase in the equilibrium constants occurs at low pH as compared with high pH. In addition the stacking tendency of ATP is more strongly favoured than that of ADP or AMP. This effect is clearly opposite to the results obtained at high pH.[10–13] The error deviations of the fitted equilibrium constants are mostly within a reasonable range, although in a few cases huge deviations occur. One reason for this behaviour may be the fact that *syn*/*anti* changes in conformation may occur, as will be discussed later. A second reason lies in the aforementioned complexation and the shift changes caused thereby, which overlap stack shifts. The estimated equilibrium constants are in reasonable agreement with previous estimates; for example at pH 8.5 for AMP K 2.1 l mol^{-1}, and for ADP and ATP K 1.3 l mol^{-1}.[10] Very different results, however, were reported for ADP and ATP 1:1 complexes with Cd^{II} at pH 6.6 (K 100 l mol^{-1}, K 17 l mol^{-1}).[10,13] Owing to the different salt concentrations, these values are not comparable with our measurements.

To determine secondary structure from relaxation measurements, it is essential to know the correlation times for tumbling and any internal motion of the molecule under investigation. As a first approximation, we assume that the molecules have spherical symmetry. This is a crude model the chief attraction of

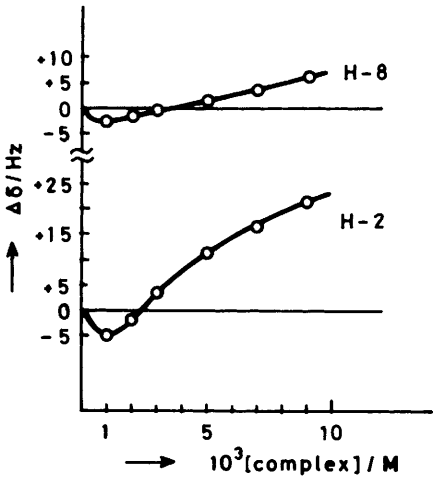


Figure 1. Shift changes (Hz) on addition of Hg^{II} to ADP

Table 1. Chemical shifts (Hz) induced by addition of 2×10^{-2} or 4×10^{-2}M Hg^{II} or Cd^{II}; AMP = ADP = ATP = 0.1M. A minus sign denotes an upfield shift; Cd(n) denotes addition of Cd to a solution of pH 8; an asterisk denotes a solution where no measurement was possible, owing to formation of a gel

		H-8		H-2	
		2×10^{-2}M	4×10^{-2}M	2×10^{-2}M	4×10^{-2}M
AMP	Hg				
	Cd				
	Cd(n)	10	*	−12	*
ADP	Hg	−3	2	−2	12
	Cd	10	15	−30	−48
	Cd(n)	13	25	−21	−37
ATP	Hg	26	37	−1	−3
	Cd	20	30	−14	−20
	Cd(n)	10	*	−50	*

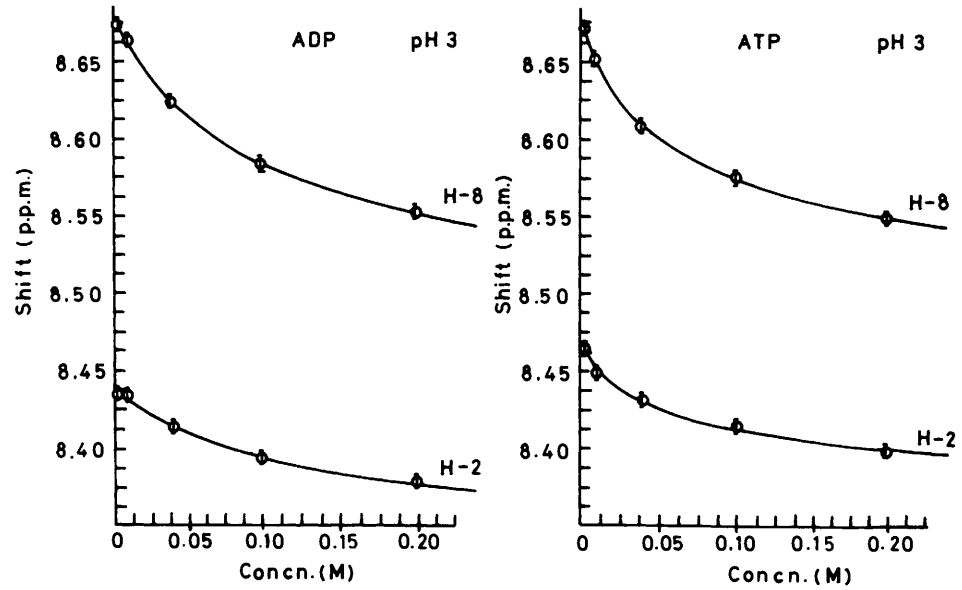


Figure 2. Chemical shifts *versus* the concentration; the points mark the measured values with their mean errors. The curves show the fit obtained using the isodesmic formulation

