Intermolecular Interaction in Systems with Energy-Rich Phosphates, II

Effect of the Protons Arising in Aqueous Hydrolysing ATP Solutions, IR Investigations

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Energy-Rich Phosphate, ATP Hydrolysis, Hydrogen Bonds Polarizable, Intermolecular Interaction, Proton Addition

IR spectra of 0.3 M aqueous solutions of $Mg_{0.5}K_{3-n}H_n$ ATP were plotted. The dependence of the spectra on the hydrolysis was investigated for three different K/H ratios of the nonhydrolysed system. Changes to bands provide information as to the protons added to the phosphate groups and base residues as a function of the initial protonation and degree of hydrolysis. More and more proton addition to the $-PO_3^{--}$ groups is observed as the initial protonation increases and as the degree of hydrolysis rises. The same applies as far as the addition of protons to the base residues is concerned to the systems with a higher initial protonation. At low degrees of hydrolysis the PO_4^{3-} ions which occur do not bind the two hydrolysis protons completely. 150% protonated PO_4^{3-} ions first form which cross-like via $OH^+ \cdots O$ hydrogen bonds, which only become more strongly protonated in the systems with a higher initial protonation at large degrees of hydrolysis. The $OH^+ \cdots O$ bonds between the 150% protonated hydrogen phosphate ions as well as the $NH^+ \cdots N$ hydrogen bonds between the base residues cause an IR continuum and are thus easily polarizable. An extremely large change to the hydrate structure within narrow hydrolysis ranges is indicated by changes to the bands of the water molecules. The biological relevance of these findings is briefly discussed in the conclusions.

Introduction

George and Rutman[1] considered the energy-rich bond from the thermodynamic point of view. The energy-rich molecule appears as the carrier of free enthalpy which under biological conditions can be made available optimally on molecule splitting. The properties of the molecules and their interaction with the environment, especially with the protons occurring on hydrolysis, give rise only in conjunction with each other to the effect of the energy-rich bond, the significant phenomenon in bioenergetics. In a paper published in 1970 George *et al.*[2] were able to show with the aid of thermodynamic data that the main portion of the free enthalpy of the phosphates made available originates from the change to the solvation enthalpy of the surrounding water structure on hydrolysis. We consider it significant in this connection that the interaction of the hydrolysis products with each other and with the surrounding medium is decisive for each process in which the free enthalpy of the hydrolysis is transferred to subsequent processes.

Solvation, protonation and processes occurring on molecule splitting are always strongly interrelated on ATP hydrolysis. The previous paper[3] clarified the nature of the interactions of each of the molecules occurring as hydrolysis products on varying the protonation degree at constant concentration. In the following the hydrolysis of the ATP in aqueous solution is discussed with the aid of IR investigations and on the basis of the above results. Hydrolysis of the ATP signifies in the following the hydrolytic splitting of the covalent phospho-ester bond in accordance with the following equation:

$$ATP + H_2O \rightarrow ADP + P\,.$$

Results and Discussion

IR spectra of 0.3 M aqueous solutions of ATP were investigated as a function of the degree of hydrolysis. The non-hydrolysed system contained a varying ratio of K^+ ions and protons, *i.e.*, $Mg_{0.5}K_{3-n}H_n$ ATP was present. Three hydrolysis series were plotted, namely with initial protonations $n = 0.5$; $n = 1.2$ and $n = 1.5$. These protonation de-

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grees were selected since with these the pH values of the systems for all degrees of hydrolysis lie in the physiological pH range, except for the system $n = 1.5$ at large degrees of hydrolysis.

The bands in the range 1300 – 800 cm^{-1} supply information concerning proton addition to the phosphate groups (Fig. 1). The ring vibrations in the range 1800 – 1300 cm^{-1} provide information with regard to the addition of protons to the base residues (Fig. 3).

