Hydration and Self-association of Adenosine Triphosphate, Adenosine Diphosphate, and their 1:1 Complexes with Magnesium(II) at Various pH Values: Infrared Investigations

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The dependences on pH of the hydrogen bonding of the K^+ salts and the 1 : 1 complexes of ATP and ADP with Mg^{2+} have been studied for aqueous solutions by i.r. spectroscopy. Water of hydration is strongly bound to the phosphate chains. Proton attachments cause rearrangements of electrons in phosphate groups and adenine residues changing mesomeric bond resonance. With 50% protonated ATP $(\mathrm{POH \cdots O^-P}) \rightleftharpoons (\mathrm{P^-O \cdots HOP})$ and with 100% protonated ATP and ADP $(\mathrm{POH \cdots N}) \rightleftharpoons (\mathrm{P^-O \cdots H^+N})$ polarizable bonds are formed. In weakly acidic media, $(\mathrm{N^+H \cdots O^-P}) \rightleftharpoons (\mathrm{N \cdots HOP})$ polarizable bonds are formed between two molecules of ATP and ADP. We assume that all these effects contribute to changes in free energy occurring during ATP hydrolysis. In more acidic solutions, the protonated phosphate chains form $\mathrm{POH \cdots OH_2} \rightleftharpoons \mathrm{P^-O \cdots H^+OH_2}$ and $\mathrm{POH \cdots OP} \rightleftharpoons \mathrm{P^-O \cdots H^+OP}$ bonds. Magnesium(II) ions interact only with the phosphate groups. Due to the decrease of the pK_a values of the terminal phosphate groups upon interactions by Mg^{2+} ions, polarizable hydrogen bonds both between the terminal phosphate groups and between protonated adenine residues and phosphate chains are formed simultaneously.

In order to understand the molecular mechanism of biological energy transfer reactions involving ATP hydrolysis, it is necessary to know more about the interaction of ATP and ADP with their environment (self- and mixed-association, hydration, metal–ion interactions). Recently two papers have dealt with the stacking interactions between the adenine residues.[1,2] Heyn and Bretz [1] determined the thermodynamic parameters at neutral or slightly basic pH, with high

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[1] M. P. Heyn and R. Bretz, *Biophys. Chem.*, 1975, **3**, 35.

[2] T. J. Gilligan III and G. Schwarz, *Biophys. Chem.*, 1976, **4**, 55.

concentrations of Mg salt, using optical methods. Gilligan and Schwarz[2] demonstrated that at low pH hydrogen bonds are formed between the protonated adenine residues and the phosphate chains, which should stabilize the stacking between the protonated bases.[3] In contrast, no detailed description, on a molecular basis, has been published on hydrogen bond formation of nucleotides over the whole pH range.

Strong hydrogen bond formation between organophosphorus compounds (self-association) has been detected by i.r. and ^{1}H n.m.r. studies.[4,5] The O–O distances in hydrogen bonded phosphate molecules, measured in crystals, are the shortest hydrogen bond lengths studied.[6] Changes in free energy of solvation might be of primary significance in regard to free energy changes upon ATP hydrolysis.[7] It is known that inorganic and nucleoside phosphates strongly interact with water of hydration.[7] The attachment of water molecules in the first and second hydration shell of phosphate groups[8] and of adenine[9] as well as the interactions with various metal ions[10] have been calculated. In biological systems, ATP forms 1 : 1 complexes with Mg^{2+}, mainly by co-ordination with the phosphate groups.[11,12]

We have studied the self-association of ATP, ADP, and their 1 : 1 complexes with Mg^{2+} and their interaction with the hydration shell by i.r. spectroscopy over a wide pH range. In a second paper, we shall discuss the association of the splitting products of hydrolysis of ATP and MgATP at various pH values.[13] Changes in the characteristic i.r. bands indicate the attachment of protons to adenine residues (in the region 1 750—1 300 cm^{-1}) and to phosphate groups (1 300—700 cm^{-1}). Changes in the OH and NH stretching vibrations in the region 3 000—2 000 cm^{-1} indicate the formation of strong hydrogen bonds. A continuous absorption in the region below 3 000 cm^{-1} demonstrates the presence of easily polarizable hydrogen bonds which are of special interest, since these bonds interact strongly with their environment.[14-16,17a]

In the following, intermolecular interactions in aqueous solutions of ATP and ADP are investigated as a function of the concentration ratios of K^+, H^+, and Mg^{2+}. A similar study on orthophosphate solutions was published previously.[18]

