ELSEVIER

Carbohydrate Research 257 (1994) 285–291

CARBOHYDRATE RESEARCH

Structure–activity relationships in the induction of single-strand breakage in plasmid pBR322 DNA by amino sugars and derivatives

Nobuhiro Kashige [a], Tadatoshi Yamaguchi [b], Akira Ohtakara [c], Masaru Mitsutomi [c], John S. Brimacombe [d], Fumio Miake [a], Kenji Watanabe [a,*]

[a] *Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma, Jonan-ku, Fukuoka 814-01, Japan*
[b] *Department of Hygiene, Miyazaki Medical College, Kiyotake, Miyazaki 889-16, Japan*
[c] *Faculty of Agriculture, Saga University, Saga 840, Japan*
[d] *Chemistry Department, University of Dundee, Dundee DD1 4HN, United Kingdom*

(Received June 25th, 1993; accepted October 13th, 1993)

Abstract

Structure–activity relationships in the induction of strand breakage in plasmid pBR322 DNA by amino sugars and their derivatives were investigated using agarose gel electrophoresis. The coexistence of a potential free aldehyde group at the C-1 position and a free amino group at the C-2 position in the molecules was indispensable for the display of DNA strand-breaking activity in both mono- and oligo-aminosaccharides. The activity was increased by the introduction of an acidic group, especially a phosphate group, at the C-6 position. The activity was also increased by the addition of Cu^{2+}. The order of activity of the amino monosaccharides tested was D-isoglucosamine > D-mannosamine > D-galactosamine > D-glucosamine, and it is suggested that this order is correlated with the portion of acyclic (*aldehydo*) form in the solution of each sugar. The possible chemical basis for DNA strand breakage by amino sugars is discussed.

1. Introduction

Some carbohydrate derivatives have been shown to break DNA, especially in the presence of Cu^{2+}, and in some cases the involvement of oxygen radicals generated in the carbohydrate solutions has been demonstrated [1]. The elucidation of the specific features of these DNA-breaking agents, particularly through

* Corresponding author.

SSDI 0008-6215(93)E0415-W

comparison with those of DNA-alkylating or -intercalating compounds, may help clarify the mechanisms of such biological phenomena as carcinogenesis, carcinostasis, mutagenesis, virus induction, virus inactivation, and aging.

In previous investigations [2,3] we found that some amino sugars such as D-glucosamine cause the in vitro inactivation of phages of all nucleic acid types (double stranded DNA, single stranded DNA, double stranded RNA, and single stranded RNA) without affecting the growth of their host cells, and that they caused the single-strand breakage of the DNA of both phage PL-1, active against *Lactobacillus casei*, and plasmid pBR322. Nanjou et al. [4,5] also reported that some amino sugars induced single-strand breakage in the RF-I DNA of coliphage ϕX174.

In the present work, therefore, we have examined the activities of some amino sugar derivatives that cause single-strand breaks in pBR322 covalently closed circular duplex DNA (ccc-DNA) in order to determine their structure-activity relationships.

2. Experimental

Materials.—Covalently closed circular duplex DNA (ccc-DNA) of plasmid pBR322 was prepared from *Escherichia coli* W3350/pBR322 as described in principle by Kupersztoch-Portnoy et al. [6]. Each batch of pBR322 DNA was analyzed for purity by agarose gel electrophoresis, and only the DNA samples containing more than 85% ccc-DNA were used. D-Isoglucosamine (1-amino-1-deoxy-D-fructose) was synthesized according to the method of Kuhn and Haas [7]. Methyl *N*-acetyl-α-L-daunosaminide, *N*-acetyl-L-acosamine, and *N*-acetyl-L-ristosamine were synthesized by the methods of Brimacombe et al. [8,9]. Methyl α-D-altrosaminide was synthesized by the method of Meyers et al. [10]. Methyl and benzyl D-glucosaminides, 3-amino-3-deoxy-D-allose, and 6-aminomethyl-3,6-dihydro-2-methoxy-2*H*-1,4-oxazin-3-ol were gifts from Dr. H. Hashimoto, Tokyo Institute of Technology. *O*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucose was prepared by the enzymatic hydrolysis of partially *N*-acetylated chitosan according to the procedure of Mitsutomi et al. [11]. *O*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucose was prepared by the digestion of partially *N*-acetylated chitosan by *Streptomyces griseus* chitinase [12]. Other reagents were purchased from Wako Jun-yaku Co., Nacalai Tesque Inc., and Sigma Chemical Co., and used without further purification.

