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AN ENQUIRY INTO THE IMPORTANCE OF SOLVATION EFFECTS IN PHOSPHATE ESTER AND ANHYDRIDE REACTIONS

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SUMMARY

Current theories to account for the greater thermodynamic reactivity of "high energy" phosphate compounds compared to orthophosphate esters in terms of intramolecular properties are examined in the light of new data for the ionization of pyro- and tripolyphosphoric acids, ADP, ATP, phosphoenolpyruvate and acetyl phosphate. The changes in ΔG^0 for hydrolysis, as reactants and products undergo successive matching ionizations, are not compatible with electrostatic repulsion being a dominant factor: nor are the changes in ΔH^0 in accord with the hypothesis that the P–O bond is weakened as the anhydride ion structure acquires a bigger negative charge.

Calculations of solvation energies for the ortho-, pyro-, and tripolyphosphate ions show these intermolecular energy terms to be large in magnitude, with minimum values ranging from 76 to 643 kcal/mole depending on charge and structure. Differences between these quantities for reactant and product species therefore constitute a major factor contributing to the thermodynamic reactivity which might be masking even greater contributions from specific intramolecular properties than theoretical calculations have so far indicated. Of all solvents likely to participate in living systems only water gives rise to solvation energies large enough to stabilize multi-charged phosphate and phosphate anhydride ions in solution.

Using these solvation energies, values have been calculated for the proton affinities of the various ions. Proton transfer from a C–H group is a very favorable reaction with ions having a doubly-charged anionic center. This finding has important implications with respect to enzyme reaction mechanisms and the transport of multi-charged anionic species through membranes.

New values are also reported for the heats of hydrolysis of pyro- and tripolyphosphate, and for $\bar{S}^0$ of the $H_2P_2O_7^{2-}$ and $P_3O_{10}^{5-}$ ions. ΔG^0 values for the hydrolysis reactions compared with those for ADP and ATP show an enhancement of the reactivity of the adenosine esters relative to their inorganic counterparts. An interesting consequence is that in going from AMP to ADP to ATP the ribose C-5 ester bond acquires "high energy" character, the hydrolysis of ATP yielding adenosine and tripolyphosphate having a ΔG^0 value more like that for the fission of an anhydride bond.

INTRODUCTION

In living organisms on earth the "high energy" phosphate compounds, especially phosphate anhydride derivatives such as ATP, play a central role conserving energy in substrate level and oxidative phosphorylation, and utilizing it in a great variety of group transfer reactions. They are involved in the breakdown and synthesis of organic molecules, in active transport, in muscle and nerve action. These processes occur in a predominantly aqueous medium with a gross pH about neutrality, and as a result the phosphate compounds are present as ions. The environment, however, may have been very different under primitive earth conditions when life began, as it may well be different in extraterrestrial forms of life, if they exist. So it is important to establish whether the "high energy" property of these compounds is purely intramolecular in origin, or whether interactions with the solvent water are of significance.

Although the question why high energy phosphate compounds are rich in energy has long been recognized as a key problem in bioenergetics, it is odd that in the chief theoretical formulations to date—opposing resonance[1], electrostatic repulsion[2], and electron distribution along the P–O–P backbone[3–5]—the water has been either ignored, or regarded solely as a continuous dielectric for the purpose of calculating repulsion energies. The very existence of ions in aqueous solution is due to their solvation energies compensating for the large amount of energy required to disrupt the crystal lattice in the dissolution of an ionic crystal, or to break the covalent bond to hydrogen and bring about electron transfer in the ionization of an acid. Even for singly-charged ions the solvation energies are similar in magnitude to strong covalent bond energies, *e.g.* Na^+ 100, and Cl^- 88 kcal/mole: and for multi-charged ions the values* are several times greater, *e.g.* Mg^{2+} 465, and S^{2-} 322 kcal/mole[6,7]. Hence even in the absence of values for the high energy phosphate compounds the conclusion is inescapable that at biological pH an important term contributing to their thermodynamic reactivity will be the difference between the solvation energies for the multi-charged reactant and product ions.

Our determinations of thermodynamic data for these reactions have now reached a stage where a preliminary assessment of the influence of adenosine substitution on the reactivity of the phosphate anhydride ions can be made, and where predictions based on electrostatic models can be tested in detail. In addition provisional values have been calculated for the solvation energies of the orthophosphate, pyrophosphate and tripolyphosphate ions which fully substantiate the above conclusion.

RESULTS

Resume of experimental data

In comparing the reactivity of the various phosphate compounds we follow the usual practice and take as reference reaction the transfer of the phosphate group to water—namely the hydrolysis.

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* The value of 263.7 kcal/mole of MORRIS[7] has been adopted for the absolute enthalpy of hydration (solvation energy) of the proton; solvation energies quoted here are rounded off to the nearest kilocalorie.

ΔG^0 for the hydrolysis of ATP has been based on literature data for the glutamine synthetase and hydrolysis reactions[8,9]: ΔG^0 for the hydrolysis of ADP has been obtained from the ATP value and literature data for the myokinase reaction[10]: extrapolation to zero ionic strength was done by an empirical procedure using activity coefficient ratios obtained in pK studies[11]. ΔG^0 for the hydrolysis of glucose 6-phosphate, acetyl phosphate and phosphoenolpyruvate have also been based on literature data[12–15]. ΔG^0 for the hydrolysis of AMP was calculated from an equilibrium constant obtained using a deaminase assay to measure the AMP concentration at equilibrium after chromatographic separation of the products. The heats of hydrolysis for glucose 6-phosphate, acetyl phosphate, phosphoenolpyruvate, AMP, ADP and ATP were measured using unbuffered solutions, $I \leqslant 0.01$, in a Beckman microcalorimeter, under conditions such that complex formation with metal ions played an insignificant role[16]. For the present purpose it has been assumed that ΔH^0 at infinite dilution would have the same values.

