The Existence of Monomeric Metaphosphate in Hydroxylic Solvent: A Positional Isotope Exchange Study

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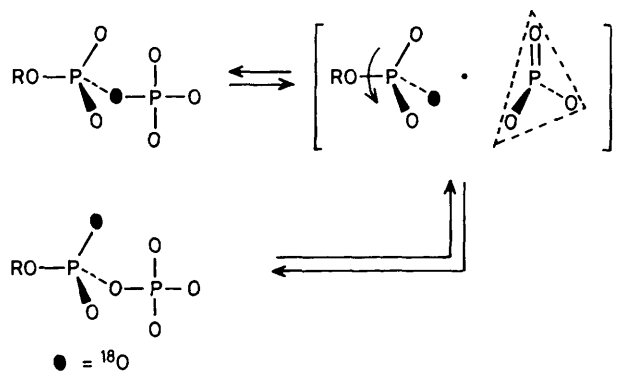
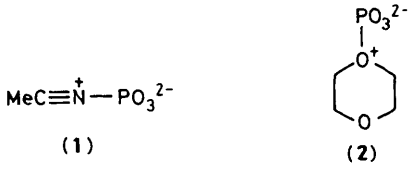
The phosphoryl transfer reactions of adenosine 5′-[α,β-^{18}O]diphosphate trianion in acetonitrile, acetonitrile–t-butyl alcohol, and neat t-butyl alcohol are all accompanied by closely similar bridge-to-non-bridge scrambling in the reisolated starting material; the simplest interpretation involves a monomeric metaphosphate intermediate even in the presence of hydroxylic solvent.

Positional isotope exchange (PIX) studies arguably represent the most sensitive probe into the existence, however fleeting, of monomeric metaphosphate.[1] Providing that the lifetime of the dissociation complex within the solvent cage is sufficient to allow bond rotation and that back reaction is significant then reactions of pyrophosphates proceeding *via* a metaphosphate intermediate will be accompanied by exchange of the bridging oxygen in the reisolated starting material, Scheme 1. These are less demanding requirements that those of the stereochemical analysis[2—7] in which monomeric metaphosphate will only signal its presence if it can tumble within the solvent cage or if it is sufficiently long-lived to escape the solvent cage



Scheme 1. Principle of the positional isotope exchange analysis.



(1) (2)

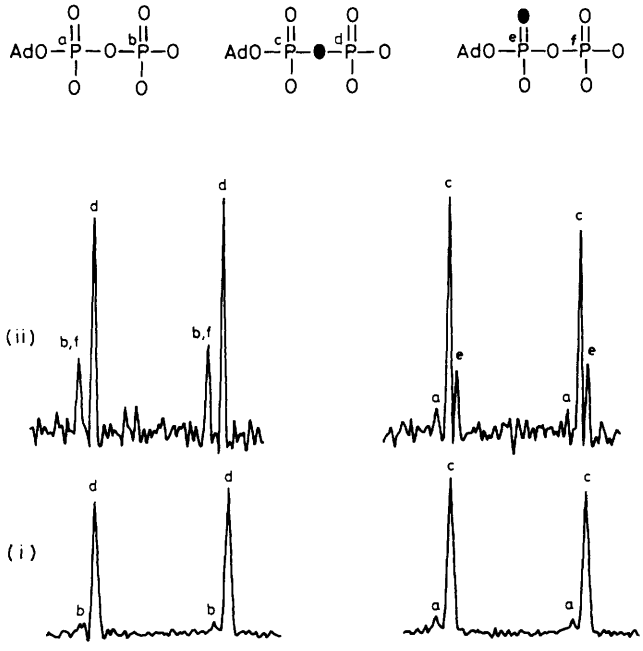


Figure 1. ^{31}P N.m.r. spectra recorded at 121.5 MHz of (i) [α,β-^{18}O]ADP; and (ii) reisolated [α,β-^{18}O]ADP after incubation of the tris(tetrabutylammonium) salt in t-butyl alcohol at 70 °C for 36 h. Spectra were obtained on a Bruker AM-300 instrument and were processed with Gaussian multiplication (Gaussian broadening 0.1 Hz, line broadening −0.5 Hz).

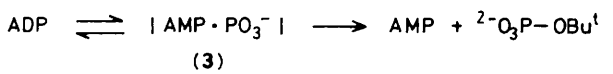
completely to allow racemisation. Previous PIX studies[1] have suggested that metaphosphate does not participate in hydrolysis reactions but may be involved in phosphoryl transfer reactions conducted in acetonitrile. However, the interpretation of the experiments in acetonitrile is complicated by the reports that this solvent can co-ordinate to monomeric metaphosphate as in (**1**).[8,9] We have now shown that positional isotope exchange accompanies phosphoryl transfer from adenosine 5′-[α,β-^{18}O]diphosphate to t-butyl alcohol even in the neat alcohol where presumably there are no alternative temporary acceptors. This observation would appear to require the participation of a metaphosphate intermediate even in an organic hydroxylic solvent.

