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Note

Evidence that a formic ester is an early intermediate in the periodate oxidation of D-ribose 5-phosphate

CARL BERNOFSKY* AND SOO-YOUNG C. WANDA

Department of Biochemistry, Tulane University School of Medicine, New Orleans, Louisiana 70112 (U.S.A.)

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Treatment of D-ribose 5-phosphate with 1 mol of periodate converts it from a substance that is reactive with the carbonyl reagent 3-methyl-2-benzothiazolinone hydrazone (MBTH) into a compound that no longer reacts with this reagent. To explain this unexpected finding, we propose that the aldehyde group of D-ribose 5-phosphate is converted into an ester by insertion of an oxygen atom between C-1 and C-2. The implication of this result to the mechanism of periodate oxidation is discussed.

Oxidation of D-ribose 5-phosphate (Rib-5-*P*) by sodium periodate under alkaline conditions is known to utilize 3 mol of periodate and lead ultimately to the formation of glycolaldehyde phosphate and formic acid[1,2]. Although periodate oxidation of carbohydrates and related compounds has been the subject of extensive research[3–7], there is no general agreement on the detailed mechanism of the reaction[8]. In this communication, evidence is presented that the first stage in the oxidation of Rib-5-*P* by alkaline periodate occurs by insertion of an oxygen atom between C-1 and C-2, which converts Rib-5-*P* from an aldehyde into a 2-formic ester. This mechanism suggests that the end products of complete periodate oxidation, glycolaldehyde phosphate and formic acid, arise by hydrolysis of a non-carbonyl intermediate containing oxygen atoms from periodate inserted at C-1–C-2, C-2–C-3, and C-3–C-4.

RESULTS

Rib-5-*P* reacts slowly at pH 4 with the carbonyl reagent MBTH to form a characteristic azine[9] that absorbs at 310 nm. At room temperature, this reaction is essentially complete in 18 h. Upon chromatography of Rib-5-*P* on Dowex 1-formate, the MBTH-reactive material that is eluted from the column coincides with the Rib-5-*P*

*To whom requests for reprints should be directed.

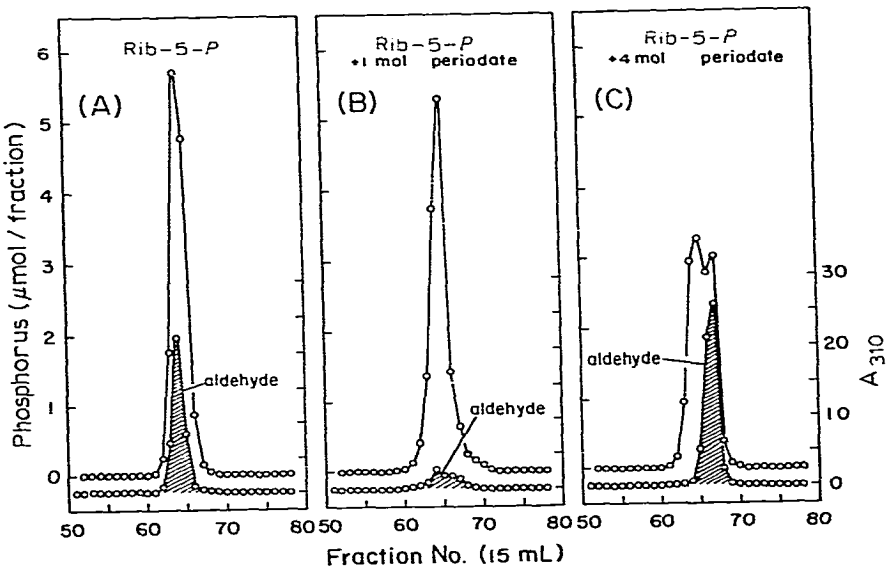

Fig. 1. Chromatography on Dowex 1-formate of Rib-5-*P* (A) and its oxidation products from 1 : 1 periodate (*B*) and 4 : 1 periodate (*C*). See Experimental section for details. Not shown in the chromatograms are the u.v.-absorbing iodate and periodate ions, which are both eluted with a peak at fraction 52 and thus are well separated from the compounds under discussion.