ΔG^0 for the hydrolyses of pyrophosphate and tripolyphosphate were calculated from similar measurements of ΔH^0 and values of ΔS^0 obtained from the determination of S^0 for the crystalline salts $Na_2H_2P_2O_7$ and $Na_5P_3O_{10}\cdot 6H_2O$, activity coefficient measurements, solubility and heat of solution studies, and a value for $\bar{S}^0(H_2PO_4{}^{1-})_{aq}$ calculated from data* in the literature[17,18].

Thermodynamic data for the secondary phosphate and the adenine ring ionizations were obtained using a potentiometric method[19], extrapolating values of pK' within the Debye–Hückel limiting region to $I = 0$. Data for the two strong primary ionizations of pyrophosphoric acid were calculated from a combination of pK' measurements using a dye-indicator method and calorimetric determinations of the heat of protonation. A similar procedure was employed for the three strong primary ionizations of tripolyphosphoric acid except that a value of ΔS^0 for the first very strong ionization, based on the variation of $\bar{S}^0$ with ionic charge for ortho-, pyro- and tripolyphosphate ions, was used together with an experimental value for the pK to estimate ΔH^0. For the strong ionization of phosphoenolpyruvic acid we have used our values for phenyl phosphoric acid in which the bonding of the phosphate is quite similar.

With these ionization data, values of ΔG^0, ΔH^0 and ΔS^0 for the hydrolysis reactions involving various ionic species have been calculated from experimental values obtained at a particular pH, see Tables I–III.

Reactivity enhanced in ADP and ATP

While the hydrolyses of inorganic pyrophosphate and tripolyphosphate obviously come into the high energy category, comparison with corresponding reactions of ADP and ATP shows there is some enhancement in the case of ADP and appreciably more with ATP—especially the pyrophosphate split. The actual increment in ΔG^0 depends on which ionic species are being compared, as does the relative importance of ΔH^0 and ΔS^0, although in all the ATP reactions the entropy term apparently plays the major role.

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* STEPHENSON[17] obtained $S^0(H_2PO_4{}^{1-})_{aq} = +21.6$ cal/degree per mole from data for KH_2PO_4. Similar calculations based on a recent value[18] for the entropy of $Na_2HPO_4\cdot 12H_2O$, give $S^0(HPO_4{}^{2-})_{aq} = -8.7$ cal/degree per mole, and hence $S^0(H_2PO_4{}^{1-})_{aq} = +20.9$ cal/degree per mole. We have adopted a value of 21.2 cal/degree per mole.

TABLE I

TRIPOLYPHOSPHATE, PYROPHOSPHATE AND GLUCOSE 6-PHOSPHATE HYDROLYSES

The symbols Glc-6-P^{2-}, Glc-6-PH^{1-} and Glc-6-PH_2 denote the conjugate base and the two conjugate acid species of glucose 6-phosphate. Solutes, 1 molal; H_2O, liquid. Temp. 25°; $I = 0$.

Reaction	ΔG^0 (*kcal/mole*)	ΔH^0 (*kcal/mole*)	ΔS^0 (*cal/degree per mole*)
$H_5P_3O_{10} + H_2O \rightarrow H_3PO_4 + H_4P_2O_7$	−8.8	−10.1	−4
$H_4P_3O_{10}^{1-} + H_2O \rightarrow H_3PO_4 + H_3P_2O_7^{1-}$	−7.8	−9.9	−7
$H_3P_3O_{10}^{2-} + H_2O \rightarrow H_2PO_4^{1-} + H_3P_2O_7^{1-}$	−6.9	−8.8	−6
$H_2P_3O_{10}^{3-} + H_2O \rightarrow H_2PO_4^{1-} + H_2P_2O_7^{2-}$	−7.3	−8.4	−4
$HP_3O_{10}^{4-} + H_2O \rightarrow H_2PO_4^{1-} + HP_2O_7^{3-}$	−7.2	−5.8	+5
*$P_3O_{10}^{5-} + H_2O \rightarrow HPO_4^{2-} + HP_2O_7^{3-}$	−10.5	−3.6	+23
$H_4P_2O_7 + H_2O \rightarrow H_3PO_4 + H_3PO_4$	−9.5	−7.6	−6
$H_3P_2O_7^{1-} + H_2O \rightarrow H_2PO_4^{1-} + H_3PO_4$	−7.5	−7.3	−1
*$H_2P_2O_7^{2-} + H_2O \rightarrow H_2PO_4^{1-} + H_2PO_4^{1-}$	−7.7	−6.8	+3
$HP_2O_7^{3-} + H_2O \rightarrow HPO_4^{2-} + H_2PO_4^{1-}$	−7.1	−5.8	+4
$P_2O_7^{4-} + H_2O \rightarrow HPO_4^{2-} + HPO_4^{2-}$	−10.4	−3.7	+22
Glc-6-$PH_2 + H_2O \rightarrow$ Glc $+ H_3PO_4$	−5.2	−4.4	+3
Glc-6-$PH^{1-} + H_2O \rightarrow$ Glc $+ H_2PO_4^{1-}$	−3.9	−2.5	+5
Glc-6-$P^{2-} + H_2O \rightarrow$ Glc $+ HPO_4^{2-}$	−3.0	−0.6	+8

* A final revision of experimental data for these reference reactions may alter the values of ΔS^0, ΔH^0 (and hence ΔG^0) slightly, but the increments from one reaction to another, based on the ionization data, would not be changed.