EXPERIMENTAL

Chemicals were purchased from Sigma (K_2H_2ATP, Ba_3ADP_2, Ba_2ATP), Boehringer (H_3ADP), and Merck ($MgSO_4$, H_2SO_4). To prepare Mg^{2+} salts, Ba_2ATP was triturated in 1 : 1, Ba_3ADP_2 in 2 : 1 aqueous solutions of $MgSO_4$–H_2SO_4. The best yield was obtained with strongly diluted solutions (0.001M) and an excess of Ba_2ATP or Ba_3ATP_2, respectively. The suspension was improved by ultrasonic treatment. The Ba_2SO_4 precipitate was removed by centrifugation and the solutions lyophilized. Subsequent enzyme tests on ATP, ADP, and AMP concentrations, carried out by Boehringer, showed $<3\%$ decomposition. The degrees of protonation, *i.e.* the $K^+ : H^+$ ratio, were obtained by mixing KOH or HCl with ATP and ADP.

All substances were checked by a number of tests. The content of water of hydration was obtained by ultimate analysis and by concentration measurements of the adenosine phosphates using the u.v. band at 260 nm, as well as by titration. The concentration of Mg^{2+} ions was determined by Mercko test no. 3338 (Merck).

The pH values of the samples were determined at 10 °C by a microcapillary device requiring only 20 µl per measurement (Dr. W. Ingold, Zürich, Frankfurt/M.).

The spectra were obtained with a Perkin-Elmer i.r. spectrophotometer, model 325 (slit program 6.5, time response 3.0 gain in the range 4 000—1 000 cm^{-1} 1.6, in the range 1 000—200 cm^{-1} 2.4). Cells with silicon windows described in ref. 19 were used. The temperature was measured directly at the samples. To prevent decomposition of the samples during measurement, all spectra were determined at 10 °C.

RESULTS AND DISCUSSION

Figure 1 shows spectra of concentrated aqueous solutions of adenosine phosphates and the influence of

TABLE 1

pK_a Values of adenosine phosphates at 25 °C (from refs 28 and 29 and references cited therein)

Acceptor	Ionic strength	ATP	ADP	MgATP	MgADP
Ribose	0.0	12.35 *	12.35 *		
Terminal	0.0	7.68	7.20	5.44	5.38
phosphate	0.1	6.53	6.44	5.21	5.30
Adenine residue	0.1	4.06	3.93	3.80	3.87 †
Phosphate		2.00	2.00		
chain		1.00	1.00		
		1.00			

* From adenosine. † At ionic strength 0.0.

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[3] F. Jordan and H. D. Sostman, *J. Amer. Chem. Soc.*, 1972, **94**, 7898.

[4] J. R. Ferraro and D. F. Peppard, *J. Phys. Chem.*, 1963, **67**, 2639.

[5] S. Detoni and D. Hadži, *Spectrochimica Acta*, 1964, **20**, 949.

[6] I. Olovsson and P.-G. Jönsson, in ' The Hydrogen Bond, Recent Developments in Theory and Experiment,' eds. P. Schuster, G. Zundel, and C. Sandorfy, North Holland, Amsterdam, 1976, ch. 8, p. 393.

[7] P. George, R. J. Witonsky, M. Trachtman, C. Wu, W. Dowart, L. Richman, W. Wichman, F. Shurayh, and B. Lentz, *Biochim. Biophys. Acta*, 1970, **223**, 1.

[8] B. Pullman, A. Pullman, H. Berthod, and N. Gresh, *Theor. Chim. Acta*, 1975, **40**, 93.

[9] A. Pullman and B. Pullman, *Quart. Rev. Biophys.*, 1975, **7**, 505.

[10] D. S. Marynick and H. F. Schaefer III, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3794.

[11] C. M. Frey and J. E. Stuehr, *J. Amer. Chem. Soc.*, 1972, **94**, 8898.

[12] C. M. Frey, J. L. Banyasz, and J. E. Stuehr, *J. Amer. Chem. Soc.*, 1972, **94**, 9198.

[13] M. Matthies and G. Zundel, unpublished work.

[14] G. Zundel, *Allg. Prakt. Chem. (Wien)*, 1970, **21**, 329.

[15] R. Janoschek, E. G. Weidemann, and G. Zundel, *J.C.S. Faraday II*, 1973, 505.

[16] R. Janoschek, E. G. Weidemann, H. Pfeiffer, and G. Zundel, *J. Amer. Chem. Soc.*, 1972, **94**, 2387.

[17] G. Zundel, in ' The Hydrogen Bond, Recent Developments in Theory and Experiments,' eds. P. Schuster, G. Zundel, and C. Sandorfy, North Holland, Amsterdam, 1976, (*a*) p. 683; (*b*) p. 739.

[18] D. Schiöberg, K. P. Hofmann, and G. Zundel, *Z. Phys. Chem. (Frankfurt)*, 1974, **90**, 181.

