Note

Induction of Strand Breakage in ϕ X174 RFI DNA by Aminosugar DerivativesSatoshi NANJOU, Shoji FUJII,
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L-Ascorbic acid and some mono- and polysaccharide derivatives inactivate various kinds of bacteriophages, and break DNA strands.^{1~5)} The DNA strand breakage is considered to be caused by oxygen radicals generated during the autoxidation of these DNA-breaking sugars, which have characteristic properties of reductone based on their endiol groups.

Aminosugars, which have an amino reductone structure, are also expected to break DNA. 2-Amino-2-deoxy-D-glucose⁶⁾ and its 6-phosphate ester³⁾ inactivated bacteriophages. However, little is known about DNA strand breakage by aminosugars. In this work, we investigated

DNA strand breakage by various aminosugars, and discussed the structural characteristics of the aminosugars causing DNA strand breakage.

Double-stranded replicative form I DNA (RFI DNA; supercoiled, covalently closed, circular duplex DNA) of bacteriophage ϕ X174 was prepared as described previously.⁷⁾ 2-Amino-2-deoxy-D-gluconic acid (D-glucosaminic acid),⁸⁾ 2-amino-2-deoxy-D-glucose oxime (D-glucosamine oxime),⁹⁾ 2-amino-2-deoxy-D-galactonic acid (D-galactosaminic acid),¹⁰⁾ 2-amino-2-deoxy-D-talose (D-talosamine),¹¹⁾ 1-amino-1-deoxy-D-fructose (D-isoglucosamine),¹²⁾ 2-amino-2-deoxy-D-mannose (D-mannosamine)¹³⁾ and 2-acetamido-2-deoxy-D-mannose (N-acetyl-D-mannosamine)¹³⁾ were synthesized according to the previously described procedure. Other chemicals were purchased from Nakarai Chemicals, Ltd., and the Sigma Chemical Co. and were used without further purification.

The reaction mixture (20 μ l) contained 300 ng of ϕ X174 RFI DNA, test agents, and 50 μ M of CuCl₂ in 50 mM Tris-HCl buffer (pH 7.1), unless otherwise noted. The reaction mixture was incubated for 3 hr at 37°C, and the reaction was stopped by the addition of 5 μ l of 0.1 M EDTA solution containing 50% (w/v) sucrose and 0.1% (w/v) bromophenol blue. The sample, a final volume of 25 μ l, was analyzed directly by agarose gel electrophoresis.

Electrophoretic analysis of the DNA in the reaction mixture and quantitative analysis of duplex DNA bands on the gels were performed as described previously.^{2,7)}

Aminosugars such as 2-amino-2-deoxy-D-glucose (D-glucosamine) and 2-amino-2-deoxy-D-mannose (D-mannosamine), induced strand breakage in ϕ X174 RFI DNA and converted it to RFII DNA and RFIII DNA in the presence of Cu²⁺ (Fig. 1). Their DNA breaking

FIG. 1. Induction of Strand Breakage in ϕ X174 RFI DNA by Aminohehexoses.

1, drug-free control; 2, CuCl₂ (50 μ M); 3, D-fructose (20 mM) + CuCl₂ (50 μ M); 4, D-fructose 6-phosphate (20 mM) + CuCl₂ (50 μ M); 5, D-isoglucosamine (1 mM); 6, 5 + CuCl₂ (50 μ M); 7, D-mannosamine (10 mM); 8, 7 + CuCl₂ (50 μ M); 9, D-glucosamine (20 mM); 10, 9 + CuCl₂ (50 μ M); 11, D-galactosamine (20 mM); 12, 11 + CuCl₂ (50 μ M); 13, D-talosamine (20 mM); 14, 13 + CuCl₂ (50 μ M); 15, RFIII DNA (linear duplex DNA) converted by *Xho*I from RFI DNA. Electrophoretic analysis was carried out by the reported procedure.^{2,7)}