The enhancement likewise appears in the values of ΔG^0, ΔH^0 and ΔS^0 for the hydrolysis of the ester bond to ribose in ADP and ATP that can be calculated from the data in Tables I and II. Comparison with AMP (see Table IV) would suggest that the ester hydrolysis of ADP is on the borderline and that of ATP well within the high energy category. In this set of reactions the change in reactivity is clearly a consequence of changes in the relative thermodynamic properties of the pairs of ions—AMP^{2-}/HPO_4^{2-}, $ADP^{3-}/HP_2O_7^{3-}$ and $ATP^{4-}/HP_3O_{10}^{4-}$. Quite apart from the oversimplification involved in attributing changes in reactivity to differences in bonding, these data demonstrate how inaccurate it is to designate the anhydride bonds in ATP as energy rich and the ester bond as energy poor on the basis that the linkage between the ribose and the first phosphate residue resembles that in AMP. The proper basis for an evaluation is the hydrolysis yielding tripolyphosphate. It may also be noted that in going from AMP to ADP to ATP both ΔH^0 and ΔS^0 contribute to the enhancement of the ester hydrolysis. Usually in a series of reactions that shows a trend in ΔG^0 values the contributions from ΔH^0 and ΔS^0 oppose each other—the one favorable the other unfavorable—so it may be inferred that in this case the observed trends must have at least a dual origin to account for the augmentation.

The extent and nature of this intriguing influence of adenosine substitution on the reactivity of the phosphate anhydrides will not be settled until there is further corroboration of the values of ΔG^0 for the ADP and ATP reactions[8,9] and some uncertainties that still remain in the pyrophosphate and tripolyphosphate data are resolved. Refinement of the measurements upon which $\bar{S}^0(H_2P_2O_7^{2-})_{aq}$ is based is unlikely to alter ΔG^0 for the pyrophosphate reference reaction by more than 0.7

kcal/mole. For the tripolyphosphate reaction the uncertainty in ΔG^0 may be as great as 1.1 kcal/mole because of the problem of extrapolating the hydrolysis heat to infinite dilution and a combination of factors involved in the determination of $\bar{S}^0(P_3O_{10}{}^{5-})_{aq}$ —the higher molecular weight of the hydrated salt, the very low mean ion activity

TABLE II

ATP, ADP AND AMP HYDROLYSES

The symbols ATP^{4-}, ADP^{3-} and AMP^{2-} denote ATP, ADP and AMP, respectively, in which the phosphate group(s) are fully ionized: $ATPH^{3-}$, $ADPH^{2-}$ and $AMPH^{1-}$, *etc.*, the conjugate acid species with respect to the phosphate group(s): Ado stands for adenosine. H^+ before the symbol indicates proton attachment to the adenine ring. Solutes, 1 molal; H_2O, liquid. Temp. 25°; $I = 0$.

Reaction	ΔG^0 (*kcal/mole*)	ΔH^0 (*kcal/mole*)	ΔS^0 (*cal/degree per mole*)
ATP: Orthophosphate split of anhydride chain			
$H^+ATPH^{3-} + H_2O \longrightarrow H^+ADPH^{2-} + H_2PO_4{}^{1-}$	−9.3	−7.1	+7
$ATPH^{3-} + H_2O \longrightarrow ADPH^{2-} + H_2PO_4{}^{1-}$	−9.9	−7.2	+9
$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + H_2PO_4{}^{1-}$	−10.8	−6.8	+13
$ATP^{4-} + H_2O \longrightarrow ADPH^{2-} + HPO_4{}^{2-}$	−10.7	−4.4	+21
ATP: Pyrophosphate split of anhydride chain			
$H^+ATPH^{3-} + H_2O \longrightarrow H^+AMPH^{1-} + H_2P_2O_7{}^{2-}$	−10.6	−8.1	+8
$ATPH^{3-} + H_2O \longrightarrow AMPH^{1-} + H_2P_2O_7{}^{2-}$	−11.4	−7.4	+13
$ATP^{4-} + H_2O \longrightarrow AMP^{2-} + H_2P_2O_7{}^{2-}$	−12.8	−6.8	+20
$ATP^{4-} + H_2O \longrightarrow AMPH^{1-} + HP_2O_7{}^{3-}$	−12.8	−5.6	+24
ADP: Hydrolysis of anhydride group			
$H^+ADPH^{2-} + H_2O \longrightarrow H^+AMPH^{1-} + H_2PO_4{}^{1-}$	−9.1	−7.8	+4
$ADPH^{2-} + H_2O \longrightarrow AMPH^{1-} + H_2PO_4{}^{1-}$	−9.2	−7.0	+7
$ADP^{3-} + H_2O \longrightarrow AMP^{2-} + H_2PO_4{}^{1-}$	−9.8	−6.8	+10
AMP: Hydrolysis of ester group			
$H^+AMPH^{1-} + H_2O \longrightarrow H^+Ado + H_2PO_4{}^{1-}$	−2.4	−1.5	+3
$AMPH^{1-} + H_2O \longrightarrow Ado + H_2PO_4{}^{1-}$	−2.9	−2.1	+3
$AMP^{2-} + H_2O \longrightarrow Ado + HPO_4{}^{2-}$	−2.3	0	+8

TABLE III

PHOSPHOENOLPYRUVATE AND ACETYL PHOSPHATE HYDROLYSES

Solutes 1 molal; H_2O, liquid. Temp. 25°; $I = 0$.