Reaction of plasmid pBR322 with amino sugars.—Mixtures (100 μL) containing 1 μg of pBR322 DNA, mostly of the ccc-DNA form, sample compound at various concentrations, and 50 mM Tris-HCl buffer (pH 7.2), with or without 1 mM $CuCl_2$, were incubated 37°C. At intervals 10-μL aliquots were mixed, to stop the reaction, with 3 μL of 22.5 mM EDTA (pH 8.0) containing 1.5% (w/v) SDS, 25% (w/v) sucrose, and 0.02% (w/v) Bromophenol Blue, and the resulting samples were subjected directly to electrophoresis on 0.8% agarose gel. The electrophoresis and

quantitative determination of each DNA species, namely ccc-DNA, nicked, open-circular DNA (oc-DNA), and full-length linear DNA (linear-DNA), on the gels by densitometry were performed as described previously [3].

3. Results and discussion

When ccc-DNA of plasmid pBR322 was treated with 100 mM D-glucosamine in Tris-HCl buffer as just described, the amount of ccc-DNA decreased with reaction time, and an equivalent amount of oc-DNA appeared, indicating that D-glucosamine primarily induces a single-strand breakage. No double-strand breakage was induced in the ccc-DNA under these conditions. The strand breakage was stimulated by the addition of Cu^{2+} to the mixture, in which case all the ccc-DNA was converted into linear-DNA via oc-DNA, and after a prolonged reaction the linear-DNA was further fragmented. Cupric chloride alone at 1 mM concentration did not effect DNA breakage. Since the D-glucosamine-induced DNA strand breakage was stimulated by Cu^{2+}, it was considered that active oxygen and, possibly to some extent, the D-glucosamine radicals generated in the process of autoxidation of the amino sugar were involved in the DNA strand breakage. The activities of various derivatives of D-glucosamine and other amino sugars in inducing ccc-DNA breakage, in the presence or absence of Cu^{2+}, are summarized in Table 1, where the relative amounts of ccc-DNA remaining after 3 h are shown as an index. D-Glucose and 2-deoxy-D-glucose, used as controls without an amino group, were not effective in the absence of Cu^{2+}, although they were slightly active in the presence of Cu^{2+}. *N*-Acetyl-D-glucosamine and D-glucosaminic acid were much less effective than D-glucosamine. Therefore, the coexistence of both the free amino group at the C-2 position and the aldehyde group at the C-1 position in the molecule was considered to be indispensable for DNA strand-breaking activity. All methyl and benzyl glycosides, for example of glucosamine, daunosamine, mannosamine, and altrosamine, were also ineffective, supporting the hypothesis of a requirement for the aldehyde group at the C-1 position. The *N*-acetyl derivatives tested were not effective, further indicating a requirement for the amino group at the C-2 position. D-Kanosamine and 3-amino-3-deoxy-D-allose, having an amino group at the C-3 position, induced a little less DNA strand breakage than D-glucosamine, which has it at the C-2 position.

