Carbohydrate Research, 103 (1982) 190–194
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Structure of disulfated disaccharides from chondroitin polysulfates, chondroitin sulfate D and K

NOBUKO SENO AND KEIKO MURAKAMI

Department of Chemistry, Faculty of Science, Ochanomizu University, Otsuka, Bunkyo-ku, Tokyo 112 (Japan)

(Received December 3rd, 1980; accepted for publication in revised form, September 8th, 1981)

Three types of chondroitin polysulfates, chondroitin sulfate D, E, and K (ChS-D, -E and -K) have been isolated from the cartilages of shark[1,2], squid[3], and king crab[4], respectively. These chondroitin polysulfates were found to differ both in the degree of sulfation and in the position of the sulfate groups. The structure of the unsaturated disulfated disaccharide, obtained from ChS-E after digestion with chondroitin ABC lyase, was found to be 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 4,6-disulfate[5]. However, the positions of the additional sulfate groups in ChS-D and ChS-K were not determined, but were thought to be either at O-2 or -3 of the D-glucopyranosyluronic acid residue[2,4,5]. Periodate oxidation of the unsaturated disaccharide disulfate (ΔDi-diS_D) and monosulfate (ΔDi-monoS_D) from ChS-D was previously studied by Suzuki[2], who reported that ΔDi-diS_D rapidly consumed 1 mol and ΔDi-monoS_D 3 mol of periodate. Based on this finding, Susuki tentatively concluded that a sulfate group is in a novel location, either at O-2 or -3 of the unsaturated glucopyranosyluronic acid residue, but the uronic acid content during oxidation was not determined.

In a preliminary experiment, however, we observed that ΔDi-diS_D rapidly consumes over 1 mol of periodate and, after an oxidation of 20 h, 3 mol of periodate without a concomitant decrease of the uronic acid content. Therefore, it appears that the use of the unsaturated disaccharides to determine, by periodate oxidation, the position at O-2 or -3 of the additional sulfate group on the uronic acid residues is open to criticism.

In this paper, the positions of sulfate groups in the saturated disulfated disaccharides, Di-diS_D and Di-diS_K, isolated from ChS-D and ChS-K after digestion with testicular hyaluronate 4-glycanohydrolase (EC 3.2.1.35), followed by chondroitin ABC lyase, were determined.

On periodate oxidation of a 2mM sample in 20mM sodium periodate at 37° in the dark, the uronic acid residue of Di-diS_D was rapidly and completely oxidized, whereas that of Di-diS_K was quite stable (Fig. 1A). After oxidation for 24 h, Di-diS_K

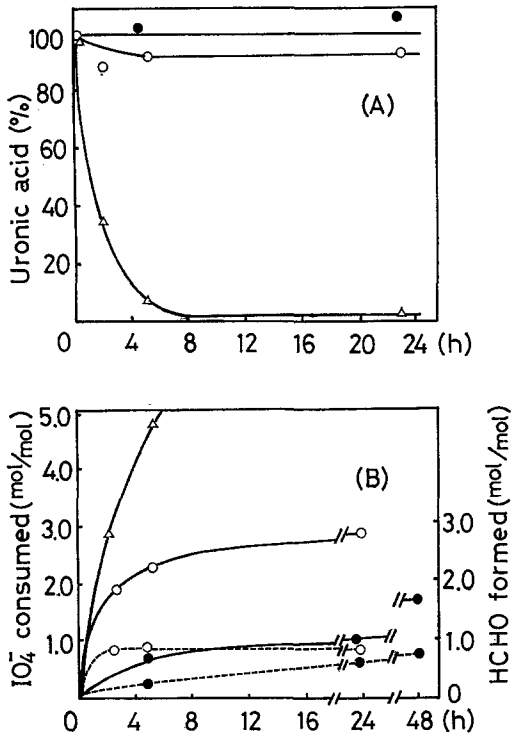
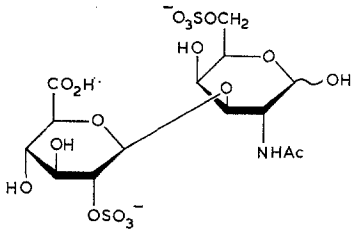


Fig. 1. Periodate oxidation of the saturated disaccharides Di-diS$_D$ (△), Di-diS$_K$ (●), and Di-monoS$_K$ (○). The 2mM sample was incubated with 20mM periodate at 37° in the dark: (A) uronic acid remaining (——); (B) periodate consumed (——) and formaldehyde formed (– – – –).