Reaction	ΔG^0 (*kcal/mole*)	ΔH^0 (*kcal/mole*)	ΔS^0 (*cal/degree per mole*)
$HOOC{\cdot}C(=CH_2)OPO_3H_2 + H_2O \longrightarrow CH_3CO{\cdot}COOH + H_3PO_4$	−15.2	−14.3	+3
$HOOC{\cdot}C(=CH_2)OPO_3H^{1-} + H_2O \longrightarrow CH_3CO{\cdot}COOH + H_2PO_4{}^{1-}$	−13.4	−12.2	+4
${}^{1-}OOC{\cdot}C(=CH_2)OPO_3H^{1-} + H_2O \longrightarrow CH_3CO{\cdot}COO^{1-} + H_2PO_4{}^{1-}$	−15.5	−8.8	+22
${}^{1-}OOC{\cdot}C(=CH_2)OPO_3{}^{2-} + H_2O \longrightarrow CH_3CO{\cdot}COO^{1-} + HPO_4{}^{2-}$	−14.8	−6.0	+30
$CH_3CO{\cdot}OPO_3H^{1-} + H_2O \longrightarrow CH_3COOH + H_2PO_4{}^{1-}$	−9.9	−8.6	+4
$CH_3CO{\cdot}OPO_3{}^{2-} + H_2O \longrightarrow CH_3COO^{1-} + H_2PO_4{}^{1-}$	−10.5	−6.4	+14

TABLE IV

HYDROLYSIS OF THE ESTER BOND TO RIBOSE IN AMP, ADP AND ATP

Solutes, 1 molal; H_2O, liquid. Temp. 25°; $I = 0$.

Reaction	ΔG^0 (*kcal/mole*)	ΔH^0 (*kcal/mole*)	ΔS^0 (*cal/degree per mole*)
$AMP^{2-} + H_2O \longrightarrow Ado + HPO_4^{2-}$	−2.3	0	+8
$ADP^{3-} + H_2O \longrightarrow Ado + HP_2O_7^{3-}$	−5.0	−0.9	+14
$ATP^{4-} + H_2O \longrightarrow Ado + HP_3O_{10}^{4-}$	−8.6	−1.9	+22

coefficient for the 1:5 electrolyte, and the extrapolation of the heat of solution of the salt to infinite dilution.

However, in the following discussion of the sequence of hydrolysis reactions for each compound, where reactant and product species undergo successive matching ionizations, the relative values of ΔG^0, ΔH^0 and ΔS^0 are the crucial issue, not their absolute magnitudes.

Ionic charge and electrostatic repulsion

It is of course the negative charges on the adjacent phosphate residues of the anhydride groups in ADP and ATP at biological pH that led to the hypothesis[2] that electrostatic repulsion makes an important contribution to their energy-rich character. Exactly the same structural feature is present in inorganic pyrophosphate and tripolyphosphate. Now if repulsion is considered from the point of view of decreasing the work required to split the anhydride group it should be apparent in the free energy term, ΔG^0. From the point of view of decreasing the P–O–P bond energy it should be apparent in the enthalpy term, ΔH^0, and hence in ΔG^0. But the trends in neither ΔG^0 nor ΔH^0 for the hydrolyses of pyrophosphate and tripolyphosphate are what would be expected if intramolecular electrostatic repulsion was a dominant factor. With increase of charge, and as a consequence increase in repulsion, ΔH^0 gets progressively less favorable throughout each sequence of anhydride ion reactions. ΔG^0 also becomes less favorable for the initial ionizations; it is then little affected by increasing charge, until finally, although it reaches its most favorable value with the most highly charged ions, the increase is due to the much more favorable value of ΔS^0 which has its origin in the very negative values of $\bar{S}^0$ for the $P_2O_7^{4-}$ and $P_3O_{10}^{5-}$ ions.

Doubt is also cast on the importance of repulsion as a dominant factor by the values for the orthophosphate ester, glucose 6-phosphate. The trends in ΔG^0 and ΔH^0 are substantially the same, and not much less in magnitude, even though internal repulsion peculiar to the anhydride ion is necessarily absent.

Data are not yet available for the strong primary phosphate ionizations of ADP and ATP, but the trends in ΔG^0 and ΔH^0 associated with the secondary phosphate ionizations show just the same characteristics as the inorganic anhydrides, see Table II. ATP presents an interesting feature. It just so happens that at 25° the values of pK^0 for the secondary phosphate ionization of ADP and orthophosphate are almost identical, hence ΔG^0 is the same for the orthophosphate split of ATP^{4-} no matter whether the products are ADP^{3-} and $H_2PO_4^{1-}$, or $ADPH^{2-}$ and HPO_4^{2-}. By a further coincidence the pyrophosphate split shows the same behavior, AMP and pyrophosphate have almost identical pK^0 values at 25°, so ΔG^0 is the same for the formation of $AMPH^{1-}$ and $HP_2O_7^{3-}$ as for AMP^{2-} and $H_2P_2O_7^{2-}$. Yet in both cases electrostatic repulsion should favor the first of the two reaction paths. However, when the contributions of ΔH^0 and ΔS^0 to ΔG^0 are considered, significant differences emerge which reflect the different contributions of the heat and entropy terms to the standard free energies of ionization.

Another comparison that can be made with data for the nucleotides is the influence of the adenine ring ionization. In the conjugate acid the ring takes on a positive charge: adenosine becomes a singly charged cation, $H^+ \cdot Ado$; AMP becomes a zwitter-ion with zero net charge, $H^+ \cdot AMPH^{1-}$; ADP and ATP become zwitter-ions with net charges of 1— and 2—, respectively, $H^+ \cdot ADPH^{2-}$ and $H^+ \cdot ATPH^{3-}$. Examination of molecular models shows that no steric hindrance or strain is involved in the conformations of AMP, ADP and ATP where the positively charged ring is in close proximity to the negatively charged phosphate group(s). A recent X-ray diffraction study of thiamine pyrophosphate, in the form of the neutral zwitter-ion octahydrate, has shown it to have this type of structure with the pyrophosphate group bent back over and above the organic portion containing the thiazole and pyrimidine rings[20].