D-Galactosamine, D-mannosamine, and D-isoglucosamine were a little more effective in DNA strand breakage than D-glucosamine. The order of activity, in either the presence or the absence of 1 mM Cu^{2+}, was D-isoglucosamine > D-mannosamine > D-galactosamine > D-glucosamine. The differences in activity among these sugars may be ascribed to differences in the ratio of the acyclic (*aldehydo*) form to cyclic form characteristic of their aqueous solutions. Hayward has reported that D-glucose, which has all hydroxy groups equatorially disposed in its pyranose chair conformation, forms only 0.0028% of *aldehydo* form. On the other hand, when one of the hydroxy groups on the stable chair conformation is axially disposed, as in D-galactose and D-mannose, more of the *aldehydo* form is

Table 1
pBR322 ccc-DNA single-strand breakage by D-glucosamine derivatives in the absence or presence of Cu^{2+}

Compound	Concn (mM)	ccc-DNA remaining [a] (%)	
		no Cu^{2+}	+ Cu^{2+}
None		100	100
D-Glucosamine (2-amino-2-deoxy-D-glucose)·HCl	100	29 [b]	0 [b]
	10	96	0
	1	99	80
D-Glucose	100	98	79
2-Deoxy-D-glucose (2-deoxy-D-*arabino*-hexose)	100	92	89
D-Glucosaminic acid	100	96	100
D-Isoglucosamine (1-amino-1-deoxy-D-fructose)·HCl	100	0	0
	10	87	0
	1	90	0
D-Galactosamine (2-amino-2-deoxy-D-galactose)·HCl	100	24	0
	10	94	0
	1	100	35
D-Mannosamine (2-amino-2-deoxy-D-mannose)·HCl	100	0	0
	10	63	0
	1	100	11
α-Aminoacetone	100	0	0
	10	58	0
	1	89	0
N-Acetyl-D-glucosamine	100	100	85
N-Acetyl-D-galactosamine	100	95	71
N-Acetyl-D-mannosamine	100	96	91
N-Acetyl-L-acosamine (3-acetamido-2,3,6-trideoxy-L-*arabino*-hexose)	100	100	74
N-Acetyl-L-ristosamine (3-acetamido-2,3,6-trideoxy-L-ribo-hexose)	100	96	95
D-Glucosamine 1-phosphate	100	48	7
	10	99	77
	1	100	100
D-Glucosamine 6-phosphate	100	0 [b]	0
	10	82 [b]	0
	1	100	10
D-Glucosamine 6-sulfate	100	14	0
	10	88	0
	1	101	33
D-Glucosamine 3-sulfate	100	63	0
	10	100	6
	1	101	91
N-Sulfo-D-glucosamine (2-deoxy-2-sulfoamino-D-glucose)	100	104	19
	10	100	88
	1	100	101
D-Kanosamine (3-amino-3-deoxy-D-glucose)	10	97	0
	1	109	59
3-Amino-3-deoxy-D-allose	10	99	29
	1	102	94
Methyl 2-amino-2-deoxy-α-D-glucopyranoside	100	100	100
Benzyl 2-amino-2-deoxy-α-D-glucopyranoside·HCl	100	100	100

Table 1 (continued)

Compound	Concn (mM)	ccc-DNA remaining [a] (%)	
		no Cu^{2+}	+ Cu^{2+}
Benzyl 2-amino-2-deoxy-α-D-mannopyranoside	100	100	100
Methyl 6-amino-6-deoxy-α-D-glucopyranoside	100	100	100
Benzyl 3-amino-4-azido-3,4-dideoxy-α-D-arabinopyranoside	100	100	100
Benzyl 3-amino-4,6-di-*O*-benzoyl-3-deoxy-α-D-altropyranoside	100	100	100
Methyl 2-amino-2-deoxy-α-D-altropyranoside	100	100	100
Methyl *N*-acetyl-α-L-daunosaminide (methyl 3-acetamido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside)	100	100	94
6-Aminomethyl-3,6-dihydro-2-methoxy-2*H*-1,4-oxazin-3-ol	100	103	95
	10	103	99
	1	102	98
Chitobiose [*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-amino-2-deoxy-D-glucose]·HCl	100	26	0
	10	75	0
	1	100	44
O-(2-Amino-2-deoxy-α-D-glucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose (GlcN·GlcNAc)	100	100	85
	10	100	100
	1	100	100
Chondrosine [*O*-(β-D-glucopyranosyluronic acid)-(1 → 3)-2-acetamido-2-deoxy-D-galactose]	10	94	0
	1	97	16
Chitotriose·HCl	10	96	0
	1	99	37
O-(2-Amino-2-deoxy-β-D-glucopyranosyl) (1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucose (GlcN·GlcNAc·GlcNAc)	10	100	100
	1	100	100
Chitotetraose·HCl	1	96	20
Chitopentaose·HCl	1	100	20
Chitohexaose·HCl	1	101	16

