Reduction of Nitroblue Tetrazolium Chloride and *in vitro* Inactivation of Viruses by D-Aldohexoses#

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In previous papers,[1,2] we reported that in the presence of Cu^{2+} (10^{-4}M) aldopentoses, D-arabinose (**1**), D-lyxose (**2**), D-ribose (**3**), and D-xylose (**4**) (Fig. 1), inactivate *in vitro* tobacco mosaic virus (TMV) and bacteriophage øX174 and that superoxide and other oxygen radicals generated by the autoxidation of these sugars are responsible for the inactivation of the viruses. Moreover, we suggested that the observed differences in the biological activity and the rate of the autoxidation reactions of the four pentoses are due to their configurational difference, since the order [(**3**) > (**2**) > (**4**) > (**1**)] is the same both in the activity and in the reaction rate, and the similar effect of configuration on the rate of the oxidation reaction and enolization of aldopentoses was reported in the literature.[3,4]

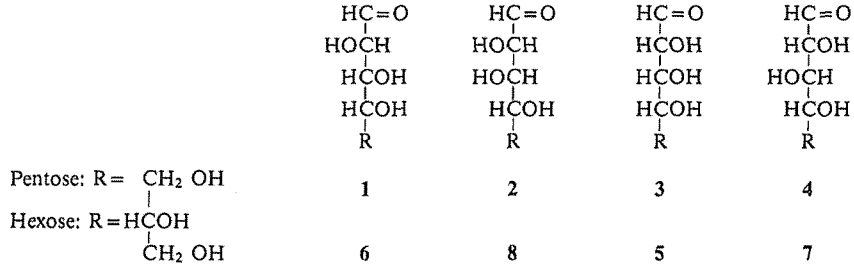

Fig. 1. Acyclic structures of D-aldopentoses and D-aldohexoses

Aldohexoses are in general less reactive than aldopentoses in oxidations (e.g. halogen oxidation of free sugars[5]) and have been reported to exhibit very weak nucleic acid-cleaving activity.[6] However, we have recently found that some partially substituted aldohexoses exhibit higher biological activities than the corresponding unsubstituted ones.[7,12] More recently Bucala *et al.*[8] reported the possibility of some common reducing hexoses being involved in nucleic acid aging *in vivo* and age-related dysfunction in gene expression *via* chemical modification or strand scission. In view of these developments, we have examined the reduction of nitroblue tetrazolium chloride (NBT) and the *in vitro* inactivation of viruses by four kinds of D-aldohexoses: D-altrose (**6**), D-mannose (**8**), D-allose (**5**), and D-glucose (**7**) (Fig. 1), which have the same configuration at C-2 and C-3, respectively, as the above-mentioned four aldopentoses, to see whether or not in the term of configuration the order in their reactivity and biological activity is the same as the corresponding aldopentoses.

Materials and Methods

Preparations of aldohexoses were from commercial sources and used without further purification.

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Sources were as follows: D-allose (**5**) and D-altrose (**6**), Sigma Chemical Corp. ; D-glucose (**7**) and D-mannose (**8**), Nakarai Chemicals, Kyoto, Grade G. R. In the experiments of NBT reduction, a HITACHI Model 200-01 Spectrophotometer was used. Experimental procedures for NBT reduction, virus inactivation reactions and assays for inactivated viruses were the same as reported in previous papers.[1,2,9,10,11]

Results and Discussion

Table 1 shows the data for the rate of reduction of NBT by D-aldohexoses in buffered aqueous solutions. NBT concentration in the present experiment was 125 μM, which is two and half times that for the reduction by aldopentoses,[1] because significant rates of reduction were not obtained at concentrations lower than 50 μM of NBT. The results show that the order of reactivity in NBT reduction by the four aldohexoses was **6** > **5** ≒ **7** > **8**. This is not in accord with the order for aldopentoses.[1]

Table 2 shows the results of bacteriophage øX174 inactivation in the presence of Cu^{2+}(10^{-6}M) by aldohexoses as well as by aldopentoses. The results clearly reveal that the ability of aldohexoses to inactivate the bacteriophage is much lower than that of aldopentoses. However, the order of the phage inactivation magnitude was **6** ≒ **7** ≒ **8** > **5**, which seems to reflect no significant difference among the sugars examined nor does it correlate with the data for NBT reduction.

Table 1. The Rate of Reduction of NBT by D-Aldohexoses*

Aldohexoses	ΔA_{560} /min
D-Allose (**5**)	8.8×10^{-4}
D-Altrose (**6**)	3.0×10^{-3}
D-Glucose (**7**)	8.3×10^{-4}
D-Mannose (**8**)	3.4×10^{-4}

* Measurements were made using 12 mg/ml of sugar and 125 μM of NBT, at pH 10.4 and 25° ± 0.2° in 0.015 M carbonate buffer, 10 min after dissolution of sugar. The reaction mixtures were equilibrated with air, and the A_{560} increased linearly during the first 20 min of reaction time. The data are the mean values of three different measurements.