If only electrostatic considerations were to apply, these conformations of AMP, ADP and ATP would be those of minimum potential energy, *i.e.* the most stable. Relative to the conjugate base species, where the adenine ring is uncharged, Coulombic attraction would be expected to introduce a substantial positive increment in ΔG^0 for the hydrolysis of AMP, and, by diminishing the internal repulsion between negatively charged phosphate groups in the anhydride chain, ΔG^0 for the hydrolyses of ADP and ATP would also be expected to assume less favorable values. Although, as can be seen in Table II, the increments are positive, they are very small, 0.5, 0.1, 0.6 and 0.8 kcal/mole for AMP, ADP, ATP (ortho split) and ATP (pyro split), respectively; and they are not at all commensurate with the hypothesis that repulsion is a dominant factor in the hydrolysis of the anhydride chain. Furthermore for the hydrolytic fission of the nucleoside bond in AMP, ADP and ATP giving adenine and ribose 5-phosphate, 5-pyrophosphate and 5-tripolyphosphate, respectively, the ionization data are such that ΔG^0 for the reaction involving the conjugate acids is actually more favorable than ΔG^0 for the reaction involving the conjugate bases in the case of AMP and ADP by 0.4 and 0.2 kcal/mole: only with ATP is it less favorable, by 0.4 kcal/mole. In view of the Coulombic attraction in $H^+ \cdot AMPH^{1-}$, $H^+ \cdot ADPH^{2-}$ and $H^+ \cdot ATPH^{3-}$ less favorable values of ΔG^0 would have been expected throughout, just as it was in the ester and anhydride hydrolyses. We infer from these small haphazard differences in ΔG^0 that the observed effect of the adenine ring ionization is not primarily a consequence of electrostatic interaction but is due to other factors.

With phosphoenolpyruvate, according to the electrostatic hypothesis[2], repulsion between the $-COO^-$ and the phosphate group should favor hydrolysis at biological pH to an extent similar to that expected for ADP (and pyrophosphate). But, as the trends in ΔG^0 and ΔH^0 in Table III show, phosphoenolpyruvate resembles pyrophosphate and ADP in not showing the expected correlation with increasing ionic charge. ΔG^0 again remains almost constant, and ΔH^0 becomes even less favorable throughout the sequence of reactions. Moreover it has been widely accepted that the high energy feature is due mainly to the reversion of the enolpyruvate formed initially on hydrolysis to the more stable keto form. In the absence of solvent interactions, ΔS^0 for a tautomeric change is small, so ΔH^0 should be the predominant term. Yet for the more ionized species the $T\Delta S^0$ term is of comparable or even greater magnitude. Only for the neutral or singly charged species is ΔH^0 the predominant term, and the tautomeric change probably the dominant factor.

The trend in ΔH^0 to less favorable values as reactant and product species become more highly charged is also shown by acetyl phosphate, where, like glucose 6-phosphate, there are no adjacent negatively charged groups in the reactant species. In this case, however, the matching ionizations are the secondary phosphate ionization of the acetyl phosphate and the carboxyl ionization of the acetic acid, so there is charge separation in the products.

This trend in ΔH^0, which appears to be quite general, is in sharp contrast to that for ΔS^0 which reaches its most favorable values for the reactions involving the more highly charged species. Solvation effects are clearly implicated because a very important factor which determines the partial molal entropies of ions is their structure making or structure breaking influence on the liquid water.

Solvation energies

As suggested at the outset in phosphate ester and anhydride reactions at least at biological pH solvation energies are likely to be contributing to the reactivity to an extent that has been little appreciated. The values of ΔH^0 for the sequential reactions in Table I, calculations of solvation energies for the orthophosphate ions, and an estimate of the solvation energy of H_3PO_4 enable us to arrive at lower limits for the solvation energies of the anhydride ions.

Calculations* of lattice energies for sodium or potassium salts of $H_2PO_4^{1-}$, HPO_4^{2-} and PO_4^{3-} using KAPUSTINSKII's equation[21–23] together with the experimental values for their heats of solution[24] give solvation energies of 76, 299 and 637 kcal/mole, respectively. The heat of solution of H_3PO_4(s) is known, $\Delta H^0 = -3.2$ kcal/mole (ref. 25), and an estimate of its heat of sublimation can be made from a variety of data in the literature, *e.g.* sublimation heats** for structurally related compounds[24–27],

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* KAPUSTINSKII's equation[21] expresses the lattice energy U_0 in terms

$$U_0 = \frac{287.2\nu z_c z_a}{r_c + r_a}\left(1 - \frac{0.345}{r_c + r_a}\right)$$

of ν, the number of ions in the molecule, z_c and z_a the charge on the cation and anion, and r_c and r_a their Goldschmidt or thermochemical radii. YATSIMIRSKII[22] obtained 2.38 Å for the radius of PO_4^{3-}, but there are insufficient thermochemical data at present to calculate values for HPO_4^{2-} and $H_2PO_4^{1-}$ (or indeed for any of the pyrophosphate or tripolyphosphate ions). However, noting that the radius decreases as the charge decreases for similar oxyanions, *e.g.* CO_3^{2-} and HCO_3^{1-} with radii of 1.85 and 1.63 Å, and SO_4^{2-} and HSO_4^{1-} with radii of 2.30 and 2.06 Å, the latter increment has been adopted as the more appropriate for the tetrahedral phosphate ions giving HPO_4^{2-}, 2.14 Å and $H_2PO_4^{1-}$, 1.90 Å.

** Tables are given of vapor pressure as a function of temperature for both inorganic and organic compounds from which the evaporation heat can be evaluated—and hence, knowing the heat of melting, the sublimation heat.

viscosity data[24,28,*] and a comparison of its evaporation rate with the rates for compounds for which the sublimation heats have been measured[29]. These various estimates agree in placing the sublimation heat of H_3PO_4 at about 23 kcal/mole, giving a solvation energy of about 26 kcal/mole.