which is simplicity. Given the thickness of the heterocyclic ring (van der Waals radius 0.32 nm) this approximation may be reasonable for short stacks, *e.g.* dimers to tetramers, but is likely to be a poor approximation for monomers and long stacks (perhaps ellipsoids of revolution would be more appropriate).

The correlation times were estimated from ^{13}C spin–lattice relaxation times[14] of C-2 and C-8 of the bases and C-1′, C-2′, and C-4′ of the ribose. These carbon atoms are relaxed entirely by dipolar interactions with their directly bonded protons (r 0.11 nm). In accordance with the values (for adenosines) published by von Goldammer and his co-workers,[15] obtained in liquid ammonia, almost identical relaxation times for the different ^{13}C atoms were obtained. The correlation time derived from such measurements depends strongly on the model used; as a spherical molecule was assumed, the derived correlation time must be regarded as an effective correlation time. As has been pointed out recently by Levy,[16] for deoxy-sugars one can also estimate a ring pucker correlation time for H-2′–H-2′ vector using the proton–proton cross-relaxation rate constant (ref. 16). The ^{13}C spin–lattice relaxation rate is given by equation (3). Table 3 gives the correlation times estimated for C-2 of the base at various concentrations.

$$T_1^{-1} \equiv R_1(^{13}C) = \gamma_C^2\gamma_H^2\hbar^2 r_{CH}^{-6}\tau_c \qquad (3)$$

With the correlation times obtained *via* ^{13}C, it is possible to determine an average effective distance between the protons of interest and the sum of nuclei which are responsible for their dipolar relaxation, using proton relaxtion times, specifically the T_1 values of H-8 and H-2 of the bases [equation (4)]. The X_i

$$T_1^{-1} \equiv R_1(^1H) = [X_1(R1)^{-6} + 3/2\sum_{i=2}^{\infty} X_i(2i-2)(R)^{-6}]\gamma_H^4\hbar^2\tau_c \qquad (4)$$

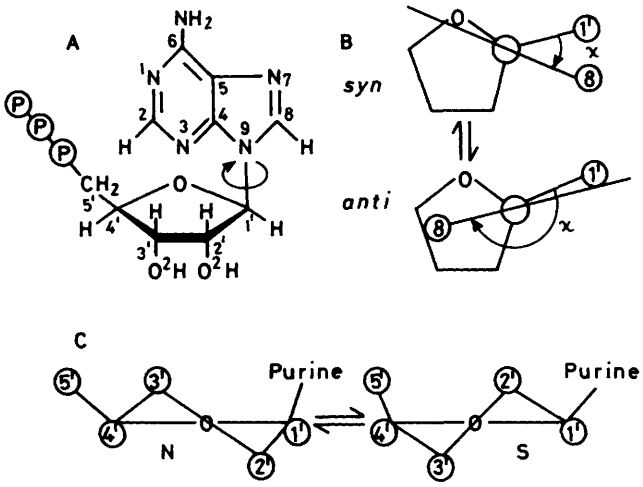
values are the mole fractions of the different stack species; $R1$ is the average of the intranucleotidic proton–proton distances; R is the average of all dipolar effective distances in the case of stack formation; τ_c is the average correlation time estimated by the ^{13}C measurements.

