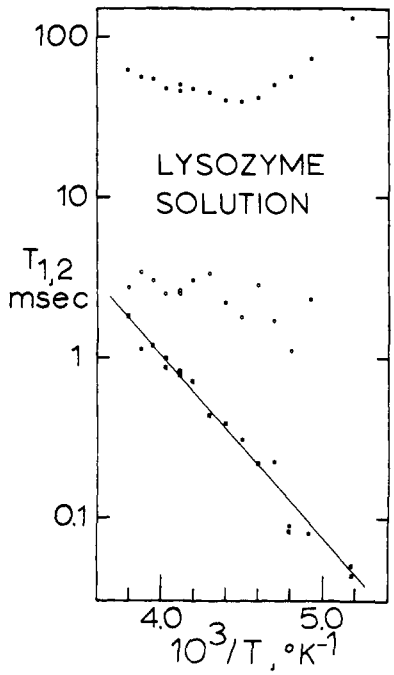

Figure 2. ^{1}H NMR relaxation times measured in frozen lysozyme solutions at 30 MHz as a function of reciprocal temperature: ●, T_1 of the major relaxation component; ○, T_1 of the minor relaxation component, ■, T_2.

proton signal relaxes with a T_1 value in the range of 50 msec and shows a clear but very broad minimum. The proton T_1 of the remaining signal is much shorter and is difficult to measure accurately as shown by the scatter in Figure 2. It is possible that the protons observed relax with two or more values of T_2. Measurements made on protein powders suggest this possibility but also indicate that extraction of the separate relaxation rates would require extremely precise data. Within the experimental errors of the present measurements, all the protons observed relax with a single T_2. The activation energy for the reorientation events causing T_2 relaxation is 5.2 kcal/mol. The relaxation spectrum remains essentially unchanged over the period of a week at low temperatures.

The very broad T_1 minimum and the ratio T_1/T_2 at the T_1 minimum implies that the Bloembergen, Purcell, Pound[9] equations must be modified to account for the observations. A log normal distribution of correlation times has been previously used with success in fitting such data.[4] Although this procedure lacks fundamental justification, it provides a useful parameterization of the data. In the present case T_2 is 360 μsec and T_1 is 39 msec at the T_1 minimum at 227°K. The width, β, of the log normal distribution describing the data is 3.8 and the second moment, σ_0^2, is 2.38×10^{10} sec^{-2}. These values are similar to other values reported in both protein crystals and more complex systems.[10,11] If the second moment is corrected for a slow motion cut off[4] in the distribution, σ_0^2 becomes 2.7×10^{10} sec^{-2} which is close to that for ice of 2.6×10^{10} rad^2 sec^{-2} [12] and similar to the values reported earlier for water adsorbed in protein systems.

Quantitative measurements of the number of protons associated with each group of protons with different relaxation properties is of importance for discussions of protein hydration. We designate as component I the 81% of the observed protons which relax with the longer T_1 values and as component II the remaining protons which relax with the shorter T_1 value. Using the spin intensity measurements to determine the amount of each relaxation component as described previously, we may conclude that the signal intensity of component I corresponds to 0.28 g of water per g of protein or 223 water molecules per protein molecule and component II corresponds to 0.06 g of H_2O per g of protein or 52 water molecules per protein molecule. The sum of components I and II is then 0.34 g of water per g of protein in good agreement with earlier reports.[1]

These data are very similar to that reported for two components of the proton relaxation rates in lysozyme crystals.[3] Therefore the crystal result is not a unique property of protein crystal systems. The general features of these frozen protein solutions are fundamentally different from the well-known liquid components in frozen electrolyte solutions.[13] The long relaxation time component in frozen 5% sodium hydroxide solution at −20°, for example, consists of a simple exponential with T_2 equal to 100 msec and T_1 equal to 139 msec. No 3-msec component is apparent and the highly liquid character of the unfrozen part of the sodium hydroxide solution is clear from the small value of T_1:T_2.

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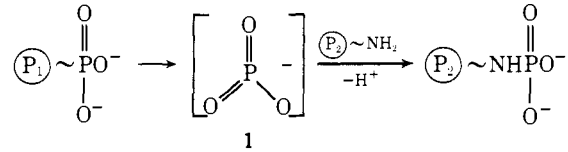
The Three Phase Test for Reaction Intermediates. Evidence for Monomeric Metaphosphate

Sir:

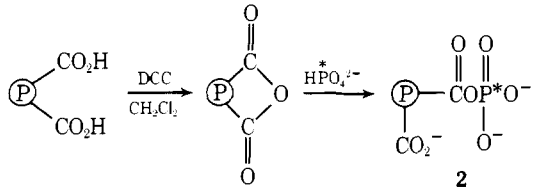
The elusive monomeric metaphosphate ion **1** occupies a central role in the hydrolysis of organophosphate compounds.[1] Alkyl, aryl, and acyl phosphates as well as phosphoramidates and halo phosphonates are all believed to generate **1**, yet incisive experimental evidence for the existence of this ion is rare. We have recently described a new method for detecting reaction intermediates in which an intermediate is generated from a suitable polymer-bound precursor and detected by trapping on a second solid phase.[2] We now report the application of this method to the detection of **1**.