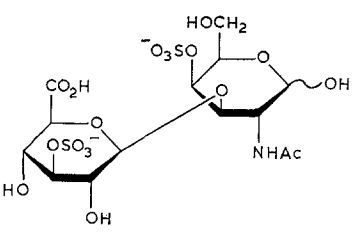
consumed nearly 1 mol of periodate and released 0.4 mol of formaldehyde, but the liberation of formaldehyde increased to 0.73 mol after 48 h (Fig. 1B). To evaluate the influence, on the oxidation, of the sulfate group at O-4 of the 2-acetamido-2-deoxy-D-galactose residue of Di-diS$_K$, Di-monoS$_K$ was oxidized under the same conditions. The uronic acid residue of Di-monoS$_K$ was also resistant to oxidation (Fig. 1A), and a rapid liberation of ~1 mol of formaldehyde was observed (Fig. 1B). Di-diS$_D$ consumed >5 mol of periodate, although the theoretical value is 2 mol. This may be the result of over-oxidation of the uronic acid and the hexosamine residues[6,7].

These results suggest that the structure of Di-diS$_D$ from ChS-D is 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid 2-sulfate)-D-galactose 6-sulfate (**1**) and that of Di-diS$_K$ is 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid 3-sulfate)-D-galactose 4-sulfate (**2**). Accordingly, the position of the additional sulfate group of ChS-D is located at O-2 of the D-glucuronic acid residues, and that of ChS-K at O-3.

On paper chromatography in butyric acid–0.5M ammonia, Di-diS$_D$ and Di-diS$_K$ separated well; however, the monosulfated disaccharides, Di-monoS$_D$ and Di-monoS$_K$,



1



2

could not be separated under the same conditions. In this solvent system, axial and equatorial isomers, such as ⊿Di-4S and ⊿Di-6S, separate well[5]. Therefore, the monosulfated disaccharides were subjected to high-voltage, paper electrophoresis, but even in borate buffer (pH 10.0), both monosulfated disaccharides migrated to the same position.

EXPERIMENTAL

Material. — Bovine testicular hyaluronate 4-glycanohydrolase (EC 3.2.1.35, 280 NFunits/mg) was purchased from Sigma Chemical Co., and chondroitin ABC lyase [EC 4.2.2.4], chondro-4-sulfatase [EC 3.1.6.9], and chondro-6-sulfatase [EC 3.1.6.10] from *Proteus vulgaris* from Seikagaku Kogyo Co. Ltd., Tokyo. Unsaturated sulfated disaccharides, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 4-sulfate (⊿Di-4S), 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 6-sulfate (⊿Di-6S), and 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid 2- or 3-sulfate)-D-galactose 6-sulfate (⊿Di-diS_D) were isolated from the digestion products with chondroitin ABC lyase of ChS-A, -C, and -D, respectively[2,5], and ⊿Di-monoS_D was obtained from ⊿Di-diS_D by treatment with chondro-6-sulfatase[5]. Saturated sulfated disaccharides, 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-D-galactose 4-sulfate (Di-4S), 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-D-galactose 6-sulfate (Di-6S), 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid 2- or 3-sulfate)-D-galactose 6-sulfate (Di-diS_D), and 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid 2- or 3-sulfate)-D-galactose 4-sulfate (Di-diS_K) were isolated, from ChS-A, -C, -D, and -K, by digestion with testicular hyaluronate 4-glycanohydrolase, followed by chondroitin ABC lyase, as described previously[4,8]. Monosulfated disaccharides, Di-monoS_D and Di-monoS_K, were obtained from the digestion products of Di-diS_D with chondro-6-sulfatase and of Di-diS_K with chondro-4-sulfatase, as reported previously[4].

Periodate oxidation of sulfated disaccharides. — Periodate oxidation of the disaccharides was carried out at 37° in the dark. At time intervals, aliquots were analyzed (after addition of 1% sodium sulfite) for any remaining uronic acid by the carbazole reaction[9], for periodate consumption by the spectrophotometric method[10] at 223 nm, and for formaldehyde formation by the chromotropic acid reaction[11].