Now if the hydrolyses of the anhydride compounds as listed in Table I were endothermic in the gas phase, the favorable (exothermic) values of ΔH^0 for the aqueous solution reactions could only arise by the solvation energies of the products exceeding those of the reactants, and since ΔH^0 is the major component of ΔG^0 for the solution reactions, the argument for the importance of solvation energies would need no further elaboration.

If the reactions were thermoneutral in the gas phase, which would be the case if the sum of the energies of the bonds broken was exactly equal to the sum of the energies for the bonds formed, then the following calculations can be made. In gas phase–aqueous solution reaction cycles, like that set out for the hydrolysis of $H_3P_2O_7^{1-}$ in Fig. 1, the only unknown is the solvation energy for the pyrophosphate anion: taking 10.5 kcal/mole as the heat of evaporation of water, and substituting the values for the remaining species given above, the solvation energy of $H_3P_2O_7^{1-}$ is found to be 84 kcal/mole. Similar cycles for the hydrolyses of the other pyrophosphate ions lead to values for their solvation energies, and using the pyrophosphate values in turn, values for the tripolyphosphate ions have been calculated (see Table V).

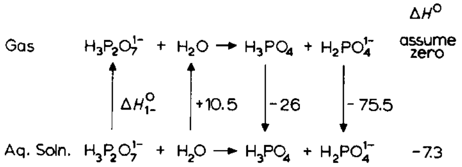

Fig. 1. Reaction cycle for the calculation of the solvation energy of the $H_3P_2O_7^{1-}$ ion.

If the reactions were exothermic in the gas phase, which would be the case if special intramolecular properties of the anhydrides contributed to their instability, examination of the calculation procedure shows that the values obtained above are minimum values for the solvation energies of the anhydride ions: the more exothermic the gas phase reaction, the larger the solvation energy.

If we have adopted a value for the solvation energy of H_3PO_4 that is too low, which could be the case if there was appreciable association of H_3PO_4 in the gas phase, this would also give a lower limit for the anhydride energies. The uncertainty is unlikely to exceed 10 kcal/mole. It is hardly feasible, in view of the physical properties of H_3PO_4, that the value adopted is too high: any error in this direction could scarcely be greater than 2 or 3 kcal/mole. With regard to the solvation energies of the orthophosphate ions, the lattice energies used in their evaluation may be too low[**] but this would also result in minimum values for the anhydride ions.

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[*] Examination of data for a number of liquids indicates that the viscosity is about 2–3 millipoises at the boiling point. If the data for orthophosphoric acid are plotted in the form log η against $1/T$, extrapolation of the linear portion below 100° (where error due to the slow reorganization of the liquid giving some polyphosphoric acids would be minimized) gives an effective boiling point of about 650°. Taking the entropy of evaporation to be about 32 cal/degree per mole, like sulfuric acid, leads to a heat of evaporation of about 21 kcal/mole.

[**] KAPUSTINSKII's equation[21] often gives results on the low side, see ref. 23.

TABLE V

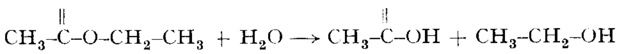
SOLVATION ENERGIES

	Solvation energy (kcal/mole at 25°)
$H_2PO_4^{1-}$	76
HPO_4^{2-}	299
PO_4^{3-}	637
$H_3P_2O_7^{1-}$	84
$H_2P_2O_7^{2-}$	134
$HP_2O_7^{3-}$	358
$P_2O_7^{4-}$	584
$H_4P_3O_{10}^{1-}$	90
$H_3P_3O_{10}^{2-}$	140
$H_2P_3O_{10}^{3-}$	191
$HP_3O_{10}^{4-}$	418
$P_3O_{10}^{5-}$	643

We are thus drawn to the conclusion that there can be no doubt that in the aqueous solution hydrolysis of anhydride ions there is an interplay between solvation energies of reactant and product species that are of a magnitude either comparable to, or, for the more highly charged ions, several times greater than the intramolecular P–O and O–H bond energies. For the ionized species of AMP, ADP and ATP the solvation energies will be modified by increments characteristic of the adenosine moiety. But in the anhydride hydrolyses of ADP and ATP, where adenosine itself is not liberated with the generation of a new hydroxyl group, we would expect the contribution of these increments to be much the same, so the overall contribution of solvation energy to ΔH^0 would be like that for the parent pyrophosphate and tripolyphosphate ions. The values of ΔH^0 for corresponding reactions of ADP and pyrophosphate, ATP and tripolyphosphate, which differ at the most by 1.5 kcal/mole, bear this out.

A similar interplay between solvation energies must occur in the hydrolysis of phosphate esters. Although the total solvation energy concerned will not be as great as that for the more highly charged anhydride ions, the difference between the energies for the ester ion and water, and the alcohol and phosphate ion, is clearly capable of resulting in a sizeable contribution to ΔH^0. In all probability solvation favors the hydrolysis, an important feature being the interaction of the two newly formed hydroxyl groups of the product species with the solvent water.

This is certainly the case with the acidic solution hydrolysis of simple esters of carboxylic acids, *e.g.* data for ethyl acetate show that in the gas phase



$$CH_3\text{–}\overset{\overset{O}{\|}}{C}\text{–}O\text{–}CH_2\text{–}CH_3 + H_2O \longrightarrow CH_3\text{–}\overset{\overset{O}{\|}}{C}\text{–}OH + CH_3\text{–}CH_2\text{–}OH$$

$\Delta H^0 = +4.6$ kcal/mole, whereas in aqueous solution, due to solvation energies of 10.7, 10.5, 12.8 and 12.6 kcal/mole (reading from left to right), $\Delta H^0 = +0.4$ kcal/mole, solvation thus favoring the reaction by 4.2 kcal/mole[30–32]. For the hydrolysis of pyrophosphoric acid a tentative estimate of the solvation energy of $H_4P_2O_7$ suggests that the gas phase reaction may be nearly thermoneutral, the favorable value of $\Delta H^0 = -7.6$ kcal/mole for the acidic solution reaction, which constitutes most of the driving force, arising largely from the greater solvation energy of the products.

