Nucleic Acid Studies on Insoluble Polymer Supports

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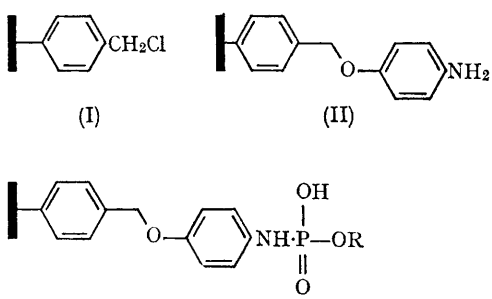
THE use of linear aminopolystyrenes as soluble polymer supports for nucleotides attached through the acid-labile phosphoramidate linkage has been reported recently by Cramer and co-workers.[1] We have been investigating related systems involving phosphoramidate formation with an insoluble amino-polymer.

The reaction of a Dow polystyrene[2] containing 12% of chloromethyl groups (I) with *p*-acetylaminophenol and subsequent removal of acetyl groups by alkaline hydrolysis gave the amino-polymer (II) carrying the functional group on 11% of styrene residues. Formation of the phosphoramidate linkage was achieved by condensation of the amino-polymer (II) with 2′,3′-*O*-anisylideneadenosine 5′-phosphate using dicyclohexylcarbodi-imide (DCC).[3] Hydrolysis of the phosphoramidate polymer (III) with detachment of the adenosine moiety was effected by treatment with 80% acetic acid at 80°. Estimation of the material released in two successive treatments and phosphorus analysis on the residual polymer showed that 23% of the amino-groups in (II) had been converted into phosphoramidate linkages, of which 50% had been cleaved. Although more efficient hydrolysis of the phosphoramidate was observed using 0·5N-HCl in dioxan/ethanol at 20° (67% release) traces of adenine were detected along with adenosine and 5′-adenylic acid in the hydrolysate.

While the use of polymer supports for synthetical studies is well established,[4] it has not yet been applied as an aid in the study of reaction mechanisms. Two proposed mechanisms for the phosphorylation reaction using DCC invoke the intermediacy of monomeric metaphosphate[5] (IV) and of cyclic trimetaphosphate[6] (V). The formation of the latter type of intermediate necessitates the interaction of at least three phosphate groups which should be subject to inhibition by appropriate distribution of the phosphate residues in a cross-linked polymer-support matrix. If, therefore, formation of such an intermediate as (V) is mandatory for phosphorylation then a marked



(I) (II)

(III) R=Anisylideneadenosine

difference should be observed between phosphorylation under equivalent conditions in heterogeneous systems comprising the hydroxyl groups of an insoluble polymer and a phosphate in solution on the one hand and an insoluble phosphorylated polymer and the alcohol in solution on the other.

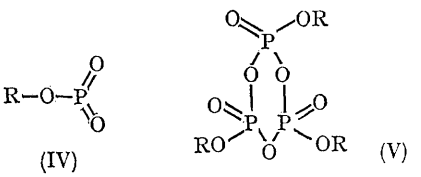
Both emulsion and "popcorn" polystyrenes containing hydroxyl functional groups were smoothly phosphorylated on treatment with DCC and β-cyanoethyl phosphate in pyridine. Ammonolysis to remove the cyanoethyl group gave a

product identical with the phosphorylated polymer (VI) obtained by treatment of the hydroxylated polymer with pyrophosphoryl chloride.[7] The attachment of a nucleoside phosphate by means of DCC proved equally viable.

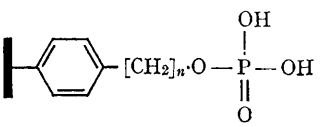
By contrast, all reactions involving the phosphate monoester of hydroxylated polymers [*e.g.*, (VI)] and a soluble alcohol with DCC in pyridine failed to produce any diester, neither was the formation of dicyclohexylurea detectable. Both emulsion and "popcorn" phosphorylated polymers proved equally inert in such a phosphorylation system and the substitution of hydrophobic groups into the nucleoside (as soluble alcohol) likewise failed to promote reaction.

These results clearly implicate multiple interaction of phosphate units in the phosphorylation process with DCC and favour the intermediacy of (V) rather than of (IV).

Two observations suggest that mesitylenesulphonyl chloride promotes phosphorylation by a process different from that involving DCC. First, the action of hydracrylonitrile and the sulphonyl chloride on a phosphorylated polymer (VI) resulted in dephosphorylation of the polymer (up to 75%) rather than formation of a phosphate diester. Secondly, Letsinger[8] has reported the esterification of a polymer-linked-nucleoside β-cyanoethyl phosphate with a second nucleoside using the sulphonyl chloride. Clarification of both processes requires further investigation but, at the present time, both suggest that a polymer-supported phosphate is activated by mesitylenesulphonyl chloride towards nucleophilic substitution by an alcohol.



(IV) (V)



(VI)[n=1,2]

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