[a] After incubation as described in the Experimental section, as percent of the starting amount. [b] From ref 3.

present (D-galactose, 0.02%, D-mannose, 0.005%) than in solutions of D-glucose [13]. Moreover it has been reported that D-fructose in solution has 0.7% of the open-chain (*keto*) form. Thus, if the relative amounts of acyclic form in solutions of amino sugars may be thought to be the same as for ordinary sugars it is understandable that D-isoglucosamine shows the highest activity.

By the introduction of such acidic groups as sulfate or phosphate at the 6 position the activity of D-glucosamine was increased. Thus, D-glucosamine 6-phosphate caused the most DNA strand breakage of all the D-glucosamine derivatives tested. It has been reported that the proportion of *aldehydo* forms in solutions of

sugar phosphates is greater than that for the parent sugars [14]. Accordingly, the behavior of D-glucosamine 6-phosphate shows that as the amount of *aldehydo* form increases, so does the DNA strand-breaking activity. On the other hand, the activity of D-glucosamine 1-phosphate was lower than that of D-glucosamine, since the former has no free aldehyde group at C-1. The 6-sulfate of D-glucosamine was much more effective than either the 2- or the 3-sulfated derivatives. The higher activity of the 6-sulfate presumably has the same basis as the activity of D-glucosamine 6-phosphate. As expected, the activity of *N*-sulfo-D-glucosamine was lower than that of D-glucosamine, since the former has no free amino group at C-2.

The DNA strand-breaking activity of some oligosaccharides was also investigated. Chitobiose, a dimer of D-glucosamine, was more effective than D-glucosamine in the presence of Cu^{2+}. However, when the C-2 amino group of chitobiose was acetylated (GlcN · GlcNAc in Table 1), DNA strand-breaking activity was reduced. The reduction of activity in this case may result from the relative orientation of the aldehyde and amino groups: the C-1 aldehyde group was free but the C-2 amino group is acetylated, and the C-2′ amino group is free but the C-1′ aldehyde group is engaged in ring formation and linkage to the C-4 hydroxy group. On the other hand, chondrosine, *O*-(β-D-glucopyranosyluronic acid)-(1 → 3)-2-acetamido-2-deoxy-D-galactose, having both C-1 free aldehyde and C-2 free amino groups, showed activity like chitobiose. Chitotriose, chitotetraose, chitopentaose, and chitohexaose, which are trimer, tetramer, pentamer, and hexamer of D-glucosamine, respectively, all at 1 mM concentration, showed DNA strand-breaking activity in the presence of 1 mM Cu^{2+}, because they have both the C-1 free aldehyde and C-2 free amino group in the reducing-terminal sugar residue. However, at all concentrations higher than 10 mM, precipitation with DNA occurred, and the precipitates would not migrate in agarose-gel electrophoresis. Therefore, it was impossible to determine their DNA-breaking activity at higher concentrations by means of the electrophoretic procedure. Moreover, in the cases of aminoglycoside antibiotics such as streptomycin, kanamycin, neomycin, gentamicin, fortimicin, micronomicin, and paromomycin, all as sulfates at the concentrations of 100 mM, no electrophoretic migration occurred when mixtures with DNA were applied to agarose gels. This is possibly due to the formation of very high molecular weight, salt-like complexes. In general the DNA strand-breaking activities of amino oligosaccharides seem to be higher than those of amino monosaccharides, especially in the presence of Cu^{2+}, and like the differences among individual monosaccharides this phenomenon may also result from the presence of higher proportions *aldehydo* forms in solutions of the oligosaccharides. One could surmise that the introduction of a glycosyl group at C-3 or C-4 of a monosaccharide leads to a conformation change favoring the *aldehydo* form.