Despite the evidence in favour of a monomeric metaphosphate intermediate in the solvolysis reactions of monosubstituted phosphates[9,10] recent kinetic,[11,12] thermodynamic,[13] stereochemical,[2,3] and positional isotope exchange studies[1] are in agreement that monomeric metaphosphate does not have a significant life-time in aqueous solution and that such reactions proceed *via* a preassociative concerted reaction.[14] The most direct evidence in favour of metaphosphate has come from studies in the gas phase[15,16] and in organic solvent such as the Conant–Swan fragmentation reaction,[17] the three phase test,[8] and the phosphorylation of hindered alcohols in acetonitrile.[18] In particular we[4,5] and others[6,7] have looked at the stereochemical course of phosphoryl transfer reactions in organic solvent and have found evidence of racemisation that would accommodate a metaphosphate intermediate. However, in the majority of the above studies solvents such as acetonitrile and dioxane have been used and the precise nature of the electrophilic species and the mechanism(s) leading to its formation and subsequent reaction remains ambiguous because of the recognised abilities of these solvents to complex with monomeric metaphosphate,[8,9] (**1**) and (**2**) respectively. To address these ambiguities we have now carried out PIX studies on the phosphoryl transfer from ADP to t-butyl alcohol in neat alcohol.

Syntheses of both bridge-labelled pyrophosphate and adenosine 5′-[β,γ-^{18}O]triphosphate (ATP) have previously been developed.[19] Extending this, we now report that reaction of bridge-labelled pyrophosphate with 5′-tosyl adenosine gives adenosine 5′-[α,β-^{18}O]diphosphate (ADP) in good yield[20] with no accompanying scrambling of the isotope, Figure 1(i). The corresponding [α,β-^{18}O]ATP is readily available from this by enzymatic phosphorylation using phosphoenol pyruvate and pyruvate kinase.

Incubation of the tris-tetrabutylammonium salt of [α,β-^{18}O]ADP in acetonitrile or in acetonitrile–t-butyl alcohol (1 : 1, v : v) at 70 °C for 36 h gave rise to significant levels of scrambling of the bridging oxygen in the reisolated ADP at approximately 70% extent of reaction. (In the case of neat acetonitrile it has been demonstrated that under these conditions phosphoryl transfer occurs to the 2′ and 3′ hydroxy groups of the adenosyl moiety.[1]) More significantly, a parallel incubation of [α,β-^{18}O]ADP in neat t-butyl alcohol under otherwise identical conditions also gave rise to the same level of scrambling of the bridging oxygen in the reisolated ADP, Figure 1(ii), with a very similar extent of reaction. A careful control experiment in which unlabelled ADP was incubated with [$^{16}O_2$,$^{18}O_2$]AMP in t-butyl alcohol under comparable conditions (70 °C, 36 h) demonstrated that no labelled ADP was produced thus excluding intermolecular phosphoryl transfer reactions as being responsible for the scrambling.

The PIX observed in acetonitrile is comparable to that reported by Lowe and Tuck.[1] They interpreted the difference between the lack of scrambling during the hydrolysis of ADP in aqueous solution and the observation of scrambling during the phosphoryl transfer in acetonitrile in terms of a concerted preassociative and a stepwise preassociative pathway respectively. There was, however, no unambiguous way to exclude scrambling occurring by return from the caged zwitterion species (**1**), providing that, with respect to phosphorylation of the phosphoryl residue of adenosine 5′-monophosphate (AMP), solvation and reaction of the zwitterion (**1**) effectively prevents return (otherwise intermolecular phosphoryl transfer



Scheme 2

would have been detected in the control experiment). Indeed, in the absence of free monomeric metaphosphate a diffusing species such as (**1**) is implied from the results of the three phase test of Rebek *et al.*[8] The involvement of a monomeric metaphosphate intermediate is thus not demanded. We believe our observation that scrambling also occurs in phosphoryl transfer reactions conducted in t-butyl alcohol, and to the same extent, provides good evidence for the metaphosphate complex (**3**), Scheme 2. The correspondence between the extents of scrambling in t-butyl alcohol, in t-butyl alcohol–acetonitrile, and in acetonitrile can most easily be understood in terms of return from a common intermediate, namely (**3**). Whether the phosphoryl transfer proceeds *via* a preassociative stepwise mechanism or *via* a liberated metaphosphate is not clear. However, for these observations to be consistent with a liberated monomeric metaphosphate intermediate, the absence of exchange of AMP into ADP in the control experiment would require that the diffusion apart of AMP and the putative monomeric metaphosphate is effectively irreversible with respect to phosphorylation of the phosphoryl residue, which could be argued on electrostatic considerations particularly if the reaction with other nucleophiles is extremely rapid.

These observations provide the first direct evidence that monomeric metaphosphate has a finite life-time in hydroxylic solvents. It will be interesting to determine the point within the scale of solvent nucleophilicity that the reaction switches to being a preassociative concerted mechanism as observed in water.

Added in proof: In support of the above conclusions, related stereochemical studies (P. M. Cullis and M. Schilling, unpublished observations; J. R. Knowles and S. Freeman, *J. Am. Chem. Soc.*, in the press) have demonstrated that phosphoryl transfer reactions in neat alcohols are accompanied by racemisation.

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