Preliminary Communications

Amine-induced Cleavage of Periodate-oxidized Nucleotide Residues

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In the course of investigating the chemical characteristics of periodate oxidized riboseyl derivatives (1), we have reinvestigated the base-catalyzed degradations of these substances reported by Whitfield (2) and by Brown, Fried, and Todd (3). In our hands, the agents used by them (ammonia and glycine, in the pH range of 9 to 10) gave incomplete yields of inorganic phosphate and aglycone residue. This occurred only when the subsequent analyses or isolations were carried out under conditions that cleave labile bonds (i.e. inorganic orthophosphate assay by any of the acid phosphomolybdate procedures; chromatography at acid or very alkaline pH values). When, however, we performed the chromatographic and other assays under conditions favorable to preserving such acid-labile linkages, or if the substances were stabilized by reduction before analysis (1), we found that little or no separation of phosphate and aglycone occurs under these conditions.

It has been shown (4-6) that there are considerable differences between primary amines, on the one hand, and ammonia, on the other, in the kinds of addition products formed with aldehydes.

On examining the reactions of amines with periodate-oxidized 5'-nucleotides (1), we have found that the formation of addition compounds with primary amines (see Fig. 1) and the subsequent decomposition of these to separate the phosphate quantitatively from the purine or pyrimidine base may be carried out within the pH range of 5 to 9 and at room temperature. Such conditions are thus applicable to such problems as the selective degradation of adenosine triphosphate in the presence of deoxyadenosine triphosphate (7) or of sequence analysis in ribonucleic acid chains.

Procedure—Nucleoside 5'-phosphates (free or in polynucleotide combination) were oxidized with slight excesses of periodate (concentrations usually above 10^-4 m) in 15 to 30 minutes at room temperature (pH about 5 to 6).1 Excess periodate, if present, was reduced to iodate with glycerol or ethylene glycol. Methylamine or other agent was added in 10- to 100-fold excess as a buffered (pH 8 to 10) acetate, formate, or chloride salt and the final pH was adjusted if necessary (the pH tends to drop as the addition reaction proceeds, as expected from the reactions shown in Fig. 1). The addition products2 (presumed to be Schiff bases) are fairly stable at pH 9.5, and consequently, this pH was maintained during their isolation. To decompose them, the pH of the solution was lowered to below 7, or kept at 7 to 8 with an appropriate buffer. The solution was then allowed to stand.

Reductions to stabilize the addition products were carried out by the addition of solid NaBH₄ in approximately 10-fold molar excess. Ion exchange chromatography for ultraviolet-absorbing as well as for phosphorus-containing fragments was carried out essentially as indicated by Cohn (8), but with particular attention to those conditions of pH and of alkaline agents that would preserve the species of interest present (see Fig. 2). Chemical analyses for inorganic phosphate were carried out by several methods (9-11).

Fig. 3 indicates the difference between "apparent Pi," that material assaying as Pi by any of the acid phosphomolybdate methods (points and curves), and "real Pi," that Pi, isolated by chromatography or remaining after borohydride reduction (values in parentheses). When aliquots are reduced with NaBH₄ or are chromatographically analyzed, the amount of real Pi, remaining is indicated by the first figures in the parentheses; these are placed on the curves at the times at which the samples were separated or stabilized.

If samples of these solutions are converted to pH <6 and allowed to stand for several hours, real Pi increases to the values shown by the second figures in the several parentheses in Fig. 3. The difference between the two figures thus shows the increase in real Pi by an incubation at pH <6, after the initial reaction has proceeded for the time shown on the figure.

Three general classes of reactions are distinguishable from our data. (a) Alkali, in the absence of amine groups, gives a straightforward cleavage of the oxidized ribose chain. (b) Ammonia and glycine at pH 9 to 10 incompletely form acid-labile complexes that are in equilibrium with the starting dialdehyde. The partial yield of degradation products upon neutralization seems to stem from the partial yield of complex formed. (c) Primary amines (and α-amino acids) at pH 7 to 8 quantitatively form complexes that are cleaved by neutral or mildly acid conditions to inorganic phosphate and aglycone without significant reconstruction to the starting dialdehyde. The cleavage leaves at least a part of the original ribose attached to the aglycone, giving it certain attributes of the corresponding nucleoside.3 The initial acid-labile amine complexes are reducible to acid-stable compounds that include all the parts of the starting dialdehyde.4

This procedure has been applied to a mixture of deoxy-ATP and ATP, giving a quantitative cleavage of the latter into an aglycone fragment and what appears to be a linear triphosphate, and to the elimination of cytidine from ApApC (kindly supplied

⁴ The addition product (II), the Schiff base (IV), and the reduction product (V), for example, retain all the adenine and phosphate of the original dialdehyde. The former decomposes readily at pH <7, the latter does not.

† It is possible that the initial cleavage is at the C-O-C linkage in the former ribose residue, with subsequent loss of the 3-carbon fragment from the phosphate.

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Nucleoside 5'-phosphates are cleaved into a phosphate fragment and an aglycone fragment by treatment of the periodate-oxidized material with a primary amine or an amino acid in the pH range of 7 to 8. Schiff base formation is quantitative at pH >9, but a quantitative yield of the two fragments appears if the pH is kept at, or is subsequently lowered to below 8. Substances that appear not to give Schiff bases or that do so incompletely, like ammonia and glycine at pH above 9, give lower yields of the products. The neutral pH range and rapidity of the reaction with primary amines at room temperature make this decomposition applicable to sequence determination in ribonucleic acids.

SUMMARY

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REFERENCES

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