TABLE I. INDUCTION OF STRAND BREAKAGE IN ϕ X174 RFI DNA BY D-GLUCOSAMINE, D-FRUCTOSE, AND D-MANNOSAMINE DERIVATIVES

Sugars (20 mM)	50 μ M CuCl ₂	Relative amount of DNA (%)			
		RFI	RFII ^b	RFIII ^b	Others
None	+	93.4	6.1	0	0.5
	—	93.5	5.9	0	0.6
D-Glucose ^a	+	89.1	10.1	0	0.8
	—	92.4	7.0	0	0.6
D-Glucose 6-phosphate-Na ^a	+	57.7	41.8	0	0.5
	—	90.8	9.0	0	0.2
D-Glucosamine-HCl ^a	+	2.3	91.5	5.8	0.4
	—	89.5	9.1	0.5	0.9
D-Glucosamine 6-phosphate-Na ^d	+	0	95.8	3.1	1.1
	—	82.9	16.1	0	1.0
N-Acetyl-D-glucosamine	+	11.4	87.0	1.2	0.4
	—	91.5	7.7	0	0.8
D-Glucosaminic acid	+	86.1	12.4	0	1.5
	—	92.2	6.7	0	1.1
D-Glucosamine oxime	+	81.0	18.6	0	0.4
	—	80.3	18.9	0	0.8
D-Fructose ^a	+	54.8	45.0	0	0.2
	—	92.6	6.9	0	0.5
D-Fructose 6-phosphate-Na ^a	+	0	77.0	22.2	0.8
	—	87.2	12.1	0	0.7
D-Isoglucosamine-HCl ^c	+	0	77.1	22.7	0.2
	—	89.2	10.1	0	0.7
D-Mannose ^a	+	90.4	9.2	0	0.4
	—	91.4	8.0	0	0.6
D-Mannose 6-phosphate-Na ^a	+	82.1	17.7	0	0.9
	—	92.2	7.5	0	0.3
D-Mannosamine-HCl ^d	+	0	87.4	12.1	0.5
	—	90.1	8.2	0	1.7
N-Acetyl-D-mannosamine	+	87.9	11.8	0	0.3
	—	92.4	7.0	0	0.6

^a Although the effects of these sugars were reported previously,²⁾ the data, which were confirmed by the control experiments, were listed for comparison.

^b RFII, open circular DNA; RFIII, linear duplex DNA.

^c The concentration is 1 mM.

^d The concentration is 10 mM.

activities were higher than those of their corresponding hexoses, hexose 6-phosphates, and ketohexoses reported by Morita and Komano.²⁾ These results indicated that DNA strand breakage was stimulated by the existence of an amino group, and the effect of an amino group in DNA strand breakage by reducing sugars was higher than that of ketone groups and 6-phosphate groups (Table I).

D-Isoglucosamine showed the highest activity in DNA strand breakage in the hexose derivatives that we tested. When the amino groups of these sugars were replaced by acetyl groups, DNA strand breakage was reduced markedly. This is consistent with the result that *N*-acetyl derivatives of hexosamine were not effective in the inactivation of *Lactobacillus* phage PL-1.⁶⁾ DNA strand

breakages by D-glucosamine oxime and D-glucosaminic acid were lower than that of D-glucosamine, and D-mannosaminic acid was less effective than D-mannosamine in DNA strand breakage. The low activity of these compounds may depend upon the lack of aldehyde groups in the C-1 position of their molecules.

In general, the lyxo-configuration caused higher DNA breakage than the arabino-configuration, as shown in Tables I and II. D-Tagatose, D-galactose 6-phosphate, and D-galactosamine were more active than D-fructose, D-glucose 6-phosphate and D-glucosamine, respectively.

In inactivating *Lactobacillus* phage PL-1,⁶⁾ D-galactosamine and D-mannosamine were less active than D-glucosamine. But under our experimental conditions,

TABLE II. INDUCTION OF STRAND BREAKAGE IN ϕ X174 RFI DNA BY D-GALACTOSAMINE DERIVATIVES, D-TALOSAMINE, AND D-TAGATOSE

Sugars (20 mM)	50 μ M CuCl ₂	Relative amount of DNA (%)			
		RFI	RFII	RFIII	Others
None	+	92.9	6.4	0	0.7
	—	93.2	6.0	0	0.8
D-Galactose ^a	+	91.0	8.5	0	0.5
	—	92.4	7.3	0	0.3
D-Galactose 6-phosphate-Na	+	4.0	92.7	2.6	0.7
	—	91.8	7.9	0	0.3
D-Galactosamine-HCl	+	0	86.1	13.4	0.5
	—	86.1	13.1	0	0.8
N-Acetyl-D-galactosamine	+	80.1	19.1	0	0.8
	—	91.2	7.6	0	1.2
D-Galactosaminic acid	+	60.5	38.9	0	0.6
	—	92.1	7.0	0	0.9
D-Talosamine-HCl	+	76.0	23.0	0	1.0
	—	92.4	7.0	0	0.6
D-Tagatose	+	6.3	91.5	2.0	0.2
	—	91.5	8.0	0	0.5

^a Although the effect of D-Galactose was reported previously,²⁾ the data, which were confirmed by the control experiments, were listed for comparison.

the activity of D-mannosamine was much stronger than D-glucosamine, and D-galactosamine was more active than D-glucosamine in the DNA strand breakage.

As Cu²⁺ increased DNA strand breakage, the breakage might be due to oxygen radicals generated in the process of autoxidation of aminosugar derivatives.

Recently it has been reported that the reaction between sugar and the amino group of nucleic acids might be a cause of aging.¹⁴⁾ Aminosugars are distributed widely in organisms as constituents of tissues. Therefore, more detailed investigations on the interaction between nucleic acids and aminosugars are required.

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