Table 2. Equilibrium constants for stack formation determined by isodesmic formulation

	AMP		ADP		ATP	
	pH 3.5	pH 8	pH 3	pH 8	pH 3	pH 8
H-8	2.48 ± 0.6	1.83 ± 0.7	8.75 ± 3.4	1.58 ± 0.9	18.2 ± 11.0	0.85 ± 0.5
H-2	2.64 ± 0.5	1.85 ± 0.7	6.16 ± 5.2	1.31 ± 0.5	19.4 ± 13.6	0.98 ± 0.3
5% H-8	3.15 ± 0.7		8.75 ± 0.4		14.4 ± 4.7	
Hg H-2	4.68 ± 0.8		5.78 ± 5.4		22.3 ± 19.2	
25% H-8	0.87 ± 0.5		6.83 ± 2.3		19.0 ± 4.8	
Hg H-2	2.48 ± 1.5		2.83 ± 0.6		18.4 ± 14.2	
5% H-8	3.77 ± 0.9		11.1 ± 4.8		14.0 ± 9.6	
Cd H-2	4.67 ± 0.8	5.96 ± 1.1	10.3 ± 8.9	1.90 ± 0.7	16.5 ± 4.1	3.03 ± 0.6
25% H-8	1.23 ± 0.7		2.54 ± 1.4		5.20 ± 2.6	
Cd H-2	2.92 ± 1.4		3.64 ± 1.9		17.3 ± 4.5	

Table 3. ^{13}C Correlation (ps) and relaxation time (s) of C-2; calculations carried out using equation (1)

	c 0.2M		*c* 0.01M	
	τ_c	T_1	τ_c	T_1
AMP	146	0.35	106	0.48
AMP + 5% Hg	138	0.37		
ADP	162	0.32	119	0.43
ADP + 5% Hg	189	0.27		
ATP	189	0.27	138	0.37
ATP + 5% Hg	232	0.22		



Scheme. Conformation and ring puckering for adenosine phosphates

The relaxation time depends not only on the correlation time, which is approximately proportional to the length of the stack, but also on the geometry of the nucleotide units,[17] and the distances between the neighbours within the stacks. Thus H-8 is relaxed intramolecularly by H-1′, H-2′, and H-3′, as well as intermolecularly by neighbouring molecules in the stack. The internuclear distances depend on the conformation, the glycosidic torsion angle χ, and the sugar puckering. Changes in *R*1 or R, which may be provoked by the aforementioned structural parameters, however, are expected to affect the relaxation time of H-8 more than H-2. This therefore accounts partially for the observed differences.

To define *R*1 we have to assume that in very dilute solutions the nucleotides are essentially monomeric, *i.e.* the internucleotide distances are almost infinite. The proton relaxation times then depend only on the average intranucleotide proton–proton distances (*R*1). According to the analysis of the stacking equilibria (see before), about 98% of AMP and 90% of ATP are monomeric at 3 mM. At higher concentrations, as stacks are formed, additonal relaxation arises from internucleotide interactions, allowing an average 'stack distance' (*R*) to be derived. Table 4a summarises the $^1HT_1$ results, and also includes the effect of varying the concentration of Hg^{II} and Cd^{II}.

An effect on T_1 due to the pseudorotational motion of the ring puckering and/or torsional motions of the glycosidic residue can be ruled out for two reasons. First, according to the results obtained by Levy[16] and von Goldammer,[15] ring puckering should not be an effective way of relaxation with riboses (*e.g.* not for ^{13}C). Second, the rotational barrier for the glycosidic torsion angle χ was measured to be approximately 26 kJ mol^{-1} (ref. 18), *i.e.* the rotaton frequency is *ca.* 10^7 cycles s^{-1} and therefore not contributing to T_1.

As can be seen, AMP shows with decreasing concentration an increasing relaxation time. ATP shows the opposite behaviour. Lowering the concentration results in a decrease of T_1 for both protons. The behaviour of ADP is even more complex: with decrease of concentration T_1 of H-2 increases whereas T_1 of H-8 decreases. Fitting these results to equation (4), in which the correlation time is taken from the ^{13}C measurements and the 'monomeric distance' (*R*1) as well as the 'stack distance' (*R*) are treated as parameters in an iterative least-squares fit procedure,[19] one obtains the values in Table 5.