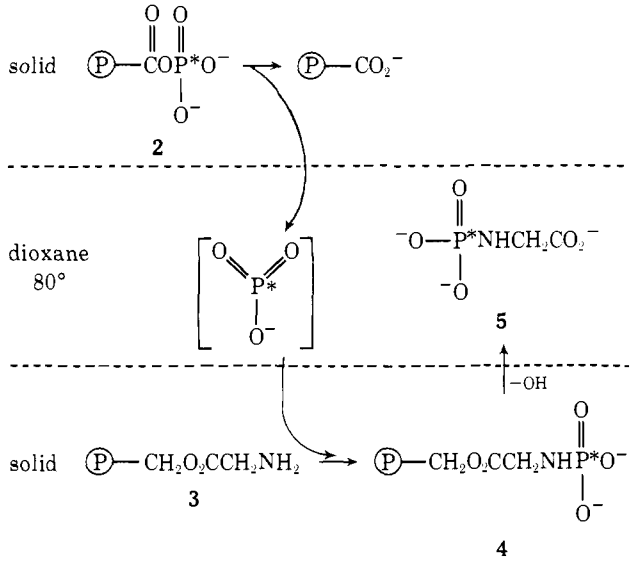
1

Ⓟ = crosslinked polystyrene

Scheme I



Scheme II



An acyl phosphate was selected as a suitable polymer-bound metaphosphate precursor. This choice was based on the work of Jencks,[3] who showed that the decomposition of acyl phosphates in media of low water or high salt concentrations produces pyrophosphate (i.e., trapping of metaphosphate by phosphate). A polymer-bound acyl phosphate linkage was therefore likely to generate metaphosphate cleanly in the aprotic medium of the polystyrene matrix. The precursor was easily prepared, although in low (10-20%) overall yield, by the reactions of Scheme I.

The polymer-bound benzoic acid[4] (ir 1720, 1670 cm^{-1}) was converted in large part to the anhydride (ir 1785, 1725 cm^{-1}) with carbodiimide, then to the radioactive acyl phosphate **2** (ir 1725, 1230 cm^{-1}) with tetramethylammonium [^{32}P] phosphate in aqueous dioxane. That the phosphate of **2** was covalently bound to rather than adsorbed on the resin was demonstrated by its failure to exchange with unlabeled phosphate in solution. The trapping agent used was the polymer-bound glycine **3** (Scheme II), prepared by established procedures of Merrifield peptide synthesis.[5]

When the polymers **2** and **3** were suspended in dioxane at 80°, phosphate transfer between the two polymers was detected generating **4** (ir 1380 cm^{-1}). Radioactivity assays indicated that the half-life of **2** is approximately 27 hr under these conditions and 70% of the released phosphate appeared on **4** while the remaining activity appeared in solution as phosphate. Saponification of **4** gave glycine *N*-phosphate, **5**, identical with an authentic sample, and isotope dilution established that 90% of the radioactivity of **4** appeared as **5**. Since direct reactions between the two resin bead surfaces have been shown to be negligible in related cases,[6] the presence of a free monophosphorylating agent in the solution between the two solid phases is established.

These results are consistent with the postulation of monomeric metaphosphate as the intermediate, but its higher oligomers, formed by disproportionation reactions within **2**, remain viable alternative possibilities as the actual phosphorylating agents. The dimer (pyrophosphate) was excluded as the intermediate in question by its inability to phosphorylate **3** under these conditions.

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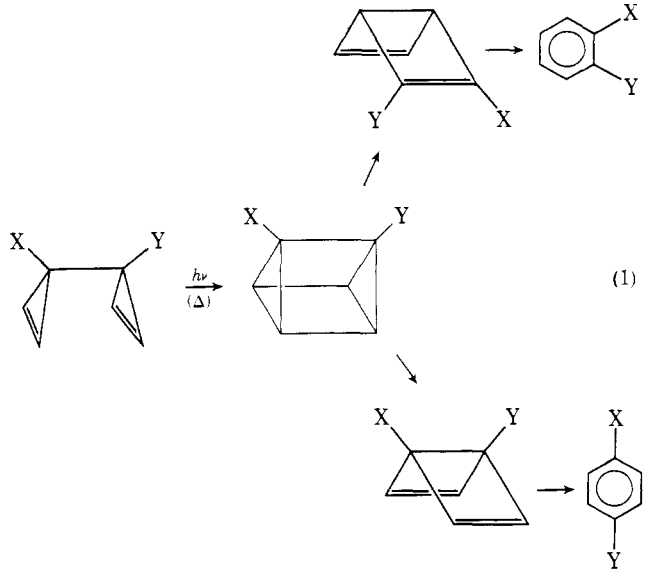
Does the Photochemical Bicyclopropenyl Rearrangement Involve a Prismane Intermediate?[1]

Sir:

In his pioneering work on both thermal and photochemical versions of the bicyclopropenyl → benzene rearrangement, Breslow suggested mechanism 1 with a prismane as the key intermediate to account for the observed ortho-para scrambling of x,y-substituents in the process of aromatization.[2] While we have dealt with the thermal (and transition metal catalyzed) case in recent papers[3-5] we have now turned to an investigation of the photochemical rearrangement whose mechanism has remained unchallenged so far.[6]

In order to facilitate product analysis by NMR, we modi-



(1)