In one of the more detailed theoretical treatments the contributions of opposing resonance and electrostatic repulsion to the energy-rich character of ATP and ADP at biological pH have been evaluated as 3.2 and 2.0 kcal/mole, respectively, in the case of ATP, and 2.6 and 1.4 kcal/mole in the case of ADP[4,5]. The contributions from just these two intramolecular factors account fully for the energy-rich character of the anhydrides. For example for ATP, $\Delta G^0 = -3.2 -2.0 -3.0$ (the base value for ester hydrolysis) $= -8.2$ kcal/mole, in agreement with the experimental value then adopted, *i.e.* about -6 to -8 kcal/mole. But we have already seen that as the anhydride ions become more highly charged the values of neither ΔG^0 nor ΔH^0 follow the trend anticipated if electrostatic repulsion played a dominant role. Furthermore, in view of the magnitude of the solvation energies of the ionic species, it is more than likely that the differences between the values for reactant and product species are of the same order as these intramolecular contributions. Not only are solvation effects important, but they may well be masking even greater intramolecular contributions to the reactivity than these theoretical calculations would suggest. In our view further advances in the theory depend not so much on additional refinements or a better choice of parameters in the calculation of electron distributions but in the improvement of the technique* for the evaluation of lattice energies of oxyanion salts[33-36], and in its application to oxyanhydride ion salts so that solvation energies could be obtained to compare with the admittedly approximate values based on KAPUSTINSKII's empirical equation.

It should be noted that the argument for the importance of solvation energies does not depend in any way on the trends in ΔG^0 and ΔH^0. It is interesting and significant that the trends are contrary to what would be expected if electrostatic repulsion was a dominant factor, but even if they were in agreement this would still not prove the case for electrostatic repulsion because solvation energy contributions could give the same result.

Different environments

The conditions under which phosphate anhydrides are capable of functioning as high energy compounds are clearly of importance in considering the origin of terrestrial life and the kind of biochemistry that might be found on other planets.

For dilute aqueous solution at 25° the standard free energy for group transfer to water is favorable throughout the entire pH range from extremely acidic to extremely alkaline solution. Temperature has little effect. Although the reactions for the unionized and the various ionized species are exothermic, to a first approximation, assuming that ΔH^0 is temperature independent, the favorable values of ΔG^0 would be decreased by only 0.6, 1.1 and 1.7 kcal/mole at 100° for ΔH^0 values of -3, -6 and -9 kcal/mole, respectively. Even with solutions containing only 40% water over the temperature range 80-110°, pyrophosphate is still extensively hydrolyzed** at

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* See WADDINGTON's comments in ref. 23 on lattice energy calculations for nitrates and carbonates[33-36].

** The value of the apparent equilibrium constant, evaluated according to the equation $K_{app} = [\text{orthophosphate}]^2/([\text{pyrophosphate}] \times [\text{water}])$, is $1.5 \cdot 10^2$ at 100° (ref. 37).

equilibrium[37]. Hence in thermal springs of high acidity associated with volcanic action the group transfer capability of the phosphate anhydrides would be little impaired. So provided inorganic pyrophosphate, tripolyphosphate, or other polyphosphates could have been formed under primitive earth conditions[38-40] they could have served as precursors of ADP and ATP in phosphorylation and pyrophosphorylation reactions.

According to our present knowledge of the atmosphere and surface conditions on other planets the only ionizing solvent besides water that would be plausible as a medium for life processes is liquid ammonia. But it is not so potent as water. While the salts of many monobasic acids are soluble, *e.g.* nitrates, cyanides, chlorides, bromides and iodides, the salts of polybasic acids are insoluble, *e.g.* carbonates, sulfates, phosphates and pyrophosphates—even ammonium carbonate and ammonium sulfate[41]. Insolubility would therefore rule out all the usual ionized species of orthophosphates and phosphate anhydride compounds from playing any role in liquid ammonia biochemistry. However, the same kind of reactions utilized in aqueous solution could be envisaged with monobasic or neutral species, for instance amides or esters. Similar considerations would apply to non ionizing solvents such as hydrocarbons, *e.g.* liquid methane.

The insolubility of the salts of polybasic acids in liquid ammonia has an interesting bearing on our main topic. Solvation of cations by ammonia is much the same energetically as solvation by water: with some cations, notably those of the transition metals, replacement of water by ammonia in the primary solvation shell occurs even in aqueous solution. Hence the lack of solubility of the salts in liquid ammonia has to be attributed to the contribution of the solvation energy of the multi-charged anionic species being too small to counterbalance the lattice energy.

Proton affinities

Knowing ΔH^0 for an aqueous solution ionization and the solvation energies of the conjugate acid and base it is easy to calculate ΔH^0 for the gas phase ionization (the proton affinity of the conjugate base being defined as this same energy quantity with no change in sign). Typical values for some familiar anions are listed in Table VI together with values for orthophosphate and phosphate anhydride ions calculated using the solvation energies in Table V. The values relating to the primary phosphate ionizations turn out to be identical, as are those relating to the secondary phosphate

TABLE VI

PROTON AFFINITIES

Values are given as kcal/mole at 25°.