Under the present hydrolysis conditions (Experimental Procedure) mainly orthophosphate hydrolysis occurs, as was shown by enzyme tests and phosphate analysis. Hence hydrolysis products with

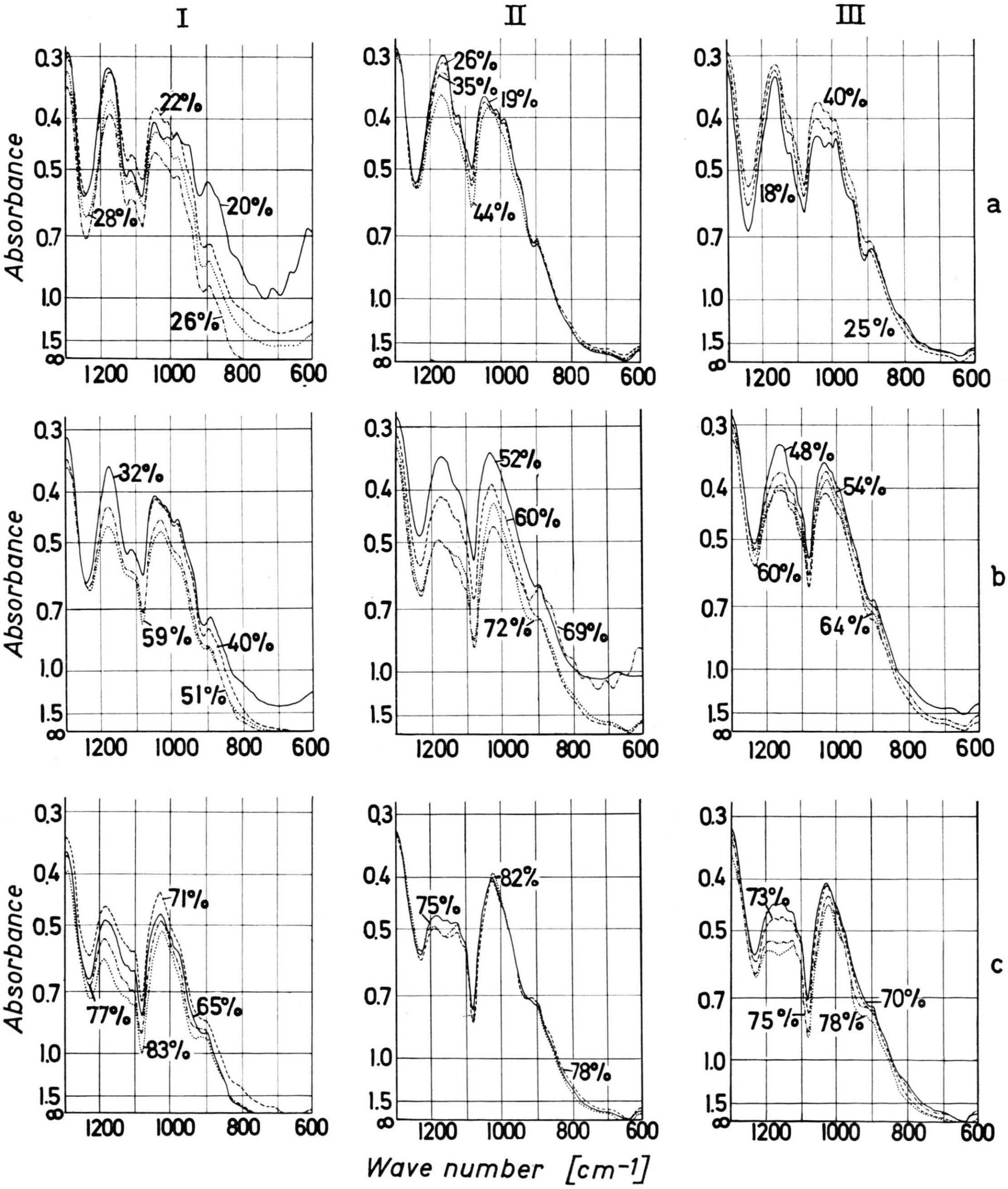


Fig. 1. IR spectra of aqueous solutions of $Mg_{0.5}K_{3-n}H_nATP$ dependent on hydrolysis. Concentration 1 ATP/170 H_2O; region of the phosphate group bands. I $n=0.5$; II $n=1.2$; III $n=1.5$.

the pK$_a$ values summarized in Table I are present in the systems. These values are changed due to the interaction in the system, as illustrated in the following. They nevertheless provide a criterium when discussing the IR results.

