Short Communication

Generation of Superoxide and
In Vitro Inactivation
of Viruses by
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Received June 9, 1982

Reductones and quinones have been shown to cause strand scission of nucleic acids and *in vitro* inactivation of viruses.¹⁾ Oxygen radicals generated by the autoxidation of the reducing agents are mainly responsible for the observed biological activity.^{1,2)} Common reducing aldoses and ketoses do not usually show virus-inactivating ability.³⁾ Recently however, we have reported^{4,5)} that some reducing sugar phosphates generate superoxide at pH 10.4 and that they exhibit phagocidal action similar to that caused by quinone antibiotics and reductones. Moreover, we have examined the virus-inactivating ability of some other moderately autoxidizable sugar derivatives, such as oxidized polysaccharides⁶⁾ and pyranosiduloses.⁷⁾ Aldopentoses are more easily oxidized at the reducing group than aldohexoses,^{8,9)} and appear to be suitable derivatives for structure-activity studies of radical-mediated inactivation of viruses by sugars. In this communication, we report that unsubstituted D-aldopentoses generate superoxide and inactivate *in vitro* tobacco mosaic virus (TMV) and bacteriophage ϕ X174 in phosphate buffer in the presence of Cu^{2+} .

As described previously,⁴⁾ the autoxidation of pentoses, D-arabinose (1), D-lyxose (2), D-ribose (3), and D-xylose (4) has been studied by

determining the rate of formazan formation ($\Delta A_{560}/\text{min}$) by superoxide (O_2^-) from nitroblue tetrazolium chloride (NBT). The *in vitro* inactivation of the two viruses was investigated by treating them with a pentose in the presence or absence of Cu^{2+} in 0.1 M phosphate buffer (pH 8.1) at 37° for 3 hr, and the virus survival ratio (%) was determined by assaying the plaque forming ability on *E. coli* C_N,⁵⁾ or by typical local lesion assay¹⁰⁾ on half-leaves of *N. tabacum* c.v. Xanthi n.c.

Table I shows the data for NBT reduction by aldopentoses in buffered aqueous solutions. The rate of reduction was dependent on the concentration of sugars (data are not shown) and inhibited by superoxide dismutase (SOD, bovine blood, see notes in Table I). The results demonstrate the ability of aldopentoses under mild conditions to generate oxygen radicals,

TABLE I. RATE OF REDUCTION OF
NBT BY ALDOPENTOSEs

Aldopentoses ^a	$\Delta A_{560}/\text{min}^b$	
	pH 10.4 at $25 \pm 0.2^\circ$	pH 8.1 at $37 \pm 0.2^\circ$
D-Arabinose (1)	3.0×10^{-4}	3.2×10^{-5}
D-Lyxose (2)	1.1×10^{-3}	2.6×10^{-4}
D-Ribose (3)	3.4×10^{-3}	6.8×10^{-4}
D-Xylose (4)	1.0×10^{-3}	1.3×10^{-4}

^a Preparations of aldopentoses were from commercial sources and used without further purification. The sources and grades were: 1 and 2, Nakarai Chemicals, Kyoto, Grade G.R.; 3, Kojin Chemical Co., Tokyo; 4, Sigma Chemical Corp. Melting points of the four preparations were the same as those cited in, "Rodd's Chemistry of Carbon Compounds," Vol. I, Part F, ed. by S. Coffey, Elsevier Publishing Co., Amsterdam, 1967, p. 229.

^b Measurements were made using 10 mg/ml of sugar and 50 μM of NBT, 10 min after dissolution of sugar in 0.015 M carbonate buffer (pH 10.4). The reaction mixtures were equilibrated with air, and the A_{560} during the first 20 min of reaction increased linearly. The data shown are the mean of five measurements. In experiments at pH 8.1, the reaction mixtures contained 20 mg/ml of sugar and 125 μM of NBT. At pH 10.4 the reaction of 3 (5 mg/ml) was inhibited by 85% and 90%, by 20 and 100 units/ml of SOD, respectively.

[†] Part of this work was presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, April 2, 1982.

FIG. 1. Inactivation of TMV by Aldopentoses. Determined by Local Lesion Assay on Half-leave of *N. tabacum*.

The survival ratio (%) represents the number of local lesions derived from sugar-treated TMV to that derived from TMV of the control run that contained no sugar. Tobacco mosaic virus (TMV-OM, $A_{260}/A_{280} = 1.16$, $70 \mu\text{g}/\text{ml}$) was treated with pentose ($10 \text{ mg}/\text{ml}$) in the presence of Cu^{2+} (10^{-4} M). For other reaction conditions, see text.

especially superoxide. The four aldopentoses apparently exhibit different rates of NBT reduction, although the observed magnitude of formazan formation was much lower than that of L-ascorbic acid and pyranosiduloses.⁷⁾

Figure 1 shows the results of the inactivation of TMV by aldopentoses. The difference in the mean survival ratios except that between D-xylose and D-arabinose could be regarded as significant by *t*-test (test of significance, 0.05), and the results suggest that the order of inactivating rate was $3 > 2 > 4 \geq 1$, which is the same as that of the rate of NBT reduction. In the absence of Cu^{2+} , the mean survival ratios appeared at 50–100% and they were statisti-

FIG. 2. Inactivation of ϕX174 by Aldopentoses.

The reaction mixture contained ϕX174 (1×10^7 pfu), sugar ($5 \text{ mg}/\text{ml}$), and Cu^{2+} (10^{-4} M). Control I, control run without sugar and Cu^{2+} ; Control II, control run containing Cu^{2+} but no sugar.

cally insignificant.

The results for ϕX174 inactivation is shown in Fig. 2. The order of sugar inactivating ability was the same for TMV inactivation and NBT reduction. In the absence of Cu^{2+} , no significant inactivation was obtained by the use of $5 \text{ mg}/\text{ml}$ of pentoses, but treatment of phage with $50 \text{ mg}/\text{ml}$ sugar gave the following survival ratios: 3, 44.5%; 2, 56.7%; 4, 70.1%; 1, 71.1%. These results revealed that aldopentoses in the presence of Cu^{2+} inactivated the viruses by oxygen radicals generated by the autoxidation of sugars. The data obtained so far on inhibition by various radical scavengers in inactivation reactions, suggest that the reaction mechanism involving hydroxyl radical and Cu^{2+} , that is similar to that reported for reductones and reducing sugar phosphates,^{4,5)} may be operative.

The order of the NBT reduction rate obtained by the four pentoses is of interest, as it may provide information on the mechanism of superoxide generation by reducing sugars. The order correlates with the order of the enoli-

zation rate of aldopentoses in the same medium obtained by Isbell *et al.*¹¹⁾ and with that of the rate of pentose oxidation by oxygen in carbonate buffer.¹²⁾ These results tentatively suggest that enolization of acyclic forms of pentoses may be a rate determining step in the reduction, if the autoxidation of α -hydroxy-carbonyl compounds proceeds through enolization followed by univalent reduction of oxygen by enolate in the absence of metal ion.^{13,14)} The specific role of copper ion in the present system, such as complexing with nucleic acids or sugars, may also be suspected.

Acknowledgments. We are grateful to Dr. J. Morita, Doshisha Women's College of Liberal Arts, Kyoto, and Dr. K. Noba, Vegetable and Ornamental Crops Research Station, Mie, for their helpful discussions and suggestions on this work. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 56560135) to N. K. from the Ministry of Education, Science and Culture of Japan.

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