[19] K. P. Hofmann and G. Zundel, *Z. Naturforsch.*, 1974, **29c**, 19.

protonation. The percentage figures represent the ratios of the concentrations of H^+ : adenosine phosphate (see Experimental section).

The pK_a values of the adenosine phosphates, which are very important for the nature of hydrogen bonds formed, are summarized in Table 1. In Figure 2, the absorbance of the continua is plotted as a function of the degree of protonation, *i.e.* the pH values of the solutions.

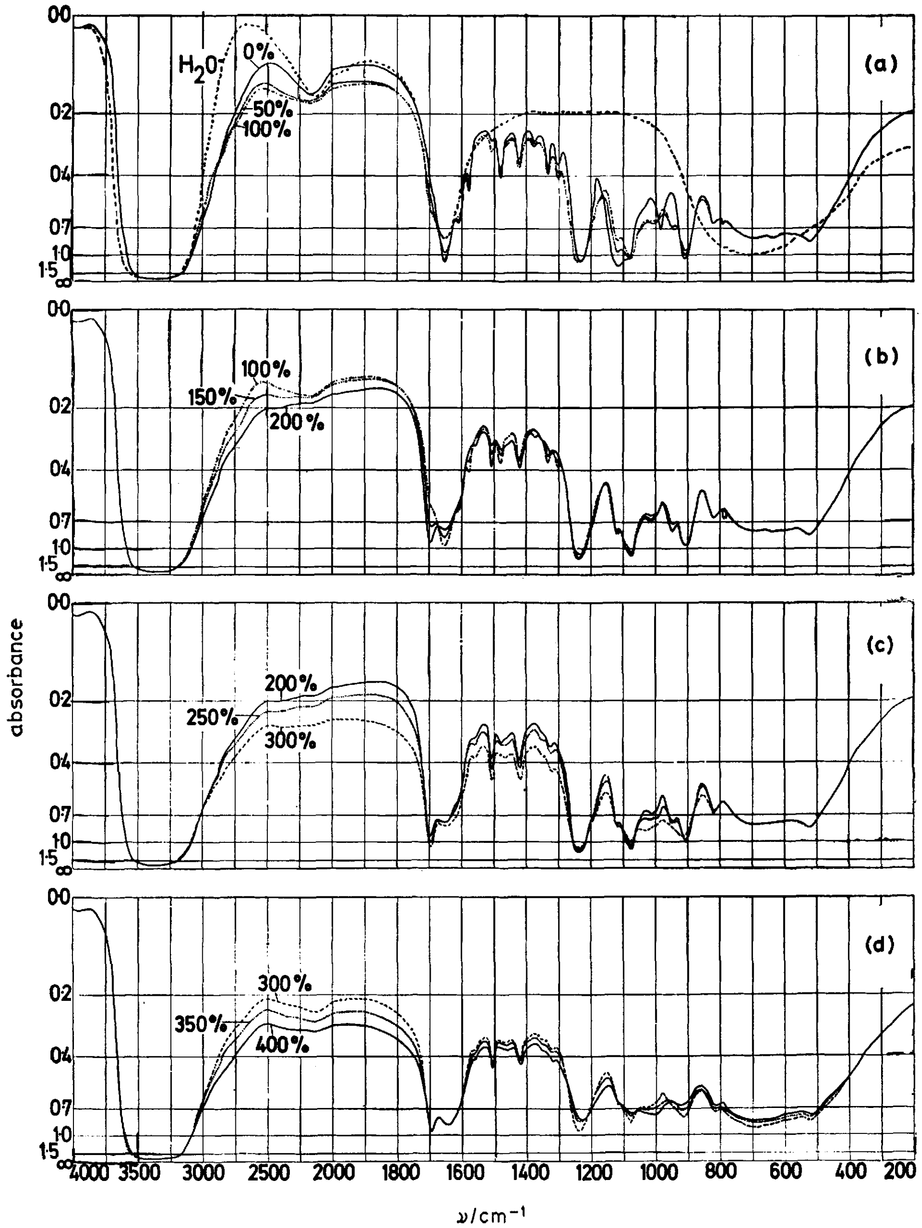

FIGURE 1 I.r. spectra of aqueous solutions of ATP showing influence of the $H^+ : K^+$ ratio, given as % protonation: (a)—(c) 1.45M, (d) 1.0M, samples at 10 °C, layer thickness 12.1 μm. (a) 0—100%, (b) 100—200%, (c) 200—300%, (d) 300—400% protonation

In Table 2, all results taken from Figures 1—3 are summarized.