Table I. pK$_a$ vlues of the hydrolysis products.

Substance	Protonated group	pK$_a$	Ref.
phosphate	PO_4^{3-}	12.0 7.2	4
ATP^{4-}	$-PO_3^{--}$	6.5	5
ADP^{3-}		5.9	6
$Mg^{2+}ADP^{3-}$		5.2	6
$Mg^{2+}ATP^{4-}$		4.5	5
ATP^{4-}	base residue	4.2	5
ADP^{3-}		4.2	6
$Mg^{2+}ADP^{3-}$		4.2	6
$Mg^{2+}ATP^{4-}$		3.7	6

Addition of protons to the phosphate groups

Dependence on the initial protonation

In the figures the initial protonation increases from left to right (I to III) and the degree of hydrolysis from top to bottom (a to c). In Fig. 2 processes occurring on proton addition are illustrated. The spectra of the $Mg_{0.5}K_{2-n}H_nADP$ solution (Fig. 4 in [3]) clarify the course of the changes to the phosphate bands on initial protonation. In Fig. 1 I a the band ν_{as} of the terminal $-PO_3^{2-}$ groups of the ATP at 1120 cm^{-1} is no longer very intense due to the fact that one part of these groups is already rearranged by the protons present in the system. The local hydrolysis induced by the Mg^{2+} ions acts in the same way. The same is indicated by the band $\nu_s PO_2^-$ of the additional PO_2^- groups at 1080 cm^{-1} arising on the rearrangement

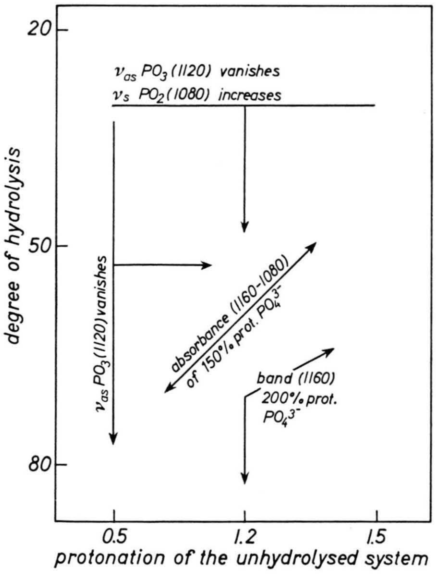
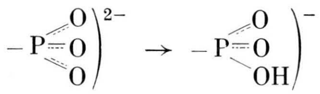


Fig. 2. Schematic representation of the addition of the protons to the phosphate groups.



$$-P\left(\begin{matrix}O\\O\\O\end{matrix}\right)^{2-} \rightarrow -P\left(\begin{matrix}O\\O\\OH\end{matrix}\right)^{-}$$

As the initial protonation increases, that is, from a I to III, the band at 1120 cm^{-1} continues to decrease and that at 1080 cm^{-1} to increase, showing that the protons are added to the $-PO_3^{2-}$ groups.

Dependence on degree of hydrolysis

In all systems the $\nu_{as} PO_3$ vibration at 1120 cm^{-1} likewise decreases as hydrolysis continues, $\nu_s PO_2$ at 1080 cm^{-1} increasing.

