Acyl transfer reactions in dipolar aprotic medium: desolvated phosphate ion as acyl acceptor in the formation of energy-rich phosphate compounds

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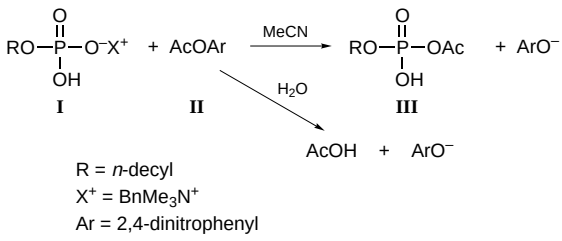
The reaction between an acetate ester and *n*-decylphosphate ions in dipolar aprotic medium provides a system which allows the facile formation of acetyl phosphate derivatives, by means of restricting the quantities of water available in the reacting system; the role of water is explained in terms of the inhibition of the nucleophilic attack of the phosphate group, which is disfavoured by its presence.

In order to promote the interconversion of different forms of energy, cells use phosphate compounds such as ATP and acetyl phosphate as the currency of energy exchange.[1] The synthesis and the hydrolysis of these compounds are catalysed by enzymes able to perform energy transduction. During the last two decades, different laboratories have dedicated their efforts to the elucidation of this fascinating puzzle.[2–5] Lehn and co-workers[4] have demonstrated that a variety of macrocyclic polyamines mediate the phosphorylation of phosphate ion, *via* an energy-rich intermediate phosphoramidate, generating energy-rich compounds such as ATP and pyrophosphate. This model system was successful as a 'mimick' of enzymes responsible for the phosphorylation of many compounds. In all cases, the enzymatic models needed, in order to make the phosphoryl transfer, an energy-rich compound to phosphorylate the macrocycle.

Here we show that a dipolar aprotic medium allows the easy reaction of organic phosphate ions with an acetate ester to generate the corresponding energy-rich acetyl phosphate compound. Specifically, we found that addition of benzyltrimethylammonium *n*-decylphosphate **I**, in anhydrous MeCN, enables its facile reaction with 2,4-dinitrophenyl acetate **II** by nucleophilic addition to the carbonyl group. Compound **I** was used in order to increase the solubility of the organic phosphate to concentrations adequate for the acquisition of kinetic and spectroscopic data. In our experiment, the solubility limit of the salt in MeCN was *ca.* 1.2×10^{-3} M. Acetyl *n*-decylphosphate **III** is then produced, and 2,4-dinitrophenolate is liberated. In aqueous solution the hydrolysis reaction is dominant, forming acetate and 2,4-dinitrophenolate ions as products (see Scheme 1).

The rate of acetyl transfer was determined at 40 °C by monitoring the formation of the 2,4-dinitrophenolate at 363 nm. The reaction is first-order in each of the reactants and proceeds quantitatively. The linear dependence of rate constants *vs.* the concentration of *n*-decylphosphate ions is maintained up to the solubility limit of the salt **I** in MeCN or MeCN–water mixtures (*ca.* 2×10^{-3} M in MeCN containing 1% water). The second-order rate constant in anhydrous MeCN is $k_2 = 13.2$ l mol^{-1} s^{-1}. The formation of **III** was followed by observation of the carbonyl region in the infrared spectra in MeCN, using a NaCl cell with a path length of 0.5 mm. While the peak corresponding to the ester carbonyl at 1786 cm^{-1} disappears, a new band appears at 1754 cm^{-1}, a reasonable position for the carbonyl stretching vibration of **III**.[6] The product could be also trapped and quantified by an adaptation of the method of Lipmann and Tuttle.[7]†



R = *n*-decyl
$X^+ = BnMe_3N^+$
Ar = 2,4-dinitrophenyl

Scheme 1

The rate of the reaction under consideration shows a dramatic dependence on the water content of the aprotic solvent (Fig. 1). We observe a decrease of 68 times the value of the rate constant for the reaction when 6% of water is added to anhydrous MeCN ($[H_2O] = 2.65$ M), with a corresponding increase in the half-life of the reaction from 46 to 3136 s. Surprisingly, addition of 10% water in dry MeCN provokes a 660-fold inhibition. These results clearly show the primordial role of water in this particular acyl transfer reaction.

To verify the influence of water in the course of the reaction between the ester and the *n*-decylphosphate, the yields of the different products described in Scheme 1 were determined (Fig. 2). The data clearly demonstrates the strong influence of water in the course of the reaction under investigation. A reduction in yield of 50% of the acetyl phosphate is triggered by addition of 5% water to MeCN. Upon addition of 30% water, acetyl phosphate production could not be detected either by IR spectroscopy or by the adaptation of Lipmann's method.[7]† In this particular case, the only products are those resulting from ester hydrolysis. Clearly, for a mild, rapid and high yielding synthesis of energy-rich compounds such as **III**, an anhydrous microenvironment is needed.