For AMP the stack distance *R* for H-2 and H-8 is smaller and therefore much more important than *R*1. However at high pH *R*1 of H-2 of AMP greatly increases. The latter situation is even more pronounced in ADP and ATP: here at high pH *R*1 for H-2 is roughly 0.1 nm longer than it is at low pH. In addition ATP yields for both protons larger stack distances *R*. Further, the behaviour of ADP is more complex, as H-8 gives an increased stack distance *R*, whereas H-2 shows a decreased stack distance. The presence of Hg or Cd also causes unexpected effects in some situations. In AMP (pH 3) the presence of either ion leads to a decrease in relaxation time; at pH 8 a strong decrease in T_1 is observed only for H-2. In ADP (pH 3), H-2 shows a remarkable increase in T_1 in the presence of Hg^{II} and Cd^{II}. This effect is most pronounced for very small ADP concentrations. At high pH the relaxation time of both protons is reduced in the presence of Cd^{II}. For ATP only at high pH and higher concentrations some metal-induced effects can be observed. The behaviour of H-8 for ADP and ATP itself at high pH, however, is highly irregular, and cannot be described within the above formalism as an equilibrium between monomers and stacks. The general effect that the T_1 values are larger at pH 8 than at pH 3 indicates the stacking to be less favourable at high pH (see also Table 2).

Discussion

The foregoing findings concerning concentration and metal-ion-dependent effects clearly reveal the importance of the

Table 4. (a) Relaxation times (T_1/s) of the base protons H-2 and H-8; (b) influence of variable amounts of salts (ratio = phosphate : metal) (phosphates *c* 0.04M)

(a)

	^{1}H T_1 Relaxation times									
10^2c	20	10	4	1	0.3	20	10	4	1	0.3
	AMP H-8					AMP H-2				
pH 3.5										
	0.88	1.10	1.23	1.41	1.55	1.90	2.89	3.53	5.00	5.05
5% Hg	0.76	1.01	1.09	1.24	1.25	1.50	2.45	2.84	4.00	4.13
25% Hg		1.01	0.99	1.08	1.17		2.29	2.17	3.07	3.89
5% Cd	0.83	0.93	1.04	1.15	1.22	1.84	2.22	2.62	2.86	4.07
25% Cd		0.89	0.99	1.08	1.21		1.77	2.24	2.47	3.90
pH 8										
	1.10	1.30	1.57	1.82	1.86	4.86	6.21	9.20	12.95	15.00
5% Cd	1.10	1.24	1.29	1.37	1.57	3.75	6.06	6.60	6.50	9.66
	ADP H-8					ADP H-2				
pH 3										
		0.68	0.43	0.32	0.30		0.35	0.44	0.68	0.91
5% Hg		0.53	0.53	0.44	0.36		0.35	0.52	0.96	1.36
25% Hg	0.57	0.55	0.48	0.29	0.22	0.43	0.55	0.75	0.81	
5% Cd		0.63	0.63	0.54	0.40		0.35	0.51	1.08	1.43
25% Cd	0.49	0.46	0.42	0.29	0.19	0.40	0.43	0.47	0.66	0.71
pH 8										
		1.20	1.51	0.83	0.97		4.09	8.31	11.25	13.43
5% Cd		0.83	0.61	0.42	0.61		2.66	5.41	5.80	9.05
	ATP H-8					ATP H-2				
pH 3										
	0.74	0.67	0.58	0.40	0.20	0.65	0.62	0.56	0.47	0.39
5% Hg	0.76	0.74	0.52	0.35	0.22	0.63	0.66	0.58	0.45	0.32
25% Hg	0.81	0.78	0.51	0.28	0.20	0.80	0.90	0.85	0.44	0.32
5% Cd	0.76	0.72	0.59	0.42	0.29	0.56	0.56	0.53	0.52	0.43
25% Cd	0.76	0.73	0.48	0.26	0.18	0.57	0.60	0.51	0.44	0.40
pH 8										
		1.08	1.24	1.03	1.17		3.10	6.75	9.66	10.82
5% Cd		0.84	0.95	0.99	1.07		1.70	3.86	8.31	10.00

(b)

	^{1}H T_1 Relaxation times			
	Hg		Cd	
	AMP			
	H-8	H-2	H-8	H-2
20:1	1.06	2.59	1.07	2.64
10:1	1.02	2.31	1.04	2.48
5:1	0.94	2.26	0.99	2.24
2:1			0.91	1.90
	ADP			
20:1	0.47	0.50	0.55	0.47
10:1	0.39	0.54	0.41	0.43
5:1	0.34	0.63	0.34	0.46
2:1	0.37	0.78		
	ATP			
20:1	0.57	0.35	0.53	0.27
10:1	0.47	0.39	0.46	0.26
5:1	0.41	0.47	0.41	0.27
2:1	0.34	0.58	0.35	0.31

internucleotidic dipolar interation for H-2 and H-8 in AMP and for H-2 in ADP. However, the unexpected behaviour of H-2 and H-8 in ATP and H-8 in ADP at pH 3 as well as the dramatic differences in H-2 relaxation (pH 3 and 8) forces one to look for additional relaxation pathways. These pathways, however, should not be accessible at high pH; on the other hand