These bands are superposed by the broad absorption of the 150% protonated phosphate ion (Fig. 1 in [3]). In the hydrolysis series I this broad band in the range 1160 – 1080 cm^{-1} appears especially marked at hydrolysis degrees between 70 and 80%. In the hydrolysis series II the same band is observed – but already less marked – at a hydrolysis degree of approx. 70% and far weaker in the hydrolysis series III at approx. 60%. The grouping (s. Fig. 5) causing this band accordingly occurs at the ever lower degrees of hydrolysis, the larger the initial protonation of the system is. The band is all the more marked when it occurs at higher degrees of hydrolysis since the inorganic phosphate content of the solution increases on hydrolysis.

In the systems II and III at hydrolysis degrees above 70% the band at 1160 cm^{-1} appears, which is characteristic for the 200% protonated PO_4^{3-} ion (*cf.* Fig. 1 in [3]).

In series c dependence on the initial protonation again becomes visible. With c I the complex formed by the 150% protonated PO_4^{3-} ions is found preferentially, and the band of the 200% protonated PO_4^{3-} ist still weak with c II and already quite strong with c III.

The addition of protons to the base residues

Fig. 3 illustrates the changes to the spectra in the range of the ring vibrations of the base residues.

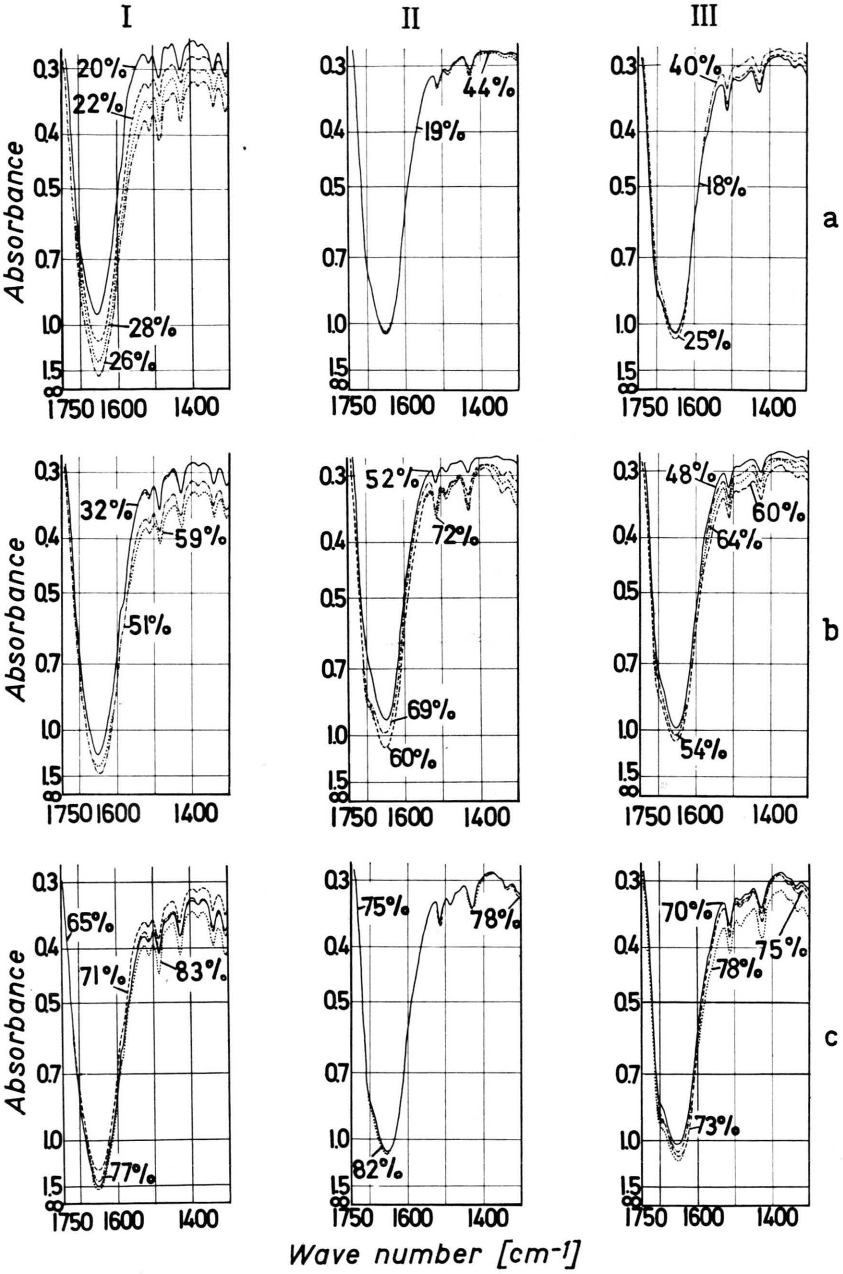


Fig. 3. IR spectra of aqueous solutions of $Mg_{0.5}K_{3-n}H_n$ ATP dependent on hydrolysis. Concentration 1 ATP/170 H_2O; region of the vibrations of the base residues. I $n=0.5$; II $n=1.2$; III $n=1.5$.

Their protonation is indicated, as shown in [3], by the occurrence of a vibration at 1690 cm^{-1} which appears as a shoulder at the intense band of the H_2O scissor vibration. As already mentioned in [3], the decrease of the band at 1480 cm^{-1} and the simultaneous increase of the band at 1520 cm^{-1} provide a further criterium for the protonation of the base residues.

Dependence on the initial protonation

