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A novel regioselective desulfation of polysaccharide sulfates: Specific 6-*O*-desulfation with *N*,*O*-bis(trimethylsilyl)acetamide

Masayoshi Matsuo, Ryo Takano, Kaeko Kamei-Hayashi and Saburo Hara

Department of Chemistry and Materials Technology, Faculty of Engineering and Design, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606 (Japan)

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ABSTRACT

Treatment of the pyridinium salts of glycosaminoglycans and galactan sulfates with *N*,*O*-bis(trimethylsilyl)acetamide (BTSA) in pyridine for 2 h at 60 °C caused specific 6-*O*-desulfation without depolymerisation or other chemical changes.

INTRODUCTION

Polysaccharide sulfates are present in most algal cell matrices, and those of animal origin are also well-known as mucopolysaccharides. Various methods for desulfation have been developed and one of the convenient methods involves dilute methanolic hydrogen chloride, as applied originally to chondroitin sulfate[1]. However, this method causes considerable depolymerisation and reduction in yield because of concomitant cleavage of glycosidic linkages. A better yield of the desulfated polysaccharides without serious decomposition can be obtained by solvolysis of the pyridinium salts in such aprotic solvents as methyl sulfoxide, *N*,*N*-dimethylformamide, and pyridine[2], or methyl sulfoxide containing a small amount of water or methanol[3–5]. The mechanism of the reaction is envisaged as a reverse of sulfation. Another advantage of solvolytic desulfation is that selective *N*-desulfation can be achieved by careful control of the reaction conditions[3]. However, versatile regiospecific chemical methods of *O*-desulfation are not known and sulfatases with any site specificity are rarely available. Therefore, the development of a method for specific *O*-desulfation would be of value in the elucidation of the biological activity of sulfated carbohydrates.

Correspondence to: Professor S. Hara, Department of Chemistry and Materials Technology, Faculty of Engineering and Design, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606, Japan.

In an investigation[6] of the silylation of methyl galactoside monosulfates, it was found that treatment of the pyridinium salts with *N*,*O*-bis(trimethylsilyl)acetamide (BTSA) or *N*,*O*-bis(trimethylsilyl)trifluoroacetamide in pyridine easily removed only the 6-sulfate group. Thus, methyl α-D-galactopyranoside 2,6-disulfate could be converted into the 2-sulfate in quantitative yield[6]. The reaction does not involve solvolysis, because desulfation did not occur when other silylating reagents such as *N*,*O*-bis(trimethylsilyl)carbamate, *N*-trimethylsilylimidazole (TSIM), and iodotrimethylsilane were used under similar conditions. We now report that the use of BTSA in pyridine is also applicable to polysaccharide sulfates, to effect specific 6-*O*-desulfation without depolymerisation.

EXPERIMENTAL

Materials.—*N*,*O*-Bis(trimethylsilyl)acetamide (BTSA) was obtained from Wako. Funoran[7] and porphyran[8] were extracted and purified from the red seaweeds, *Gloiopeltis complanata* and *Porphyra yezoyensis*, respectively. Dermatan sulfate, chondroitin sulfate, and chondroitinase ABC were obtained from Seikagaku Kogyo Co., and pullulan from Showa Denko Co.

Desulfation with BTSA.—An aqueous solution of the sodium salt of the polysaccharide sulfate (200 mg) was passed through a column (1 × 10 cm) of Amberlite IR-120 (H^+) resin at 4°C, and the eluate was neutralised with pyridine, then lyophilised to give the pyridinium salt (~ 220 mg). The pyridinium salt (220 mg) was soaked in dry pyridine (20 mL), BTSA (4 mL, 20 mol/mol equiv of total hydroxyl groups including that sulfated) was added, and the mixture was kept for 2 h at 60°C to give a clear solution. The excess of reagent and the silyl ester were decomposed by the addition of water (20 mL), the mixture was dialysed, the retentate was adjusted to pH > 7 with NaOH, the solution was dialysed immediately, and the retentate was lyophilised to give the sodium salt of the polysaccharide sulfate (140 mg).