as determined by phosphorus analysis (Fig. 1A), showing that the column does not modify the MBTH-reactivity of the Rib-5-*P*. After Rib-5-*P* has reacted with 1 mol of periodate at pH 9, the resulting product migrated chromatographically in nearly the same position as unchanged Rib-5-*P* (Fig. 1B); however, at least 75% of the Rib-5-*P* had been converted into a non-aldehydic compound, as judged from the diminished reactivity of the column fractions with MBTH. To test whether alkaline conditions alone were responsible for the loss of aldehyde reactivity, 3mM Rib-5-*P* was incubated at 30° with 20mM sodium hydrogencarbonate–sodium hydroxide, pH 10, and periodically assayed with MBTH. There was no decrease in azine formation during 6 h, indicating that there was no alkali-induced loss of the aldehyde group.

Fig. 1C shows that, when Rib-5-*P* is treated with an excess of periodate (4 mol) at pH 9, there is substantial liberation of glycolaldehyde phosphate, although most of the material still exists as a non-carbonyl intermediate. Glycolaldehyde phosphate was identified by cochromatography of the isolated aldehyde (Fig. 1C) with an authentic sample prepared by periodate oxidation of glycerol 1-phosphate, and by the identical (rapid) reaction of both aldehydes with MBTH. Although azine formation from glycolaldehyde phosphate is complete within minutes, the chromatogram shown in Fig. 1C was assayed with MBTH for 24 h to ensure detection of all carbonyl-containing products. The peak that migrates near the same position as Rib-5-*P* but shows no MBTH reactivity (Fig. 1C), appears to be a periodate-oxidation intermediate that has consumed up to 3 mol of periodate (as determined spectrophotometrically), but does not exhibit the carbonyl group.

DISCUSSION

The essential finding of the present work is that an equimolar amount of periodate converts Rib-5-*P* from an aldehyde into a substance that is a non-aldehyde. There are several ways in which oxidative attack at C-1 could accomplish this. Oxidation to D-ribonic acid 5-phosphate, for example (Fig. 2), would yield a product that does not react with MBTH. However, D-ribonic acid 5-phosphate has one more negative charge than Rib-5-*P* and would have markedly different chromatographic properties, and even if the periodate-reactive form of Rib-5-*P* were the cyclic hemiacetal, the corresponding lactone produced by oxidation at C-1 would be hydrolyzed at pH 9 to the free carboxylate anion. In contrast, the isolated, periodate-oxidation product behaves on Dowex 1-formate as if it has the same charge as Rib-5-*P*. Thus, oxidation to D-ribonic acid 5-phosphate does not appear to occur.

It is also possible to rule out oxidative cleavages in the carbon chain. Cleavage at C-1–C-2 or C-2–C-3 of Rib-5-*P* hemiacetal would yield the 3-formic ester and 1,2[1]-dialdehyde, respectively (Fig. 2). These structures contain aldehyde groups that would be reactive with MBTH, and thus they can be eliminated from consideration. Similarly, cleavage at C-3–C-4 would give rise to glycolaldehyde phosphate, which we have shown is not formed from Rib-5-*P* with an equimolar amount of periodate (Fig. 1B), although it is formed with an excess of periodate (Fig. 1C).

The only reasonable alternative is the conversion of Rib-5-*P* into the 2-formic ester (Fig. 3). Such a product should not react with MBTH, and, consistent with this, we found ethyl formate to be unreactive with MBTH. A mechanism for the formation of the 2-formic ester is presented in Fig. 3, indicating the insertion of an oxygen atom from periodate between C-1 and C-2 of Rib-5-*P*. If this mechanism were generally valid, it would mean that the periodate oxidation of vicinal glycols would proceed initially by oxygen insertion to yield hemialdal intermediates[6]. Such hemialdals have been previously detected as products of the periodate oxidation of nucleosides and nucleotides[12,13], but their origin has been believed to be through dehydration of initially-formed dialdehydes. The present work suggests that hemialdals may actually be the precursors of the dialdehydes and other products that arise from periodate oxidation of carbohydrates and related substances.