Table 5. Average distances (nm) calculated according to equation (4)

	H-8		H-2	
	$R1^a$	R^b	$R1^a$	R^b
pH 3				
AMP	0.228	0.213	0.278	0.237
AMP + Hg (20:1)	0.218	0.212	0.270	0.233
AMP + Hg (4:1)	0.214	0.214	0.255	0.234
AMP + Cd (20:1)	0.216	0.218	0.265	0.235
AMP + Cd (4:1)	0.215	0.206	0.260	0.220
pH 8				
AMP	0.230	0.215	0.321	0.261
AMP + Cd (20:1)	0.220	0.225	0.305	0.277
pH 3				
ADP	0.177	0.300	0.214	0.178
ADP + Hg (20:1)	0.188	0.235	0.233	0.178
ADP + Hg (4:1)	0.180	0.282	0.214	0.198
ADP + Cd (20:1)	0.189	0.247	0.238	0.186
ADP + Cd (4:1)	0.187	0.300	0.206	0.200
pH 8				
ADP			0.325	0.224
ADP + Cd (20:1)	0.187	0.334	0.303	0.215
pH 3				
ATP	0.182	0.260	0.185	0.247
ATP + Hg (20:1)	0.184	0.297	0.185	0.254
ATP + Hg (4.:1)	0.175	0.350	0.192	0.278
ATP + Cd (20:1)	0.186	0.296	0.187	0.253
ATP + Cd (4:1)	0.175	0.350	0.188	0.248
pH 8				
ATP			0.311	0.202
ATP + Cd (20:1)	0.208	0.208	0.318	0.205

[a] Monomer distance. [b] Stack distance.

they must be responsible for the unexpected concentration dependence. In view of the arguments already used, one is left only with the various phosphate groups and the protons at positions 5′ and 5″ as a source of H-2 relaxation at pH 3. Nevertheless one has to remember that the cited *syn/anti* isomerisation as well as the glycosidic rotations are highly unlikely to induce any changes in T_1 of H-2. Furthermore, the 5-protons can be assumed to play a less important role. This is obvious from their distances to H-2 and can also be seen by comparison of adenosine, AMP, ADP, and ATP. The dipolar relaxation of H-2 must be therefore heteronuclear *via* ^{31}P and to a smaller extent homonuclear *via* the ribose protons. From another point of view, however, this then requires that ADP and ATP are mostly (AMP probably to a lesser extent) in the *syn* conformation at low pH. This conformational hypothesis is supported (compare also the results for uridine[20] as well as investigations on different nucleosides[21–24]) by the observation that T_1 is pH-dependent, thereby reflecting the well known fact that in neutral solutions the conformation is largely *anti*.[25,26]

The relaxation behaviour of H-8, *e.g.* the unexpected concentration dependence [Table 4(a) and Table 5] in ADP and ATP, and of H-2 in ATP with and without metal ions cannot yet be explained in a simple way. In any case the ribose protons will play an important role. The complex formation of the purine bases with metal ions (well documented by the shift measurements) will not produce a large effect, as the gyromagnetic constant γ is low for Hg^{II} and Cd^{II}. Stack formation certainly is of some importance. Finally ring puckering will, if effective,

Table 6. ^{31}P Shift changes ($\Delta\delta$/Hz) and T_1 relaxation times (s) influenced by metal ions; T_1 values measured for AMP, ADP, and ATP are in the lower part of the table. The values correspond (from left to right) to the α-, β-, and γ-phosphate groups

	Hg			Cd			Cd(n)		
AMP	5			20			−30		
ADP	—	—		24	80		23	70	
ATP	—	5	5	12	55	40	10	56	62
AMP	2.1			5.0			1.4		
ADP	1.0	1.0		0.7	0.7		0.6	0.6	
ATP	0.6	0.4	0.4	0.6	0.3	0.3	0.7	0.4	0.4

	pH 3			pH 8		
AMP	3.6			0.6		
ADP	1.3	1.3		1.2	1.2	
ATP	1.0	0.8	1.1	0.9	0.6	0.9

contribute to the relaxation of H-8. The strange concentration-dependent behaviour of H-8, however, needs some additional explanation. We argue that changes in the glycosidic torsion angle are responsible for these effects. Alterations of the torsion angle may be induced at higher concentrations to allow a more efficient stack geometry.