ATP and ADP Solutions.—0% *Protonation*. Compared with pure water, with the ATP and ADP solutions the large wavenumber slope of the broad band in the region 3 500—2 500 cm^{-1} is shifted *ca.* 30 cm^{-1} toward smaller wavenumbers (Figure 1a). Furthermore, this band is strongly broadened toward smaller wavenumbers. This change, as with orthophosphate,[18,20] demonstrates that water molecules are attached to the oxygen atoms of the phosphate groups *via* strong hydrogen bonds (B_1 in Table 3). The energy surface in these hydrogen bonds is largely asymmetrical, since ΔpK_a for the water–terminal phosphate group amounts to $15.8 - 7.2 = 8.6$.[21] It may be assumed that water

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[20] A. C. Chapman and L. E. Thirlwell, *Spectrochima Acta*, 1964, **20**, 937.

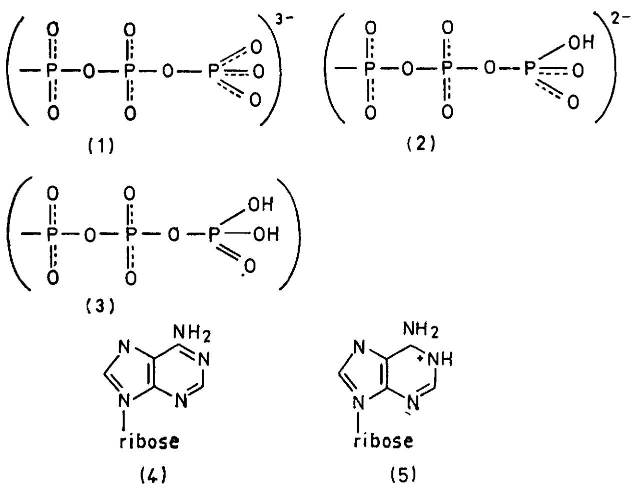
[21] 'Handbook of Chemistry and Physics,' ed. R. C. Weast, C.R.C. Press, Cleveland, 55th edn., 1974—1975.

TABLE 2

Changes with proton attachment in aqueous ADP and ATP solutions. The formulae are given for ATP but not ADP. Assignments are from refs. 20, 22, 31, and 35

	Protonation 0%		$0\% \rightleftharpoons 100\%$	100%		$100\% \rightleftharpoons 200\%$	200%		$200\% \rightleftharpoons 300\%$	300%	
	ADP	ATP		ADP	ATP		ADP	ATP		ADP	ATP
Strongly bound water of hydration molecules	3 000—2 500 cm^{-1} (see text)		(P$^-$O ··· HOH) bonds (B$_1$) remain but their strength decreases slightly	3 000—2 500 cm^{-1}		(P$^-$O ··· HOH) bonds (B$_1$) (POH ··· OH$_2$) and additionally (N$^+$H ··· OH$_2$) bonds	3 000—2 500 cm^{-1} 2 700w, br 2 380w, br		(POH ··· OH$_2$) bonds are formed (B$_5$)	Continuum	
Proton attachment to phosphate groups see Figure 1 and 3)	1 215s masked	1 235s 1 085sh	$\left.\begin{matrix}\nu_{as}\\ \nu_{s}\end{matrix}\right\}PO_2^-$ $\left.\begin{matrix}\nu_{as}\\ \nu_{s}\end{matrix}\right\}PO_2^-$ (1) $\rightleftharpoons$ (2)	1 224s 1 080s	1 240s 1 095sh 1 075s		1 224s 1 080s	1 240s 1 095sh 1 075s	$\left.\begin{matrix}\nu_{as}\\ \nu_{s}\end{matrix}\right\}PO_2^-$ $\left.\begin{matrix}\nu P{=}O\\ \nu_{as}PO_2^-\end{matrix}\right\}$ $\nu_s PO_2^-$ (2) $\rightleftharpoons$ (3)	1 224s 1 080m	1 240s 1 090sh 1 072s
	1 112s 1 015w	1 118s 988m	$\left.\begin{matrix}\nu_{as}\\ \nu_{s}\end{matrix}\right\}PO_3^{2-}$ ν (ribose) νP–O–H	1 110m 975m	1 120m 945m	Phosphate bonds remain almost unchanged	1 110m 975m	1 120m 945m	ν(ribose) ν (ribose) ν P–OH $\left.\begin{matrix}\nu_{as}\\ \nu_{s}\end{matrix}\right\}P(OH)_2$	1 110m 1 050m, sh 980m	1 120m 1 030m
	936s	915s	νP–O–P νP–O–P	925m	905s		925m	905s	ν P–O–P ν P–O–P	935s	912s
Proton attachment to adenine residues (see Figures 1 and 3)	1 655m 1 611w 1 580w 1 510vw 1 480w	1 655m 1 610w 1 578w 1 510vw 1 480w	Some protons are found at N(1) of the adenine residues before 100% protonation due to the (P$^-$O ··· H$^+$N) boundary structure of (B$_3$)	1 655m 1 611w 1 580w 1 510vw 1 480w	1 695w, sh 1 655m 1 610w 1 578w 1 510vw 1 480w	(4) $\rightleftharpoons$ (5)	1 695m 1 645s * 1 560vw 1 510w 1 480vw	1 695m 1 645s * 1 555vw 1 510w 1 480vw	Adenine bonds remain almost unchanged	1 695m 1 645s * 1 560w 1 510w	1 695m 1 645s * 1 555w 1 510w
Formation of easily polarizable hydrogen bonds (see Figure 2)			(POH ··· O$^-$P) $\rightleftharpoons$ (P$^-$O ··· HOP) bonds (B$_2$) (ATP only) preferentially with 50% protonation (POH ··· N) $\rightleftharpoons$ (P$^-$O ··· H$^+$N) bonds (B$_3$) preferentially with 100% protonation	Continuum in the region. 3 000— 1 700 cm^{-1}, at 50% protonation, also at smaller wavenumbers (ATP)		(N$^+$H ··· O$^-$P) $\rightleftharpoons$ (N ··· HOP) bonds (B$_4$) are formed	Continuum in the region 3 000— 1 700 cm^{-1}		(POH ··· OH$_2$) $\rightleftharpoons$ (P$^-$O ··· H$^+$OH$_2$) bonds (B$_5$) are formed (POH ··· OP) $\rightleftharpoons$ (P$^-$O ··· H$^+$OP) bonds (B$_6$) are formed	Intense continuum in the whole wavenumber range below 3 000 cm^{-1}	