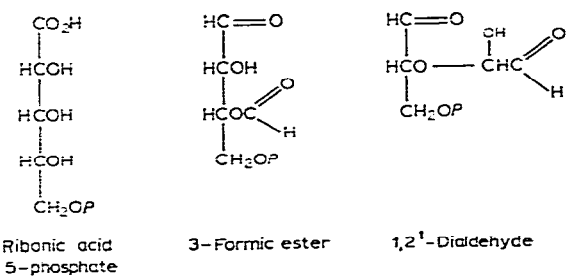

Fig. 2. Examples of initial periodate-oxidation products of Rib-5-*P* ruled out by the present work. See text for details.

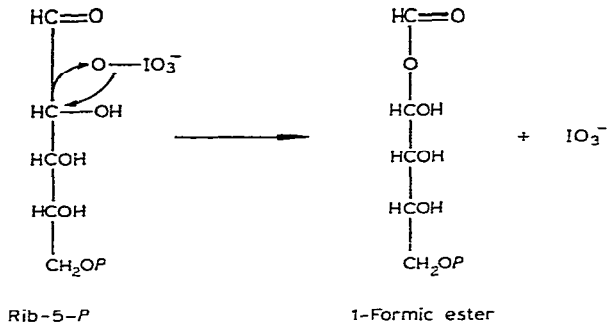

Fig. 3. Mechanism of periodate oxidation, depicting conversion of Rib-5-*P* into the non-aldehydic 1-formic ester. Polarization of the 1-aldehyde group may help favor C-1–C-2 as the initial site of oxygen insertion from periodate in preference to the C-2–C-3 and C-3–C-4 sites.

EXPERIMENTAL

Periodate oxidation. — D-Ribose 5-phosphate (20 μmol) was shielded from light and stirred for 1 h at 30° with either 0, 20, or 80 μmol of sodium metaperiodate in a final volume of 1.3 mL. Prior to use, the periodate (0.1M final) was adjusted to pH 9 with 5M ammonium hydroxide.

Chromatography. — Aliquots, equivalent to 16 μmol of Rib-5-*P*, were chromatographed on a column (0.9 × 64 cm) of Dowex 1-formate, using the same apparatus and conditions previously described[10]. The column was equilibrated with 5mM ammonium formate and eluted at 1 mL/min with a gradient generated with a Buchler Varigrad containing 250 mL of ammonium formate in each of 7 chambers as follows: 0.005M (chambers *1*, *2*, and *4*), 0.05M (chamber *3*), 0.5M (chamber *5*), and 1.0M (chambers *6*, and *7*).

Aldehyde assay. — Aldehydes were determined with 3-methyl-2-benzothiazolinone hydrazone (MBTH) by a modification of the procedure of Paz *et al.*[9]. Mixtures contained 100 μmol of sodium succinate–succinic acid, pH 4.0, 1 μmol of MBTH, and 0.5 mL of sample in a final volume of 2.0 mL. The mixtures were kept in the dark for 18–24 h at room temperature and assayed at 310 nm against a reference containing water in place of the sample. Absorbance values from chromatographic fractions were multiplied by 30 to express results as total aldehyde per 15-mL fraction. Assays of 3mM Rib-5-*P* incubated in 20mM sodium hydrogencarbonate–sodium hydroxide, pH 10, were conducted with 0.1 mL aliquots and twice the amount of succinate buffer.

Phosphorus determination. — Aliquots (2.0 mL) of column fractions were wet-ashed and analyzed for orthophosphate as previously described[11]. Results are expressed as total phosphorus per 15-mL fraction and represent column recoveries of 85%.

Chemicals. — Sodium metaperiodate was obtained from Baker Chemical Co. (Phillipsburg, N.J.), D-ribose 5-phosphate and DL-glycerol 1-phosphate were from

Sigma Chemical Co. (St. Louis, Mo.), and 3-methyl-2-benzothiazolinone hydrazone hydrochloride was from Aldrich Chemical Co. (Milwaukee, Wis.). Other chemicals were reagent grade, and solutions were prepared with Pyrex-distilled water that had previously been deionized.

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