In Table 4(b) a situation predominantly determined by the complexation of Hg^{II} and Cd^{II} is shown. The fact that an increasing Hg concentration is followed by an increase in the T_1 of H-2, whereas T_1 of H-8 decreases in ADP and ATP, can be explained by assuming Hg complexation in the neighbourhood of H-2. This complexation forces the phosphate groups into a position where they are no longer able to relax H-2. Thus the findings derived from the chemical shift measurements are corroborated. For Cd the stacking and the formation of the metal–N-7 bond probably play the more important roles.

The additional formation of metal–phosphate bonds can be detected by ^{31}P n.m.r. investigations, although the use of ^{31}P chemical shifts to elucidate the co-ordination sites of MgATP has generated controversial results.[27–29] Our feeling is that $\Delta\delta$ together with T_1 results will inevitably shed light upon the details of the complexation process. Nevertheless one has to realize that the T_1 values are influenced by the increased stacking tendency. This, however, causes some masking of the relaxation effects due to the metal ions. In any case, the chemical shift and T_1 values in ADP and ATP reflect the complexation of the α,β- and β,γ-phosphate groups respectively, in a straightforward manner. Our results are thus different from those in ref. 30, as the γ-phosphate group in ATP is more shifted by the complexation of Hg and Cd than is the β-phosphate group. One reason for this finding could be the fact that the Cd concentrations used in our studies are much smaller than those in ref. 30. Another interesting effect occurs with AMP and Cd at high pH. Here a high-field shift and an increase in T_1 are observed. Taking the proton results into account, one has to assume that AMP Cd complexation does not include the phosphate group. In addition both experiments show that Cd is much more strongly bound by the phosphate groups than is Hg (compare Table 6).

To give a more complete survey the relaxation behaviour of adenosine itself, which is unaffected by phosphates, has been measured. The large T_1 values of H-2 are shown in Table 7. Comparing these values with those of ADP and ATP at high pH corroborates our conclusion concerning the importance of the phosphate chain for H-2 relaxation.

In principle, it should be possible to show a dipolar

Table 7. ^1H T_1 Relaxation times (s) estimated for adenosine (pH 3.5)

	0.05		0.02		0.005		0.0025	
c/M	H-8	H-2	H-8	H-2	H-8	H-2	H-8	H-2
	1.90	7.80	2.16	8.37	2.27	9.00	2.33	9.50
5% Hg	1.71	5.94	1.86	6.83	2.38	9.19	2.55	10.3
5% Cd	1.80	6.01	2.01	7.28	2.36	8.21	2.58	11.7

Table 8. Nuclear Overhauser enhancements (pH 3); a dash indicates a situation where, owing to the proximity of the HDO signal, an unambiguous determination of the enhancement was impossible

		H-1′	H-2′	H-3′	
AMP	H-8	0	3.0	7.0	*c* 0.2M
	H-2	0	0	0	
	H-8	5.0	—	8.5	*c* 0.01M
	H-2	0	—	2.0	
ADP	H-8	4.0	12.4	7.2	*c* 0.2M
	H-2	0	4.6	3.3	
	H-8	5.5	9.5	10.1	*c* 0.01M
	H-2	2.0	4.6	2.7	
ATP	H-8	0	14.3	4.7	*c* 0.2M
	H-2	0	3.2	0	
	H-8	4.2	9.8	8.5	*c* 0.1M
	H-2	2.0	2.1	2.1	
	H-8	5.1	—	4.9	*c* 0.01M
	H-2	2.7	—	3.2	
	H-8	11.5	—	—	*c* 0.003M
	H-2	0	—	—	

interaction between the phosphates and the H-2 by observation of a heteronuclear Overhauser effect (n.O.e.). This experiment, however, failed. We attribute this to the fact that, owing to the increased equilibrium constant at pH 3, the stacks under investigation tend to be of a size for which the mobility (*e.g.* for higher concentrations) is in a region of roughly zero n.O.e. Our opinion coincides with arguments used in refs. 22, 23, and 26, and is supported by the fact that at neutral pH (decreasing equilibrium constant) n.O.e. can be detected.

