Formation of Acetyl Phosphate by the Action of X-Rays on Monoethyl Phosphoric Acid in Aqueous Solution

Our earlier work on the chemical action of X-rays on the nucleic acids in aqueous systems[1] has led us to suggest that labile compounds, including labile phosphate esters, are formed on irradiation, and that these may play some part in the breakdown of the polynucleotides. Following some observations on the effects of the radiation on relatively simple phosphate esters (for example, glycerophosphates, phosphoglyceric acid), we have suggested that, in general, oxidative attack at a $>$ CH(OH) (secondary alcohol) group by the free radicals can lead to the formation of a $>$ C=O (carbonyl) group, and that, if the latter is α- or β- to a phosphate group, a more or less labile ester is produced[2].

In order to elucidate further the chemical mechanism by which labile phosphate esters are formed in these circumstances, a systematic investigation of the effects of X-rays on a number of phosphate esters has been undertaken.

We are now reporting some observations on one of the simplest phosphate esters, namely, monoethyl phosphoric acid. Here one might expect that oxidative attack by the radicals on the primary phosphoryl group (—$CH_2OPO(OH)_2$) would lead, by a relatively straightforward process, to the corresponding aldehyde, together with inorganic phosphate. On the other hand, we may apply, in the presence of oxygen, a mechanism which we have recently found to be operative in the attack on certain simple aliphatic compounds[3]; this involves reaction of the organic radical primarily formed with molecular oxygen, which can lead to an organic hydroperoxide.

In the case of monoethyl phosphoric acid, the hydroperoxide resulting from attack on the primary phosphoryl group would have structure (I), subsequent decomposition of which could lead to acetyl phosphate (II):

$$\underset{\text{(I)}}{CH_3CH(O_2H).O.PO(OH)_2} \xrightarrow{(-H_2O)} \underset{\text{(II)}}{CH_3CO.O.PO(OH)_2}.$$

We have now demonstrated that this latter mechanism does, in fact, also hold for this system, since we have been able to show that irradiation of monoethyl phosphoric acid with X-rays in the presence of oxygen gives acetyl phosphate, among other products. This conclusion is based on the following experimental evidence.

(i) Slow liberation of inorganic phosphate after irradiation.

(ii) A positive Lipmann reaction[4] for acetyl phosphate, namely, formation of the corresponding hydroxamic acid which gave the characteristic ferric salt in acid solution (ferric acethydroxamate was identified by its absorption spectrum).

(iii) A difference in the yields of inorganic phosphate as determined separately by the methods of Lowry and Lopez[5] and Berenblum and Chain[6]; whereas the former is suitable for the determination of inorganic phosphate in the presence of acetyl phosphate[5], under the conditions of the Berenblum–Chain procedure, it is known that acetyl phosphate is rapidly hydrolysed[7].

A typical experiment was as follows: 100 ml. of a solution of monoethyl phosphoric acid (0·1 per cent calcium salt), adjusted to *p*H 5·5 and saturated with oxygen (1 atm.), were irradiated with X-rays (200 kV.) with a total dose of 7×10^4 r. (integral dose-rate = 2,340 r./min.). Under these conditions, 15 μmoles of acetyl phosphate per 100 ml. were produced. At the same time, some acetaldehyde was formed and about 5 μmoles of inorganic phosphate; somewhat more inorganic phosphate than acetaldehyde is to be expected, owing to the slight decomposition of the acetyl phosphate during irradiation.

These results show that one of the major processes occurring under these conditions is that leading to acetyl phosphate. In agreement with the mechanism outlined above, we have found that the formation of acetyl phosphate requires the presence of molecular oxygen.

These observations would suggest that certain other compounds containing the primary phosphoryl group (—$CH_2OPO(OH)_2$) may give, on irradiation in the presence of oxygen, the corresponding acyl phosphate to a smaller or greater extent.

Thus, labilization of organic phosphate esters can be due to the production of carbonyl groups (α- or β- to the phosphate) and/or, under certain conditions, to the formation of acyl phosphates. Carbonyl groups can be produced by OH radicals (generated by the action of the ionizing radiation on water, namely, $H_2O \rightsquigarrow H + OH$), and their formation is therefore not dependent upon the presence of oxygen in solution, although, for reasons discussed previously[2,3], oxygen should, in general, enhance the yield of the carbonyl compound. On the other hand, for the formation of the acyl phosphate, as described above, the presence of molecular oxygen is essential.

This appears to be the first instance in which acetyl phosphate is formed by a relatively simple oxidation process in aqueous solution.

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A Trough for Paper Chromatography consisting of Segments

The sensitivity of paper chromatography is such that lack of reproducibility often hampers the systematic approach. In order to facilitate measurements under identical conditions, we are using a glass trough which consists of four equal-segments.

Each segment can hold duplicate strips of paper of 7 cm. × 40 cm., or one can apply two sheets of paper of 40 cm. × 40 cm. if three small cuts are made on the top edge (see Fig. 1). To mark the movement of liquid flowing from one segment, 1 c.c. of