As the protonation increases, the protonation of the base residues also rises. This tendency is easy to follow in Fig. 3 (series I to III).

Dependence on the degree of hydrolysis

Due to the slight initial protonation, sufficiently high overall protonation is never reached on hydrolysis with series I for the base residue to be involved to any considerable extent.

The following is clear with series II: At low degrees of hydrolysis a part of the protons arising on protonation is first added to the base residue, for the intensity of the shoulder at 1690 cm^{-1} increases. This occurs more and more until the phosphate ion is protonated further from 150 to 200% commencing from 50 – 60% hydrolysis. From then on the ring protonation does not increase further.

In the hydrolysis series III the base residue is already largely protonated even with the initial substance. With hydrolysis degrees above 70% the protonation of the base residue decreases again somewhat, which is connected with the protonation of the phosphate ions from 150 to 200%.

The formation of polarizable hydrogen bonds

Changes to the background, that is, a continuous IR absorption, indicate the presence of easily polarizable hydrogen bonds [7–9]. On account of their polarizability these hydrogen bonds interact strongly with their environment [7, 8, 10–12]. Easily polarizable hydrogen bonds are very often hydrogen bonds of type $(BH\cdots B)^+$ which can be illustrated by this structure and by the boundary structure $(B\cdots HB)^+$. According to the findings in [3], the formation of easily polarizable $OH^+\cdots O$ bonds between the hydrogen phosphate ions and of such $NH^+\cdots N$ bonds between the base residues is to be expected in the systems investigated here. Hence investigating the changes to the background could indicate important features of the intermolecular interactions occurring during hydrolysis.

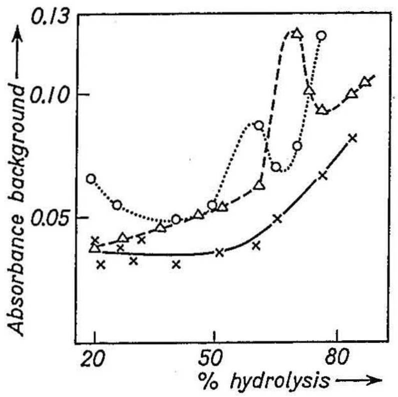


Fig. 4. Aqueous solutions of $Mg_{0.5}K_{3-n}H_n$ ATP. Absorbance of the background at 2600 cm^{-1} plotted over the degree of hydrolysis. —— $n=0.5$; — — — $n=1.2$; ···· $n=1.5$.