It was considered from these findings that the amounts of the acyclic form in amino sugars bearing both free aldehyde and free amino groups are related to their DNA strand breaking activities. In this connection it is of interest that α-aminoacetone, which has a structure in common with the acyclic forms of D-glucosamine and D-isoglucosamine, showed a DNA strand-breaking activity higher than that of D-glucosamine. Since it is known that α-(primary amino)

carbonyl compounds such as α-aminoacetone form dihydropyrazines by dimerizing self-condensation [15], it seems possible that dihydropyrazine derivatives formed from the amino sugars, existing in their acyclic forms as α-(primary amino) carbonyl compounds, may be involved in the DNA strand breaking activity of these sugars, and the detailed mechanism should be of interest.

It is hoped that studies of the kind reported here will be useful for determining the structural features of amino sugars that are indispensable for their activity. These studies may also lead to the invention of more effective DNA-breaking amino sugars by modification of their chemical structures, even though the detailed mechanism of DNA strand breakage by amino sugars remains to be established.

Acknowledgment

We are grateful to Dr. Hironobu Hashimoto, Department of Life Science, Tokyo Institute of Technology, for providing valuable samples.

References

[1] N. Kashimura and J. Morita, *J. Synth. Org. Chem. Jpn.*, 42 (1984) 523–535.
[2] K. Watanabe, T. Sasaki, N. Kashige, K. Ishibashi, Y. Nakashima, and M. Hayashida, *Agric. Biol. Chem.*, 49 (1985) 63–70.
[3] K. Watanabe, N. Kashige, Y. Nakashima, and K. Sumoto, *Agric. Biol. Chem.*, 50 (1986) 1459–1465.
[4] S. Nanjou, S. Fujii, K. Tanaka, K. Ueda, and T. Komano, *Agric. Biol. Chem.*, 48 (1984) 2865–2867.
[5] S. Nanjou, S. Fujii, K. Tanaka, K. Ueda, and T. Komano, *Agric. Biol. Chem.*, 49 (1985) 459–466.
[6] Y.M. Kupersztoch-Portnoy, M.A. Lovett, and D.R. Helinski, *Biochemistry*, 13 (1974) 5484–5490.
[7] R. Kuhn and H.J. Hass, *Liebigs Ann. Chem.*, 600 (1956) 148–155.
[8] J.S. Brimacombe, R. Hanna, and L.C.N. Tucker, *Carbohydr. Res.*, 136 (1985) 419–425.
[9] J.S. Brimacombe, R. Hanna, M.S. Saeed, and L.C.N. Tucker, *J. Chem. Soc., Perkin Trans. 1*, (1982) 2583–2587.
[10] W.H. Meyers and G.J. Robertson, *J. Am. Chem. Soc.*, 65 (1943) 8–11.
[11] M. Mitsutomi, A. Ohtakara, T. Fukamizo, and S. Goto, *Agric. Biol. Chem.*, 54 (1990) 871–877.
[12] A. Ohtakara, H. Matsunaga, and M. Mitsutomi, *Agric. Biol. Chem.*, 54 (1990) 3191–3199.
[13] L.D. Hayward, *Carbohydr. Res.*, 53 (1977) 13–20
[14] K.J. Schray and S.J. Benkovic, *Acc. Chem. Res.*, 11 (1978) 136–141.
[15] G.B. Barlin, in A. Weissberger and E.C. Taylor (Eds.), *The Chemistry of Heterocyclic Compounds*, Vol. 41, Wiley, New York, 1982, pp 11–27