* H_2O scissor vibration.


(1) (2) (3) (4) (5)

Formulae referred to in Table 2.

molecules are also bound *via* weak hydrogen bonds to the 2′-OH and 3′-OH groups of ribose, to the nitrogen atoms, and to the amino-groups of the adenine residues. The O–H stretching vibrations of these hydroxy-groups should be observed near 3 400 cm^{-1}, and those of the N–H groups near 3 300 cm^{-1}.[22] These bands are, however, masked by the intense band of the stretching vibrations of all other water molecules present.

0—100% *Protonation.* First, the protons are attached to the terminal phosphate groups, as demonstrated by the changes of the characteristic phosphate bands (for details see Table 2).

The steady increase of the background absorbance at 1 900 cm^{-1} with increasing protonation (Figure 2), not observed at smaller wavenumbers, might be caused by the formation of easily polarizable (POH ··· N) $\rightleftharpoons$ (P$^-$O ··· H$^+$N) bonds (B$_3$ in Table 3) between one protonated phosphate group and the lone pair of N(1) with slightly asymmetrical energy surfaces (ΔpK_a —2.35).* With the higher concentrated ATP solutions, the vibrations of the adenine-H$^+$ residues (1 695 and 1 510 cm^{-1}) arise before 100% protonation is reached. This indicates that the (P$^-$O ··· H$^+$N) proton boundary structure has perceptible weight. A double minimum energy surface is present in these bonds, as would be expected from the observed continuum. Investigations of orthophosphate–nitrogen base interactions have shown that intermolecular hydrogen bonds of type B$_3$ are still polarizable.[23]

Besides these bonds, with the more concentrated ATP solutions (1.45M) at *ca.* 50% protonation, hydrogen bonds (B$_2$) (POH ··· O$^-$P) $\rightleftharpoons$ (P$^-$O ··· HOP) seem to be formed, favoured by the small amount of water of hydration molecules (22 per mole ATP and per 3.5 moles K$^+$ ions). These bonds are largely symmetrical (ΔpK_a 0) and therefore also cause a continuum at smaller wavenumbers, which is illustrated by the curve of the continuous absorbance at 1 400 cm^{-1} showing a maximum (Figure 2). At 1 900 cm^{-1}, the contributions of the two types of polarizable hydrogen bonds to the

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* The symmetry of the energy surface is determined by the difference of the pK_a values of donor and acceptor (ΔpK_a). With largely symmetrical hydrogen bonds, continua may be observed in the whole wavenumber range below 3 000 cm^{-1}. With asymmetrical hydrogen bonds with double minima, continua may be found, which, however, do not extend as far toward smaller wavenumbers.[17b]

[22] L. J. Bellamy, ' The Infrared Spectra of Complex Molecules,' Methuen, London, 1975.