The background at 2600 cm^{-1} is plotted in Fig. 4 in dependence on the degree of hydrolysis. The following shows that the changes to the background can basically be explained due to superposition of the absorption of the above-mentioned types of polarizable hydrogen bonds.

Polarizable hydrogen bonds between hydrogen phosphate ions

In the system $n=1.2$ and $n=1.5$ maxima of the IR continuum are observed at about 70% or at about 60% hydrolysis, respectively. These maxima occur with the hydrolysis degrees at which the PO_4^{3-} ions are 150% protonated. The investigation [3, 12a] of the phosphate ion in aqueous solution showed that at 150% protonation the phosphate ions cross-link via polarizable hydrogen bonds (Fig. 5 a), which is also indicated in this case by a maximum of the IR continuum. We therefore also ascribe the maximum observed in the hydrolysis systems to easily polarizable $OH^+\cdots O$ hydrogen bonds between the hydrogen phosphate ions. Hence these can be illustrated by the proton boundary structures shown in Fig. 5 a. The proton fluctuates in the polarizable hydrogen bonds. This fluctuation of the protons explains the strong broadening of the band of the 150% protonated phosphate ions at 1080 cm^{-1}.

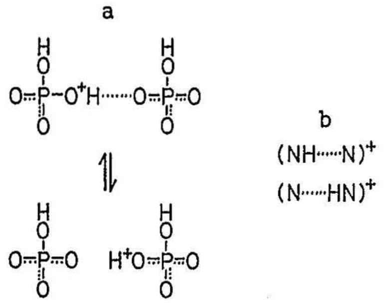


Fig. 5. Proton boundary structure of easily polarizable hydrogen bonds. a. Between hydrogen phosphate ions; b. between base residues.

The rise of the absorbance of the continuum in the system $n=0.5$ could likewise be caused by such hydrogen bonds, for it was shown that the groupings illustrated in Fig. 5 a form at these degrees of hydrolysis. The lower the initial protonation is, the higher the degrees of hydrolysis at which these groupings form.

Polarizable hydrogen bonds between the base residues

In system $n = 0.5$ no change to the background apart from the above-discussed one is observed. Accordingly no $NH^+ \cdots N$ hydrogen bonds form, which conforms with the finding that the base residues in this system are not protonated.

In system $n = 1.2$ the protonation of the base residues increases during hydrolysis. Hence the rise of the continuum as the degree of hydrolysis increases is ascribed to the formation of $NH^+ \cdots N$ hydrogen bonds (s. Fig. 5 b).

Finally, in system $n = 1.5$ the decrease of the background as the hydrolysis degree increases at low degrees of hydrolysis may be due to the fact that in the initial system $NH^+ \cdots N$ bonds which are present are ruptured and unsymmetrical hydrogen bonds are formed. The steep rise of the continuum at large degrees of hydrolysis is probably caused by the fact that $H_5O_2^+$ groupings already form (see here [13, 14]).

The question as to whether, apart from these symmetrical, easily polarizable hydrogen bonds, unsymmetrical polarizable hydrogen bonds form between different acceptors must be clarified by detailed investigation of the course of the continuum at different wave number values.

Bands of the hydration water and intermolecular interaction

Previous measurements have already shown that the torsional vibration of the H_2O is influenced by the formation of polarizable hydrogen bonds, since the transitions in the polarizable hydrogen bonds couple with the intermolecular vibrations [9, 14]. Investigating the PO_4^{3-} and the $(PO_4^{3-} + MgADP^-)$ system indicated the same.

Observing the broad band of the torsional vibration at 700 cm^{-1} shows that extremely large changes to the water bands occur within small ranges as hydrolysis proceeds.