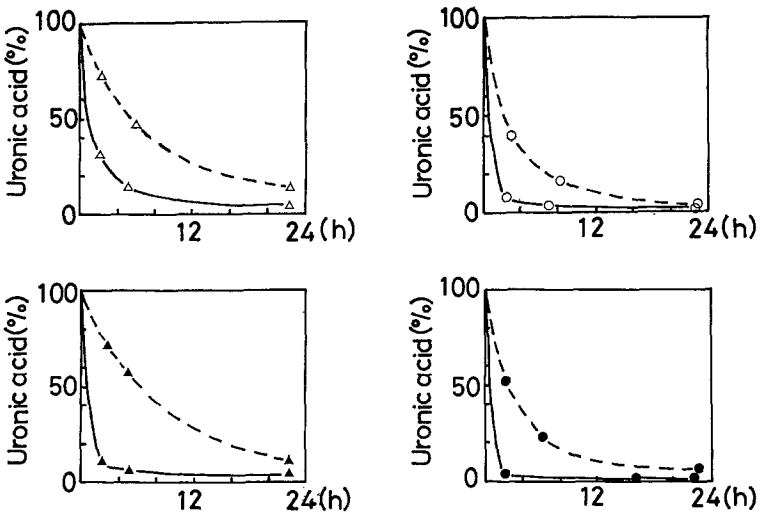


Fig. 2. Periodate oxidation of the sulfated disaccharides ⊿Di-4S (△), Di-4S (▲), ⊿Di-6S (○), and Di-6S (●): 0.2mM sample was incubated with 2mM periodate (———) or 2mM sample with 20mM periodate (——) at 37° in the dark.

When the effect of the concentrations of both sample and periodate on the oxidation of the sulfated disaccharides ⊿Di-4S, ⊿Di-6S, Di-4S, and Di-6S was compared, the rate of oxidation of the uronic acid residue in the 2mM sample with 20mM sodium periodate was much higher than that in the 0.2mM sample with 2mM periodate (Fig. 2), in spite of the fact that the molar ratio of periodate to disaccharide was the same (10:1). The effect of concentration was larger for disaccharide 4-sulfates than for disaccharide 6-sulfates. On the basis of this result, the first conditions were selected for the periodate oxidation of the disaccharides having a sulfated uronic acid residue.

Paper chromatography and paper electrophoresis of sulfated disaccharides. — Descending paper chromatography of sulfated disaccharides was performed on Toyo No. 51A paper (60-cm long) in 5:3 (v/v) butyric acid-0.5M ammonia[2]. High-voltage, paper electrophoresis of sulfated disaccharides was performed on Toyo No. 51A paper (60-cm long) in 0.1M acetic acid–pyridine buffer (pH 5.0) at 40 V/cm for 1 h, or in borate buffer [pH 10.0; containing boric acid (7.45 g) and sodium hydroxide (4.0 g) in water (1 L)][12] at 40 V/cm for 3 h. The disaccharides were detected by staining with the silver nitrate reagent.

ACKNOWLEDGMENT

This work was supported, in part, by research grants from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1 T. SODA, F. EGAMI, AND T. HORIGOME, *Nippon Kagaku Zasshi.*, 61 (1940) 43–49.
2 S. SUZUKI, *J. Biol. Chem.*, 235 (1960) 3580–3588.

3 Y. KAWAI, N. SENO, AND K. ANNO, *J. Biochem. (Tokyo)*, 60 (1966) 317–321.
4 N. SENO, S. YAMASHIRO, AND K. ANNO, *Biochim. Biophys. Acta*, 343 (1974) 423–426.
5 S. SUZUKI, H. SAITO, T. YAMAGATA, K. ANNO, N. SENO, Y. KAWAI, AND T. FURUHASHI, *J. Biol. Chem.*, 243 (1968) 1543–1550.
6 R. W. BAILEY AND J. B. PRIDHAM, *Adv. Carbohydr. Chem.*, 17 (1962) 125–138.
7 J. R. TURVEY, *Adv. Carbohydr. Chem.*, 20 (1965) 194–199.
8 N. SENO, F. AKIYAMA, AND K. ANNO, *Biochim. Biophys. Acta*, 362 (1974) 290–298.
9 Z. DISCHE, *J. Biol. Chem.*, 167 (1947) 189–198.
10 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind. (London)*, (1957) 1216.
11 J. C. SPECK AND A. A. FORIST, *Anal. Chem.*, 26 (1954) 1942–1944; N. SENO, *Seikagaku*, 33 (1961) 529–532.
12 H. BOUVENG AND B. LINDBERG, *Acta Chem. Scand.*, 10 (1956) 1283–1286.