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Preliminary communication

Autoxidation and phagocidal action of some reducing sugar phosphates*

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In the course of our study of the interaction of various carbohydrate derivatives with bacteriophage φX174 (a small, single-stranded, DNA phage), we have found that some reducing sugar phosphates undergo autoxidation more rapidly, and, *in vitro*, inactivate the phage more effectively, than other sugar phosphates and common reducing sugars in buffered, aqueous solutions. As both autoxidation and phagocidal action are inhibited by some free-radical scavengers, free radicals derived from oxygen are considered to be primarily responsible for the inactivation of the phage. Major properties of the two reactions are reported in this communication.

Table I shows the survival ratio (%) of φX174, as determined by the measurement of plaque-forming units (p.f.u.) on *Escherichia coli* C_N, when the phage was treated with a 0.5% solution of different sugar phosphates for 3 h at 37°. The sodium salts of D-fructose 6-phosphate, 2-amino-2-deoxy-D-glucose 6-phosphate, D-ribose 5-phosphate, and D-fructose 1,6-bisphosphate inactivated the phage much more effectively than other phosphorylated (or non-phosphorylated) sugars. The inactivation reaction caused by D-fructose 6-phosphate was further shown to be completely inhibited by the addition of catalase (EC 1.11.1.6, bovine liver), Tiron (4,5-dihydroxy-1,3-benzenedisulfonic acid disodium salt, a scavenger for superoxide[1]), or 1,4-diazabicyclo[2.2.2]octane (DABCO, a scavenger for singlet oxygen[2]), and stimulated by Cu^{2+}, as shown in Table II. These results suggest that oxygen-derived free-radicals formed by the autoxidation of reducing sugar phosphates cause damage to the phage. Evidence has also been obtained that DNA is a target molecule of the present inactivation-reaction[3]. Possible interaction of sugar phosphates with coat proteins of the phage[4], which may also result in inactivation, may be excluded by the observed ability of inactivated phage particles to adsorb to the host cell-wall[5].

As a means of correlating the phagocidal action of reducing sugar phosphates with their reactivity in autoxidation, the reduction of Nitro Blue tetrazolium chloride (NBT), as monitored by measurement[8,9] at 560 nm of formazan formation, was investigated. Fig. 1

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TABLE I

INACTIVATION[a] OF BACTERIOPHAGE φX174 BY SUGAR PHOSPHATES

Sugar derivatives[b]	*Survival ratio*[c] *(%)*
2-Amino-2-deoxy-D-glucose 6-phosphate	9.0
D-Ribose 5-phosphate	11.0
D-Fructose 6-phosphate	10.0
D-Fructose 1,6-bisphosphate	21.6
D-Glucose 6-phosphate	94.8
D-Galactose 6-phosphate	84.7
D-Mannose 6-phosphate	106.6
D-Glucitol 6-phosphate	79.6
D-Glucose 1-phosphate	102.8
D-Glucose	72.2
D-Galactose	78.2
D-Fructose	69.2
2-Acetamido-2-deoxy-D-glucose	71.9
myo-Inositol 2-phosphate[d] (cyclohexylamine salt)	84.8

[a] φX174 (5×10^8 p.f.u./mL) was incubated with 5 mg of sugar phosphate/mL in 0.05M Tris-HCl buffer (this is identical to starvation buffer[6], except that the concentration of Tris is increased to 0.05M), pH 8.1, containing 5 g of KCl, 1 g of NaCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, and 1 mM $CaCl_2$ per L for 3 h at 37°. An aliquot was withdrawn, diluted with the buffer, and assayed for infectious phage by the agar overlayer method of Adams[7]. [b] Sugar phosphates used were sodium salts from Sigma Chemical Company, unless otherwise specified. [c] The survival ratio (%) is the ratio of the number of plaque-forming units at 3 h to that at zero time. [d] *myo*-Inositol 2-phosphate was a gift from Dr. K. Asada, Kyoto University.

