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Note

Action of ionizing radiations on a hyaluronate tetrasaccharide

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Hyaluronic acid and other glycosaminoglycans are particularly sensitive to the effects of γ-radiation, and the associated molecular changes have been studied, especially by viscometry[1,2]. The considerable reduction in viscosity of a hyaluronate solution after very small doses of ionizing radiations can be ascribed[3] to changes in the shape, deformability, or internal structure of the molecule rather than to major changes in molecular weight. There are indications that certain hyaluronic acid solutions react with hydrated electrons[4], and chemical changes following γ-irradiation of aqueous solutions could be due to the action of e_{aq}^- in addition to the dominant influence of HO radicals.

E.s.r. evidence[4] and recent work[5] on the γ-irradiation of solid oligosaccharides indicates that initial removal of H-1 leads to a series of processes resulting in glycosidic scission, the introduction of keto groups at C-5, and the formation of deoxy groups at C-2. Detailed studies[6,7] of the γ-irradiation of cellobiose in aqueous solution have clearly demonstrated that the HO radical-induced scission of the glycosidic bond is not a simple hydrolysis, but proceeds by way of radicals formed at C-1′, C-5′, and C-4 with subsequent alterations in the structure of one subunit. Von Sonntag and his colleagues have concluded[8] that the nature of the bond in the disaccharide does not materially affect the mechanism. However, no direct information is available on hyaluronic acid, where the substituted groups are significantly different from the unsubstituted polysaccharides at C-2 and C-5.

We now report on the products of γ-irradiation, in aqueous solution and in the solid state, of the hyaluronate tetrasaccharide [**1**, β-D-GlcA*p*-(1 → 3)-β-D-GlcNAc*p*-(1 → 4)-β-D-GlcA*p*-(1 → 3)-β-D-GlcNAc*p*] produced by the action of testicular hyaluronidase on hyaluronic acid. Our principal observations centre on the mobilities in paper chromatography of the reducing saccharides formed and on their response to the Elson–Morgan reagents[9,10] and to ninhydrin.

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TABLE I

P.C.DATA FOR THE PRODUCTS OF IRRADIATION OF THE HYALURONATE TETRASACCHARIDE **1**

Compound	R_{GlcNAc} *values*					
	Solvent A		*Solvent* B			
	Standards[a]	*Products*[a]	*Standards*[a]	*Products*[a]	*Standards*[b]	*Products*[b]
1	0.21	0.21	0.23	0.23	0.23	0.23
2	0.32	0.31 (*A*)	0.40	0.40 (*A*)	0.40	0.40 (*A*)
3	0.45	0.45 (*B*)	0.55	0.55 (*B*)	0.55	0.55 (*B*)
D-Glucuronic acid	0.39	0.40 (*C*)	0.68	0.68 (*C*)		
2-Acetamido-2-deoxy-D-glucose	1.00		1.00		1.00	
2-Amino-2-deoxy-D-glucose	0.76		0.62			

[a]Detection with silver nitrate. [b]Detection with Elson–Morgan reagents.

The immediate formation of a violet colour with the Elson–Morgan reagents indicates the presence of a 2-acetamido-2-deoxyglucose residue at the reducing end of a molecule[11,12], and a positive reaction with ninhydrin indicates the presence of free NH_2 groups. Three products were recognised after irradiation of **1** in the solid state or in aqueous solution (Table I), none of which gave a positive reaction with ninhydrin reagent and were, therefore, considered not to contain a GlcN moiety.

The product (*A*) of lowest mobility exhibited R_{GlcNAc} values in two solvent systems identical to those of the trisaccharide, β-D-GlcNAc*p*-(1 → 4)-β-D-GlcA*p*-(1 → 3)-β-D-GlcNAc*p* (**2**). Both **2** and *A* gave an immediate violet colour with the Elson–Morgan reagents. Only loss of GlcA from the non-reducing terminus of **1** could yield an Elson–Morgan-positive trisaccharide.

A second irradiation product (*B*) had R_{GlcNAc} values identical with those of the less-mobile disaccharide[13] (*N*-acetylhyalobiouronic acid, **3**) formed by cleavage of the (1 → 4)-β-D-glycosaminidic bond of **1** by bacterial hyaluronidase. *B* also resembled *N*-acetylhyalobiouronic acid in giving an immediate colour with Elson–Morgan reagents. *N*-Acetylhyalobiouronic acid could arise from **1** either *via* **2** followed by scission of GlcNAc from the non-reducing terminus, or directly by scission of the central glycosidic bond. Since neither 2-acetamido-2-deoxyglucose nor 2-amino-2-deoxyglucose was detected, the latter route is the most probable.

The detection of glucuronic acid (*C*) is consistent with the formation of **2** (or a closely related compound) by cleavage of GlcA from the non-reducing end of **1**. If the scission of this linkage proceeded by established mechanisms[7], the trisaccharide product would possess a modified 2-acetamido-2-deoxyglucose moiety, but our technique might not be capable of detecting such a small change.

The foregoing data indicate radiolytic scission of a glucuronidic linkage and of the central glycosaminidic linkage of **1** in aqueous solution and in the solid state.

Similar processes could contribute to the liberation of reducing groups, as occurs in the γ-irradiation of hyaluronic acid[14].

Recently, Bradbury and von Sonntag have studied the γ-radiolysis of 2-acetamido-2-deoxyglucose in aqueous solution[15] and in the solid state[16]. A range of products was found, but it was significant that the *N*-acetyl group proved relatively difficult to remove and the radiolytic pathway initially involved[15] radicals produced by the removal of H-1 to H-6. Such degradation would only occur subsequently to the glycosidic cleavage processes reported herein.

EXPERIMENTAL

General

A 10,000-Ci ^{60}Co-source was used for irradiation of samples (5 mg) of the tetrasaccharide in aqueous solution (mM) equilibrated with argon (3.12×10^{19} eV.ml^{-1}), and in the solid state in air (2.09×10^{21} eV.g^{-1}). Aliquots from irradiated solutions were concentrated to one-tenth volume, and solid samples (5 mg) were dissolved in water (5 ml) prior to descending p.c. on Whatman No. 1 paper for 18 and 40 h with *A*, 1-butanol–pyridine–water (42:25:40); and *B*, 1-butanol–acetic acid–water (48:12:25). Detection was effected with *A*, alkaline silver nitrate[17]; *B*, Elson–Morgan reagents[9]; and *C*, 0.1% ninhydrin in 1-butanol[10]. Mobilities are expressed as R_{GlcNAc} values.

O-(β-D-Glucopyranosyluronic acid)-(1 → 3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-*O*-(β-D-glucopyranosyluronic acid)-(1 → 3)-2-acetamido-2-deoxy-β-D-glucopyranose (**1**) was prepared from a digest of sodium hyaluronate with testicular hyaluronidase[11] (EC 3.2.1.35) and had $[\alpha]_D^{25}$ −42° (initial), −51° (equil., 3 h; *c* 1.2, water); lit.[11] −41° (initial), −53° (equil.). P.c. of **1** showed it to be pure and to be identical with an authentic sample kindly supplied by Dr. Weissmann. *O*-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-*O*-(β-D-glucopyranosyluronic acid)-(1 → 3)-2-acetamido-2-deoxy-β-D-glucopyranose (**2**) was prepared from a digest of **1** with liver β-D-glucuronidase[18] (EC 3.2.1.31). P.c. confirmed that **1** had been converted into an Elson–Morgan-positive product (**2**) and D-glucuronic acid.

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