As shown in Fig. 2, the results for TMV inactivation were very similar to the phage inactivation. In this case, no statistically significant difference (t-test, degree of confidence, 0.95) was found among the four hexoses (see foot notes of Fig. 2), and all of them showed much lower activity than 8D-ribose.

Morita *et al.* reported that the order of the degradation magnitude of supercoiled øX174 DNA by aldoses is **3** > **1,2,4** > **6,7,8** in the presence of Cu^{2+}(10^{-4}M).[6] The present results together with Morita's data suggest that aldohexoses which have different abilities to reduce NBT do not necessarily differ significantly in their virus nactivating abilities. The slight difference observed for NBT reduction by aldohexoses may be noteworthy. While this reactivity does not conform with that for the aldopentoses, yet interestingly it is in accord with the order of the relative rate of enolization (**6** > **5** ≒ **7** > **8**), reported by Isbell.[3] Consequently,

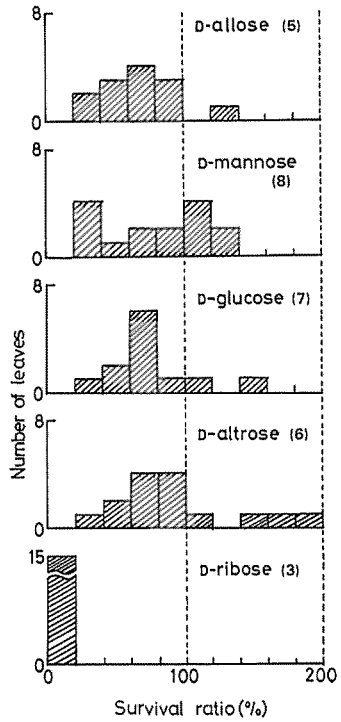

Fig. 2. Inactivation of TMV by D-aldohexoses determined by local lesion assay on half-leaves of *N. tabacum*.

The survival ratio (%) represents the number of local lesions derived from sugar-treated TMV to that derived from TMV of the control run which contained no sugar. TMV was treated with hexose (12 mg/ml) in the presence of Cu^{2+}(10^{-4}M) at 37° for 3 hr in 0.1 M phosphate buffer (pH 8.1). The mean values of the survival ratios were as follows: **5**, 74.0 ± 15.9; **6**, 91.2 ± 23.3; **7**, 80.8 ± 19.9; **8**, 79.2 ± 18.6; **3**, 7.2 ± 2.7 %.

Table 2. Inactivation of Bacteriophage øX174 by D-Aldoses in the Presence of $Cu.^{2+}$ *

Aldoses	Survival ratio (%)
Control	80.5
D-Arabinose (1)	10.2
D-Lyxose (2)	1.2
D-Ribose (3)	0.02
D-Xylose (4)	7.7
D-Allose (5)	70.5
D-Altrose (6)	33.0
D-Glucose (7)	28.8
D-Mannose (8)	24.1

* The reaction mixtures contained 1.7×10^8 pfu/ml of øX174, 5.0mg/ml of aldopentose or 6.0mg/ml of aldohexose, and 10^{-6}M of Cu^{2+} in 0.1 M phosphate buffer (pH 8.1), and were kept at 37° for 3 hr. Control: control run without sugar and Cu^{2+}.

the configurational dependence of the ability of aldohexoses to generate oxygen radicals and to inactivate viruses, if it exists, may be different from that for aldopentoses. For both confirmation and stereochemical explanation of the observed differences in reactivity and biological activity of aldohexoses, further studies are required on the substituded aldohexose derivatives with higher reducing ability, and these are now in progress in our laboratory.

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摘　　要

D-アルドヘキソースによるニトロブルーテトラゾリウムクロリドの還元とウイルスの *in vitro* 不活化

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これまでの研究により，D－アラビノース(1)，D－リキソース(2)，D－リボソース(3)およびD－キシロース(4)が，室温，緩衝液中ニトロブルーテトラゾリウム（NBT）を還元し，またCu^{2+}の存在下でウイルスを不活化することがわかったので，これらの糖とC－2，C－3位の立体配置が同じであるD－アルトロース(6)，D－マンノース(8)，D－ロース(5)およびD－グルコース(2)（いずれも市販品）のNBT還元力，バクテリオファージφX174およびタバコモザイクウイルス（TMV）の不活化反応（リン酸緩衝液中，pH 8.1, 37℃, 3時間）を検討した。その結果，NBT還元力は，ペントースと異った反応性順位（6〉5〉7〉8〉）が得られ，これはIsbellらによって報告されたアルカリ条件下のヘキソースのエノール化速度とほぼ一致した。ウイルス不活化力は，いずれのヘキソースもD－リボースより小さかったが，ヘキソース間では有意差は認められなかった。