1. The integral absorbance of the torsional vibration of the water (at 700 cm^{-1} [15]) changes, for instance with $Mg_{0.5}K_{2.5}H_{0.5}$ATP in the range of 20 – 26% degree of hydrolysis, by about two orders of magnitude. The same is true in the other systems in some hydrolysis ranges.
2. In the case of slight absorbance of the band the latter possesses a structure and its maximum lies at 710 cm^{-1}, with strong absorbance it lies at 650 cm^{-1}; both values for solutions of 5 °C.
3. The H_2O combination vibration [11] shifts parallel to the torsional vibration from 2160 cm^{-1} toward larger wave numbers.
4. The change to the integral absorbance of the H_2O scissor vibration follows that of the torsional vibration (Fig. 3), except in the hydrolysis ranges in which the formation of polarizable hydrogen bonds is indicated by the occurrence of a continuous absorption.

In the latter case the absorbance change is explained by a coupling of the torsional vibration with transitions in the polarizable hydrogen bonds. In the other ranges, however, there is no such connection.

In these ranges the band shift of the torsional vibration and that of the combination vibration toward larger wave numbers as well as the intensity change of the scissor vibration signify the formation of a hydrated structure with stronger hydrogen bonds. Fox and Martin [16] studied this with pure water at the band at 2160 cm^{-1}, investigating the temperature sensitivity. The magnitude of the change to the transition moment of the torsional and of the H_2Oscissor vibrations in the ATP solutions shows that large ranges of the hydration structure are affected by slight alterations of the degree of hydrolysis (see also ref. 16 a).

Conclusions

According to these results, the PO_4^{3-} ions arising on hydrolysis do not first of all bind both hydrolysis protons during the hydrolysis. In fact, 150% protonated PO_4^{3-} ions first form which cross-link via $OH^+ \cdots O$ hydrogen bonds. The formation of these hydrogen bonds in correlated to the formation of $NH^+ \cdots N$ bonds between base residues.

Accordingly, on ATP hydrolysis polarizable hydrogen bonds are formed which interact with their environment via relatively extensive forces. Moreover, the hydrate structure changes most remarkably, too.

These changes must be connected with a correspondingly large change to the dielectric properties of the systems. The hydrolysis of the ATP hence controls the intermolecular interactions in the system investigated. Since hydrolysing ATP is present in most biological systems, it is clear that the effects observed are of biological significance.

This corresponds to the supposition on the part of George *et al.*[2] that changes to the solvation enthalpy are primarily of significance as regards the change to the free enthalpy on ATP hydrolysis.

Hydrolysing ATP is present in many biological systems, for instance in the muscle proteins. Herbst and Piontek[17] showed that the medium in these proteins becomes briefly acidic during the contraction process. This signifies a possible relevance of the present study for the biological events in a special system, for when the intermolecular interaction and thus the dielectric properties of a medium change, the forces between molecules embedded in this medium also change.

Experimental Procedure

The substances supplied by the following firms: K_2H_2ATP Sigma Chem. Comp. St. Louis, USA; Mg_2ATP K K Lab. Plain View, New York, USA (supplied by Serva Heidelberg) (NB: this is not the di-Mg salt!); H_4ATP Fluka AG, Buchs (Switzerland). All crystallized initial substances were subjected to ultimate analysis in order to preclude contamination and in order to take the crystal water into consideration.

All work was carried out under nitrogen to avoid the sample solutions from coming into contact with the CO_2 in the atmosphere. The required cation-proton ratios were obtained by mixing the above-mentioned substances. The salts with more K^+ than K_2H_2ATP were made by neutralizing with KOH. The concentration was determined by the UV absorption at 260 nm[18]. The K^+ ion content was determined gravimetrically via the complex formation with tetraphenyloborate.

The solutions were stored at 40 °C in bottles which were completely sealed by a membrane. The spontaneous hydrolysis occurring thereby was interrupted every 24 hours, a sample removed and a spectrum of this plotted at 5 °C. During the IR measurement the hydrolysis degree of a parallel sample was determined enzymatically.

The samples were located in the cell described in [3] during spectra plotting.

The layer thickness of the sample was reproducible to $\pm 0.05\ \mu m$.

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