Recently, de Meis has suggested[5] that energy-rich phosphate compounds can be synthesized on the enzymatic surface without the need of energy at a hydrophobic enzyme site. Indeed, the binding-change mechanism that is extensively used to describe ATP synthesis by ATP synthases[2] indicates that

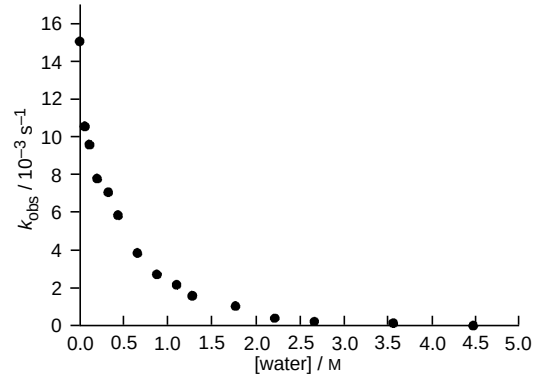


Fig. 1 Effect of water on the first order rate constant for the reaction of 2,4-dinitrophenyl acetate (3.0×10^{-5} M) with benzyltrimethylammonium *n*-decylphosphate (1.57×10^{-3} M) in MeCN. The value corresponding to anhydrous MeCN was calculated using the experimental second-order rate constant.

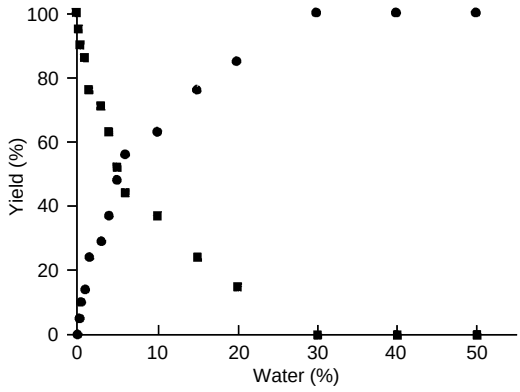


Fig. 2 Effect of water on the yields of (■) acetyl phosphate and (●) hydrolysed ester for the reaction between 2,4-dinitrophenyl acetate and benzyltrimethylammonium *n*-decylphosphate in MeCN

ATP synthesis occurs spontaneously at the catalytic sites, and energy is needed only to release the ATP produced.

Analysis of the experimental results described above, although for a model system, is relevant in terms of the chemistry of living systems. The fundamental reason why water promotes the inhibition of the acyl transfer reaction is probably the fact that solvation of the phosphate anion decreases the nucleophilicity of this compound, allowing the simultaneous appearance of the ability of this compound to act as a general base in hydrolytic reactions.[8,9] An alternative explanation involving slow nucleophilic attack followed by relatively fast hydrolysis of the acyl phosphate was experimentally excluded, since **III** was stable under our experimental conditions. Indeed, the hydrolysis of acyl phosphates has been proven to be independent of solvent composition in water–MeCN mixtures, with a rate constant of *ca.* 7.3×10^{-5} s^{-1}.[10] Thus, the predominance of the hydrolysis, or the formation, of a particular energy-rich compound is intrinsically related to the environment in which the reaction is taking place. Accordingly, the reversibility of these particular reactions should be achievable in normal solutions, under appropriate conditions, such as those described in this work. By analogy, if we are allowed to extrapolate these conclusions to enzyme catalysed reactions, a hydrolytic reaction which is spontaneous in aqueous media may well not be so on the surface of the enzyme (*e.g.* in a hydrophobic pocket).

The experimental fact that the reaction in anhydrous MeCN differs significantly from that in the presence of even small amounts of water, and completely from that observed in aqueous systems, shows, in our understanding, that the study of reactions of biochemical importance in purely aqueous systems may not adequately contribute to the understanding of enzyme catalysis. For reactions occurring in the hydrophobic pocket of a particular enzyme, water is certainly not an adequate standard state for the comparison of equilibrium and/or rate constants.[6] A typical example of this is that ionic intermediates are sometimes favoured in reactions occurring in polar solvents, but are considered to be of high energy in non-polar systems.[8,11] It may well be that this handling of the equilibrium thermodynamics of reactions is one of the factors that enzymes and nature use to promote the synthesis of energy-rich compounds.

In conclusion, we believe that the role of water, and particularly of its absence, in biochemically important events is a question still little explored. The present model represents, to the best of our knowledge, the first organic system that resulted in the facile formation of energy-rich compounds and, we hope, this fact will shed some light on the operational mode of some of the enzymes responsible for energy transduction. It provides an example that allows us to rationalize how water (or its absence) in the microenvironment of the active-site of an enzyme can determine both rate accelerations and changes in equilibrium constants.

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Footnotes and References

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† The yield of acetyl phosphate was determined by mixing compound **I** (46.8 μmol) with 2,4-dinitrophenyl acetate (11.7 μmol) in MeCN (25 ml), with a known water concentration. The reaction mixture was then stirred at 25 °C in a stoppered flask until completion of the reaction. The solvent was removed and water (1 ml) was added to the residue. This aqueous solution was added to a mixture of aqueous hydroxylamine (1 ml) [all solutions were identical with those originally described by Lipmann and Tuttle (ref. 7)] and acetate buffer (1 ml), and the mixture was left to stand for 10 min. Then aqueous HCl (1 ml) and aqueous ferric chloride (1 ml) was added. The solution was filtered and the UV–VIS spectrum of the purple solution, caused by complexation of the quantitatively formed hydroxamic acid with Fe^{3+}, was determined at 500 nm (ε = 925 cm^{-1} M^{-1}).

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