[23] M. Matthies and G. Zundel, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 831.

continuum are superimposed on each other. The remaining negative charge at the phosphate groups is screened by the cations, as shown by ultrasonic relaxation measurements.[24] The fact that such easily polarizable (POH ··· O^-P) $\rightleftharpoons$ (P^-O ··· HOP) bonds are formed is confirmed by observing the band changes of the PO_3^{2-} groups (988 and 1 118 cm^{-1}) which occur to a large extent below 50% protonation. This demonstrates that the proton interacts with two phosphate groups. Analogous easily polarizable hydrogen bonds were found earlier with aqueous solutions of orthophosphate at 50 and 150% protonation.[18] This type of orthophosphate association was recently confirmed by ^{31}P nuclear Overhauser effect results.[25]

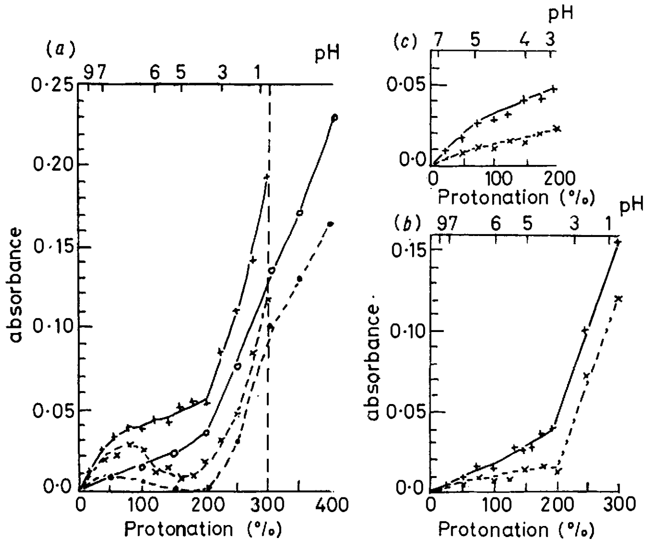


FIGURE 2 Absorbance of the continuum and dependence on degree of protonation at two different wavenumbers (—— 1 900 cm^{-1}, ---- 1 400 cm^{-1}), (a) ATP, + or × 1.45M, ○ or ● 1.0M, (b) ADP 1.0M, (c) MgATP 0.85M

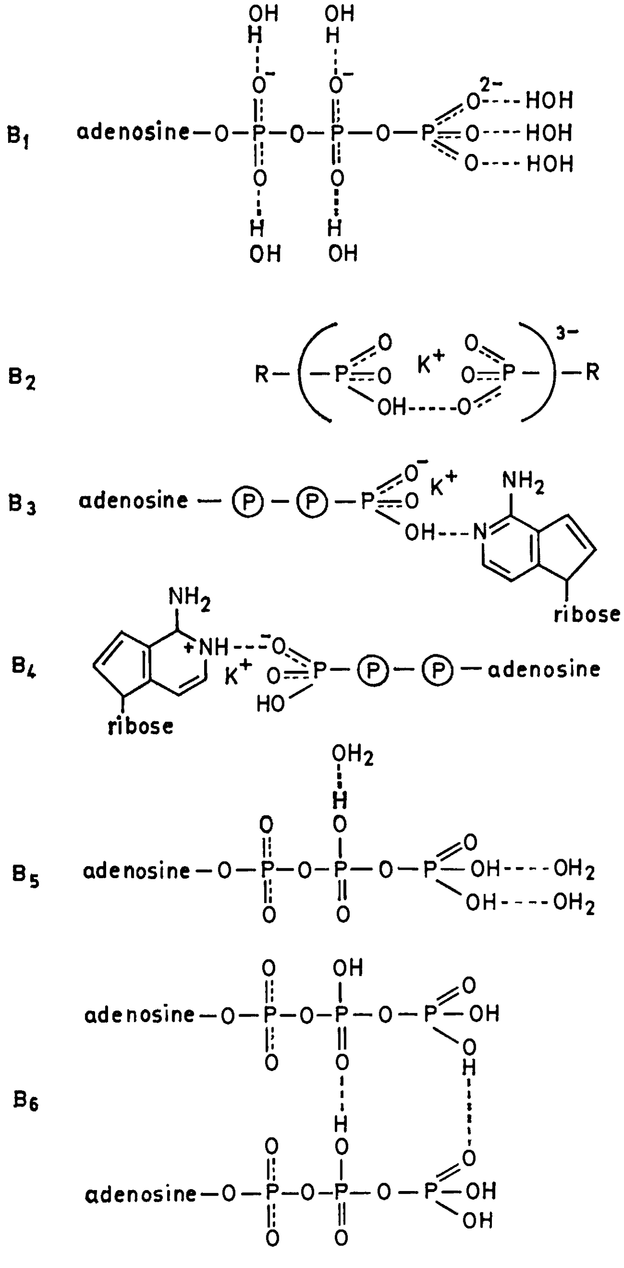
100—200% *Protonation*.—With increasing protonation (more acidic solutions), the protons are attached to the N(1) atoms of the adenine residues [decrease of bands at 1 655 and 1 480 cm^{-1}; increase of bands at 1 695 and 1 510 cm^{-1} (see Table 2)].