TABLE II

EFFECT[a] OF SOME RADICAL SCAVENGERS AND METALS ON THE INACTIVATION OF φX174 BY D-FRUCTOSE 6-PHOSPHATE, SODIUM SALT

Scavengers for reduced oxygen species (concentration)	*Inhibition*[b] *(%)*	*Metals*[c]	*Survival (%)*
None	0	none	37.8
Catalase (10 μg/mL)	100	$CuCl_2$	3.56
Tiron (1 mM)	100	$CuSO_4$	3.36
DABCO (0.1 M)	100	$FeSO_4$	22.3
Isopropyl alcohol (1 M)	32	$MnCl_2$	48.9
D-Mannitol (0.1 M)	20	$CoCl_2$	55.3
		$ZnCl_2$	20.2

[a] φX174 was treated with 19 mM D-fructose 6-phosphate (5 mg/mL) in 0.05 M Tris-HCl, pH 8.1, for 3 h at 37° in the presence of radical scavenger or metal. [b] Inhibition (%) was computed from the ratio of the survival in the presence of scavenger to that in the absence of scavenger (30% at 3 h). [c] Concentration of metal, 0.1 mM.

shows the rate of reduction of NBT by various reducing sugars in sodium carbonate buffer (0.03M, pH 10.4) at 25°. Reaction mixtures contained the indicated concentrations of sugar, 10 μM NBT, and 100 μM EDTA, and were equilibrated with air. As shown in Fig. 1, the three sugar phosphates that exhibited high phagocidal abilities also showed high reducing values. D-Glucose did not reduce NBT under the conditions employed. The observed higher reactivity of D-fructose 6-phosphate with NBT, as compared with that of D-fructose or D-glucose 6-phosphate, was also confirmed by examining the effect on the reduction of alkaline phosphatase (EC 3.1.3.1, calf intestine), or phosphoglucose isomerase (EC 5.3.1.9, yeast), as shown in Fig. 2. The results also exclude the possibility that impurities or degraded products in the preparations of sugar phosphates could have reacted with the NBT. Fig. 3 shows the effect of superoxide dismutase (EC 1.15.1.1, bovine blood) and catalase on reduction of NBT by D-fructose 6-phosphate. It was also found that Tiron,

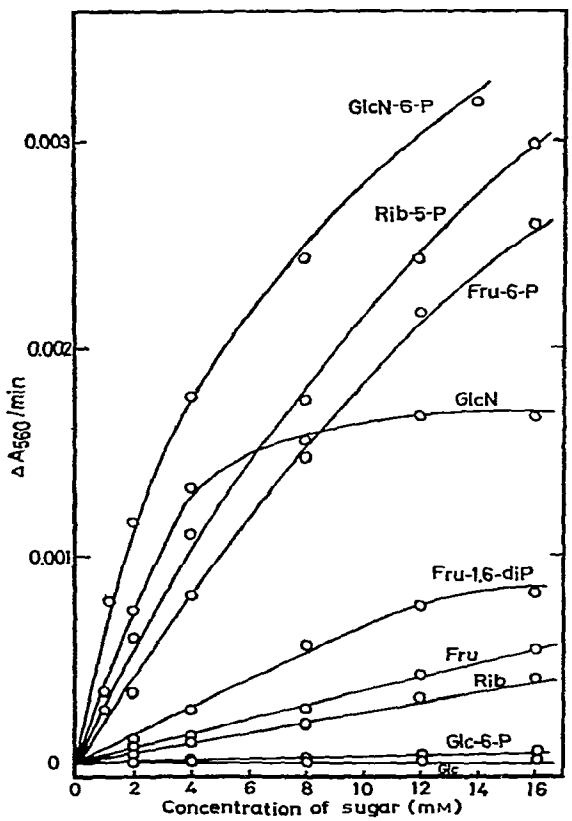

Fig. 1. Rate of reduction of NBT as a function of the concentration of reducing sugar. (The reaction rates were obtained by measuring the increase of absorbance at 560 nm during the first 5 min of reaction GlcN-6-P, 2-amino-2-deoxy-D-glucose 6-phosphate; Rib-5-P, D-ribose 5-phosphate; Fru-6-P, D-fructose 6-phosphate; GlcN, 2-amino-2-deoxy-D-glucose HCl; Fru-1,6-diP, D-fructose 1,6-bisphosphate; Fru, D-fructose; Rib, D-ribose; Glc-6-P, D-glucose 6-phosphate; Glc, D-glucose.)