Finally ^1H–^1H n.O.e. measurements were performed to gain further insight into the existence of dipolar contributions between the ribose protons and the adenine protons. As can be seen, H-8 in almost all of phosphorylated forms of adenosine shows dipolar interactions with H-2′ and H-3′. Only for ADP is there a significant dipolar interaction with H-1′, in concentrated solutions. Dilute solutions (of ATP), however, show a reasonable amount of n.O.e. between H-1′ and H-8. This finding indicates that the glycosidic torsion angle is concentration-dependent. One can surmise that this is to allow the best possible stack formation. Clearly it also demonstrates that the *syn* conformation is favoured. Total *syn*/*anti* conformational changes can be ruled out in view of the T_1 measurements.

H-2 is not affected in AMP, except by a very small contribution from H-3′. In ADP and ATP, however, H-2 retains a small but unequivocal dipolar interaction *via* H-2′ and H-3′. The observation that both H-2 and H-8 are simultaneously influenced is consistent with the aforementioned fact that a dominant but not exclusive orientation (*syn*) exists at pH 3. The n.O.e. due to the 5′- and 5″-protons cannot be explained in a straightforward manner. For example in ATP the 5′- and 5′-protons show at low concentrations interactions with H-8 (3.4%) and with H-2 (1.8%), whereas at high concentrations only H-8 (3.7%) is affected. Furthermore, no effect at high pH and low concentration and only small effects in highly concentrated solutions (pH 9, H-8 1%) can be observed. Unfortunately no solutions containing Cd or Hg showed

nuclear Overhauser enhancement. As stated before, we explain this fact by the increase in stacking tendency which causes an increase in the correlation time. This is the reason for the dramatic decrease of n.O.e. (compare refs. 22—24 and 26).

Experimental

The T_1 measurements were performed using the standard inversion-recovery method.[31] The mean deviation of the T_1 values is ±5%. All samples used were degassed by bubbling N_2 through the solutions. The adenosine phosphates purchased from Sigma (nos. A2754, A7894) and Boehringer (nos. 102 199, 236 675, and 519 979) were used without further purification. In order to make sure that no paramagnetic ions were in the solutions of the samples, reactions with 1,5-diphenylthiocarbazone were performed.

The samples were dissolved in 99.8% D_2O (Stohler) and studied at 22 °C. The n.O.e. measurements (steady state) were performed on and off resonance; at least six independent experiment cycles were combined and the mean deviation was ±10%.

Conclusions

It has been shown that the relaxation behaviour of H-2 and H-8 of the adenosine phosphates is affected by different mechanisms. Among those are the *syn/anti* conformation equilibrium, which at low pH is shifted towards the *syn* form, and changes in the glycosidic torsion angle. In the case of AMP and ADP (H-8), stack formation contributes to some extent to relaxation, by increasing the correlation time and *via* intermolecular dipole–dipole relaxation. In any case, however, H-2′ and H-3′ and to a minor extent H-1′, as well the 5′- and 5″-protons, are responsible for the relaxation. H-2 in ADP and ATP, and to a lesser extent in AMP, experiences in addition a strong and significant relaxation from the phosphate chain. A contribution due to ring puckering could not be detected.

Mercury(II) and cadmium(II) exhibit specific binding to ADP and ATP as well as to AMP (Cd) according to our shift and relaxation measurements. Cadmium seems to be complexed by the base (N-7) and the phosphate groups, whereas Hg is coordinated to the phosphate chain and the N-1 side of the base. In addition both ions increase the stacking tendency. Although effects from stacking mask complexation effects, observation of both chemical shifts and relaxation times for ^1H, ^{31}P, and ^{13}C allow a rather detailed understanding of the complexation of Hg and Cd ions to the adenosine phosphates.

Acknowledgements

We are indepted to Dr. Andrew Lane, Stanford Magnetic Resonance Lab. for discussions and his critical review of the manuscript. The XL 200 spectrometer was put at our disposal by the Fonds zur Förderung der wissenschaftlichen Forschung which we gratefully acknowledge.

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Received 3rd *December* 1985; *Paper* 5/2111