An additional increase of the background is observed at larger wavenumbers (Figure 2). The positively charged adenine rings can form hydrogen bonds with the negatively charged phosphate chain of other molecules. As shown with the model substances,[23] these (N^+H ··· O^-P) $\rightleftharpoons$ (N ··· HOP) bonds (B_4 in Table 3) are still polarizable (ΔpK_a −2.15). Hence, these bonds cause the observed continuous absorption.*

This type of H-bonding was previously observed by other methods, with crystals of $Na_2H_2ATP,3H_2O$ by *X*-ray analysis [26] and by the c.d. measurements of Gilligan and Schwarz.[2] (N^+H ··· N) $\rightleftharpoons$ (N ··· H^+N) bonds were found with aqueous purine solutions when one proton is present per two purine molecules, *i.e.* with 50% protonation,[27] as demonstrated by a maximum of the absorbance continuum. Such a maximum is not found with ATP

TABLE 3

Structures of possible hydrogen bonds in aqueous solutions of ATP; Ⓟ = phosphate group. In the case of hydrogen bonds with fluctuating proton only one proton boundary structure is given



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* In the past it was supposed that this increase of the continuum were caused by [N(1)$^+$H ··· N(3)] hydrogen bonds.[19] These bonds are, however, not sufficiently symmetrical to be polarizable.

[24] Th. Funck, personal communication.

[25] T. Glonek, *J. Amer. Chem. Soc.*, 1976, **98**, 7090.

[26] O. Kennard, N. W. Isaacs, W. D. S. Motherwell, and G. D. Watson in Jerusalem Symp. on Quantum Chem. and Biochem., eds. E. D. Bergman and B. Pullman, Academic Press, New York, 1972, vol. IV, p. 114.

[27] W. Sessler and G. Zundel, *Chem. Phys. Letters*, 1972, **14**, 356.

solutions at 150% protonation, showing that no appreciable amount of $(N^+H \cdots N) \rightleftharpoons (N \cdots H^+N)$ bonds is present.

Very weak broad bands are observed at 2 700 and 2 380 cm^{-1} (Figure 1). These bands can be ascribed to strong hydrogen bonds formed by N^+H, as well as by OH groups of the terminal phosphates with water molecules. Similar bands are observed with aqueous solutions of protonated nitrogen bases.[17b]

200—300% (*ADP*) *and* 200—400% (*ATP*) *Protonation*. In more acidic ATP and ADP solutions (pH 3) the intensities of the antisymmetric and symmetric stretching vibrations of the PO_2 groups decrease and very broad P–OH bands appear (Figure 1d, Table 2), indicating further protonation of the phosphate chains.

A very steep increase of the absorbance of the continuum is found with increasing degree of protonation in the whole wavenumber region below 3 000 cm^{-1} (Figure 2). This is a characteristic feature of phosphoric acid solutions and is caused by the very easily polarizable hydrogen bonds B_5 and B_6.[18] The studies on crystals show that such hydrogen bonds are very short.[6]

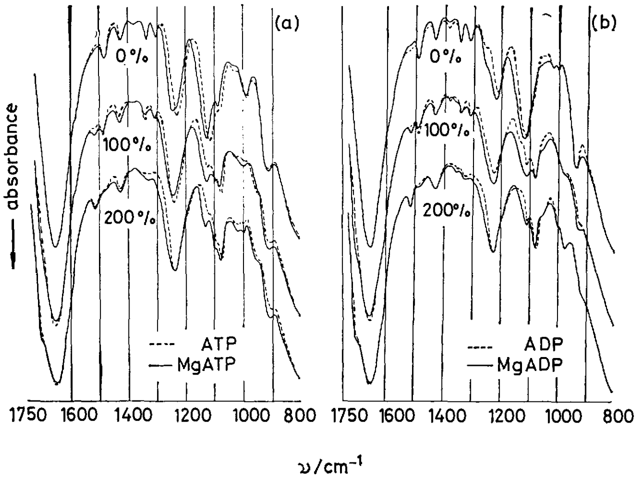

FIGURE 3 Comparison of i.r. spectra of (a) ATP and MgATP, (b) ADP and MgADP. 0.25M, 10 °C, layer thickness 12.1 μm

Interaction of Mg^{2+} *with ATP and ADP*.—With Mg^{2+}, the characteristic phosphate bands shift *ca.* 7 cm^{-1} toward larger wavenumbers (Figure 3) indicating the formation of a complex of Mg^{2+} with the phosphate chain. At all degrees of protonation, no shifts of the vibrations of the adenine residues are observed. Hence, the interactions of Mg^{2+} with the adenine residues are negligible. This is in good agreement with the results of other authors.[11,28,29]