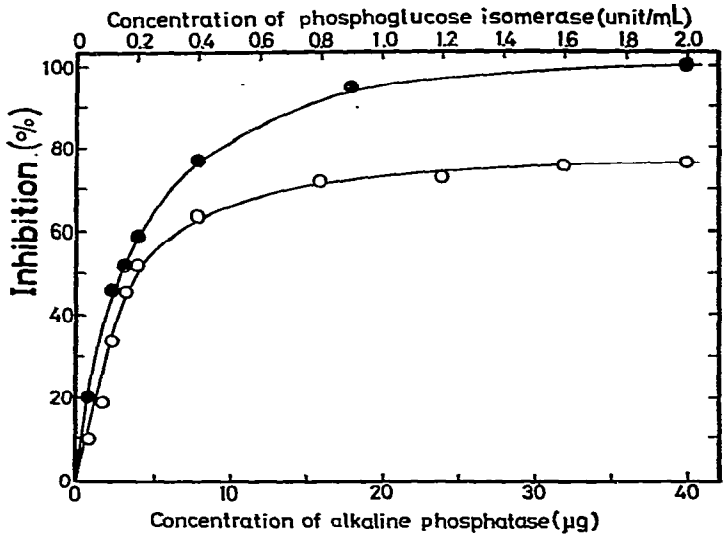

Fig. 2. Inhibition by phosphoglucose isomerase (—•—), or alkaline phosphatase (—o—), of reduction of NBT by D-fructose 6-phosphate, Na salt. (The reaction conditions were the same as described for the experiment described in Fig. 1, except that the reaction mixtures contained 10 mM D-fructose 6-phosphate, Na salt, and the indicated concentrations of the enzymes.)

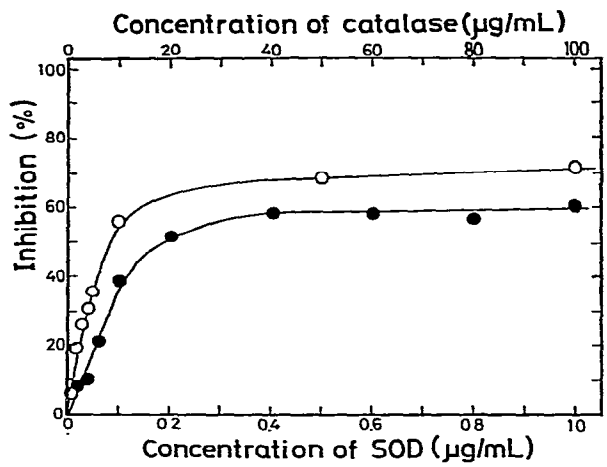

Fig. 3. Inhibition by superoxide dismutase (SOD, —o—), or catalase (—•—), of reduction of NBT by D-fructose 6-phosphate, Na salt.

or Cu^{2+}, inhibits the reaction, whereas D-mannitol (a scavenger for hydroxyl radicals) or DABCO did not have much effect on the reaction (data are not shown). These results constitute evidence that superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide are generated in the autoxidation, and are responsible for the major part of the reduction of NBT.

Superoxide is known to (*a*) undergo disproportionation, to yield hydrogen peroxide, and (*b*) react with hydrogen peroxide, to generate hydroxyl radical and singlet oxygen[10]. It is therefore suggested that some, or all, of these oxygen-derived species are involved in the observed damage to the phage DNA. If this is the case, the present inactivation-reaction is of interest, in that the phage, or its DNA, is labile enough to react with such concentrations of free radicals as are generated here, because modification or strand scission of DNA by free radicals has been observed mainly in the autoxidation of compounds having low redox potentials, such as quinones, enediols, and thiols[11,12]

Although a great deal of data has been accumulated on the oxidation of reducing sugars with oxygen under alkaline conditions[13], there has been less study of the autoxidation of reducing sugar phosphates in buffered aqueous solutions. The lability of reducing sugar phosphates under strongly alkaline conditions, and their tendency to undergo, *via* an enediol intermediate, saccharinic acid formation and a reverse aldol reaction have been reported[14,15]. Lorimer *et al.*[16] reported the enzymic oxidation by molecular oxygen of a D-*erythro*-2-pentulose bisphosphate. The effect of phosphates on the nonenzymic browning reaction of hexoses with amino acids has also been studied[17,18]

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