Singly charged anionic center				*Doubly charged anionic center*			
F^-	370	HCO_3^{1-}	335	CO_3^{2-}	514	$HP_3O_{10}^{4-}$	488
Cl^-	335	$H_2PO_4^{1-}$	311	SO_4^{2-}	464	$P_3O_{10}^{5-}$	488
Br^-	324	$H_2P_2O_7^{2-}$	311	HPO_4^{2-}	488		
I^-	315	$H_3P_3O_{10}^{2-}$	311	$HP_2O_7^{3-}$	488		
OH^-	385	$H_2P_3O_{10}^{3-}$	311	$P_2O_7^{4-}$	488		

ionizations, which is a result of our assumption that the gas phase hydrolyses were thermoneutral reactions. But, as we have indicated, this assumption leads to minimum values for the solvation energies of the anhydride ions, and these proton affinities are also likely to be minimum values. Furthermore, if our estimates of the solvation energies for the orthophosphate ions are on the low side, all the values for the proton affinities would be lower limits.

As can be seen in Table VI proton affinities for an anionic center bearing a single negative charge lie in the range 310–385 kcal/mole, while those for centers with two negative charges are substantially greater, ranging from 460 to 515 kcal/mole. It has long been recognized that ionization can be observed in solution only if these very adverse energy changes for the gas phase ionization are counterbalanced by solvation energy terms. Proton affinities also determine whether proton transfer can occur, and in this connection the magnitude of the values raises some interesting questions in biochemistry and biophysics.

Ions in protein crevices and membranes

It is not uncommon to see enzyme reaction mechanisms based on substrate binding at reactive sites within a hydrophobic crevice of the protein. Transport of substrates through membranes likewise involves exposure to an essentially organic structure. The initial solvated state of the substrate and the extent to which desolvation and eventually resolvation occur is seldom considered. Clearly it should be: and in the case of substrates where there are two negative charges on an anionic center there is an additional problem.

The acidic ionization of a C–H bond yields a carbanion. For CH_3^- and $C_2H_5^-$, the carbanions formed from CH_4 and C_2H_6, the proton affinities are 390 and 388 kcal/mole, respectively*. In the absence of water of solvation (or another solvent) gas phase energetics must prevail. Hence, while proton transfer to any of the anions in Table V with a singly charged center is energetically unfavorable, *e.g.*

$$R\text{-}CH_3 + Cl^- \longrightarrow RCH_2^- + HCl \qquad \Delta H^0 \text{ is approx. } +53 \text{ kcal/mole}$$

proton transfer to any of them with a double charged center is extremely favorable, *e.g.*

$$R\text{-}CH_3 + HPO_4^{2-} \longrightarrow RCH_2^- + H_2PO_4^{1-} \qquad \Delta H^0 \text{ is approx. } -100 \text{ kcal/mole}$$

These anions cannot co-exist with C–H bonds in an organic environment. There are several ways this impasse can be avoided: for instance prior formation of the conjugate acid species with its singly charged anionic center, or the formation of a metal–ion complex. Production of the carbanion on entry into the crevice or membrane, reaction of the carbanion with water to regenerate the C–H bond, and passage of the conjugate acid, is equivalent to the first of these possibilities. But, if the molecular architecture of the protein or membrane permits it, an even more simple answer is that desolvation occurs to a very slight extent, if at all.

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* Proton affinities of CH_3^- and $C_2H_5^-$ have been calculated from bond dissociation energies of 101 and 96 kcal/mole for CH_4 and C_2H_6, respectively[42], electron affinities of 26 and 23 kcal/mole for the CH_3 and C_2H_5 radicals, respectively[43], and a value of 315 kcal/mole for the ionization potential of the hydrogen atom[25].

CONCLUSIONS

Our somewhat fanciful title was chosen to focus attention on the neglected role of solvation effects by recalling the symbol "squiggle-P", still frequently used to denote the bonding of the reactive phosphate groups in high energy phosphate compounds. The use of this symbol and with it the term "energy-rich phosphate bond" led directly to the type of theoretical explanation outlined above. The underlying hypothesis, that the enhanced reactivity of the anhydrides originates in some unique intramolecular property or properties, depends on the argument that since ester and anhydride hydrolyses can be represented by the same general equation,

$$\mathrm{AOB} + \mathrm{H_2O} \longrightarrow \mathrm{AOH} + \mathrm{BOH}$$

in which A–O (or O–B) and O–H bonds are both broken and reformed, differences in reactivity can only arise through differences in bond properties.

We hold to the view that this argument is fallacious, and by not taking into account the reaction conditions, *i.e.*

$$\mathrm{AOB_{(aq)}} + \mathrm{H_2O_l} \longrightarrow \mathrm{AOH_{(aq)}} + \mathrm{BOH_{(aq)}}$$

the significance of solvation effects has been almost completely overlooked for many years[44,45]. The magnitude of the solvation energies of the ions, despite the approximate nature of the calculations, can leave no doubt as to their importance in deciding the value of ΔH^0, and hence ΔG^0. Furthermore the major contribution of the $T\Delta S^0$ term to ΔG^0 for certain reactions, notably ionization[9] and complex formation with metal ions[46], clearly has its origin in ion–solvent interactions.

We do not deny that the anhydride species have special intramolecular properties that are absent in simple orthophosphate esters, but suggest that the extent to which they contribute to the magnitude of ΔG^0 and ΔH^0 is still very much an open question. And we would certainly not maintain that solvation effects were solely responsible for the enhanced reactivity of the anhydrides in the way that the symbol squiggle-P and the term energy-rich phosphate bond, useful though they may be in descriptive biochemistry, give the impression it is due to bond properties alone. We would rather emphasize the joint role of intramolecular properties and interactions with the solvent water, and the necessity of taking both into consideration for the development of a satisfactory theory. It is a formidable problem because the nature of liquid water and the solvation process are still imperfectly understood[47,48]. Nevertheless theories dealing only with intramolecular properties are open to the criticism 'de nier ce qui est, et d'expliquer ce qui n'est pas', or, in more colloquial language, throwing the baby out with the bath water.

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