Brintzinger[30] discussed 'localized hydrolysis' as the reason for these band shifts, especially in the case of Cu^{2+} and Zn^{2+} complexes. 'Localized hydrolysis' was suggested, since the band shifts with bivalent ions are very similar to those observed upon addition of protons. If considerable 'localized hydrolysis' would occur with Mg^{2+}, at low degrees of protonation no significant changes with the bands of the phosphate group would be expected. The usual changes due to proton addition are, however, observed even at small degrees of protonation. Thus, with Mg^{2+} ions, 'localized hydrolysis' can only be of minor importance.*

Figure 3 shows that the shift of $\nu_s(PO_2^-)$ at *ca.* 1 080 cm^{-1} still occurs with higher degrees of protonation, *i.e.* when the terminal phosphate group is protonated. Brintzinger[31] discussed the interaction of bivalent metal ions with the phosphate groups only for the non-protonated group. The shifts, especially those of the $\nu_s(PO_2^-)$ vibration, indicate a similar equilibrium for the protonated group. Hence, the Mg^{2+} ions are also bound to the phosphate chain when one proton is present there.

Influence of Mg^{2+} *on the Formation of Hydrogen Bonds.*—The broadening of the O–H stretching vibrations of water of hydration molecules toward smaller wavenumbers can also be observed with the Mg^{2+} complexes. Thus, water of hydration attached at the phosphate groups *via* strong asymmetrical hydrogen bonds (B_1) is still observed. The presence of Mg^{2+} does therefore not strongly influence the interaction of the phosphate groups with water of hydration.

In Figure 2c, the absorbance of the background is plotted with the degree of protonation as parameter. Over the whole range of protonation, an increase in background absorbance is observed. This increase is stronger at 1 900 cm^{-1}, *i.e.* at larger wavenumbers, than at 1 400 cm^{-1}.

Upon the approach of the pK_a values of the terminal phosphate group and the $N(1)H^+$ group due to the influence of Mg^{2+} ions (see Table 1), hydrogen bonds of the types B_2—B_4 can be formed simultaneously over the whole region of protonation and cannot be distinguished. None of the three types of hydrogen bonds is sufficiently preferred in one or other region of protonation. Magnesium(II) ions favour the proton transfer in the hydrogen bonds between phosphate chains and adenine residues. Therefore, these bonds are more easily polarizable (ΔpK_a −0.75) than those formed when no Mg^{2+} is attached to ATP or ADP (ΔpK_a −2.35).

Concluding Remarks.—It was postulated by George *et al.*[7] that differences in solvation energy are of most importance when considering energy-rich bonds. With regard to the strong bonding of water molecules, small differences in free energy may occur which become available with hydrolysis. Hence, the phosphorylation equilibrium of the ATP–ADP system probably depends strongly on solvation. This may be of importance in explaining the fact that the ATP synthesis proceeds in the medium of mitochondrial or thylacoid membranes.

Furthermore, mesomeric bond resonance in the

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[*] In Figure 4 of ref. 19, the % figures are not correct. Obviously, one additional proton was present in these solutions per ADP molecule. Hence, the interpretation given there that strong 'localized hydrolysis' proceeds with ADP is wrong.

[28] R. C. Phillips, *Chem. Rev.*, 1966, **66**, 501.

[29] R. M. Izatt, J. J. Christensen, and J. H. Rytting, *Chem. Rev.*, 1971, **71**, 439.

[30] H. Brintzinger, *Biochim. Biophys. Acta*, 1963, **77**, 343.

[31] H. Brintzinger, *Helv. Chim. Acta*, 1965, **48**, 47.

phosphate groups and adenine rings, and especially the formation of various types of hydrogen bonds, strongly depend on pH. All these effects are the molecular explanation of the pH dependence of the ATP–ADP phosphorylation equilibrium studied earlier by George and Rutman.[32] In the meantime, many investigations have shown that protons are involved in phosphorylation processes.[31-35]

In summary, the following molecular effects contribute to changes of the free energy discussed by George *et al.*: [7,32] (1) different bonding of hydration water molecules; (2) changes of the mesomeric bond resonance within the phosphate groups indicated by the bands of these groups; (3) the formation of various types of easily polarizable hydrogen bonds.

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[32] P. George and R. J. Rutman, *Progr. Biophys.*, 1960, **10**, 1.

[33] H. T. Witt, *Quart. Rev. Biophys.*, 1961, **4**, 365.

[34] A. T. Jagendorf and E. Uribe, *Proc. Nat. Acad. Sci. U.S.A.*, 1966, **55**, 170.

[35] L. C. Thomas and R. A. Chittenden, *Spectrochimica Acta*, 1964, **20**, 489.