As controls, the pyridinium salt of the polysaccharide sulfate (100 mg) was treated similarly in pyridine (10 mL) without BTSA, and also with TSIM (2.8 mL). The latter treatment gave a clear solution. In addition, a solution of the pyridinium salt of the polysaccharide sulfate (100 mg) in Me_2SO (10 mL) was treated with BTSA (4 mL), the mixture was kept for 5 h at 80°C, then treated as described above.

Sulfate analysis.—The polysaccharide sulfate (300 μg) was hydrolysed by 3 M HCl (1 mL) for 18 h at 100°C. The hydrolysate was filtered through a membrane filter (0.45 μm), and the sulfate content was analysed by HPLC (Waters, ILC-1), using an ion-exchange column (Waters, IC Pak Anion, 4.6 mm × 5 cm) and elution with borate–gluconate buffer (1.3 mM sodium tetraborate, 1.5 mM sodium gluconate, 6 mM boric acid, 1.25% glycerol, and 12% acetonitrile) at 0.8 mL/min. The sulfate ions were detected by a conductivity detector (Waters, 430).

NMR Spectroscopy.—The ^{13}C NMR spectra (75.4 MHz) of 10% solutions of polysaccharide sulfates in D_2O were recorded at 80°C with a General Electric QE-300 spectrometer. Spectral widths of 10 kHz and relaxation delays of 1.0 s were used. Chemical shifts were measured in ppm from internal MeOH and converted into values related to sodium 3-trimethylsilyl-1-propanesulfonate (conversion factor, 51.6). For chondroitin sulfate, distortionless enhancement by the polarisation transfer (DEPT) pulse technique[9] was used at 80°C.

Digestion with chondroitinase.—A 0.4 M Tris-HCl buffer (20 μL, pH 8.0) containing 0.4 M sodium acetate, 0.1% of bovine serum albumin, and water (120 μL) was added to an aqueous 1% solution (20 μL) of dermatan sulfate or chondroitin sulfate. Chondroitinase ABC (5 U/mL, 20 μL) was added to each mixture which was incubated for 2 h at 37°C.

Chromatography of enzyme digests.—Each digest of chondroitinase ABC was analysed by HPLC (Irica 852), using an amine-bound silica column[10] (Shimadzu-PNH_2, 4.0 × 250 mm) and elution with 16 mM sodium dihydrogenphosphate in a 60-min linear gradient from 16 mM to 0.5 M sodium dihydrogenphosphate at 1 mL/min. The elution profiles were monitored at 232 nm.

Gel filtration of polysaccharide sulfates.—An aqueous 3% solution (10 μL) of polysaccharide sulfate was analysed by HPLC (Shimadzu, LC-5A), using a gel-filtration column (Tosoh TSKgel G4000PWXL, 7.8 mm × 30 cm) and elution with 0.2 M potassium sulfate. The elution profiles were monitored by a refractive index detector (Shimadzu, RID-2A).

RESULTS AND DISCUSSION

Funoran, porphyran, dermatan sulfate, and chondroitin sulfate were each treated with BTSA. The sulfur contents of the polysaccharides before and after the treatments are shown in Table I.

Funoran, which has the repeating unit[7] → 3)-β-D-Gal*p*6S-(1 → 4)-3,6-anhydro-α-L-Gal*p*-(1 → , was desulfated on treatment with BTSA. Likewise, most of the sulfate groups in porphyran, which has the repeating unit → 3)-β-D-Gal*p*-(1 → 4)-α-L-Gal*p*6S-(1 → , were removed. However, dermatan sulfate, which consists mainly of the repeating unit → 4)-β-D-Glc*p*A-(1 → 3)-β-D-Gal*p*NAc4S-(1 → , was largely

TABLE I

Sulfur contents (wt%) of polysaccharides before and after treatments with silylating reagents

Sample	Native	With BTSA[a]	Without BTSA[a]	With TSIM[a]	With BTSA[b]
Funoran	6.2	0	6.0	6.1	4.0
Porphyran	4.4	1.1	4.3	4.2	–
Dermatan sulfate	5.1	4.7	5.0	5.1	4.6
Chondroitin sulfate	6.6	2.2	6.5	6.4	–

[a] In pyridine at 60°C for 2 h. [b] In methyl sulfoxide at 80°C for 5 h.

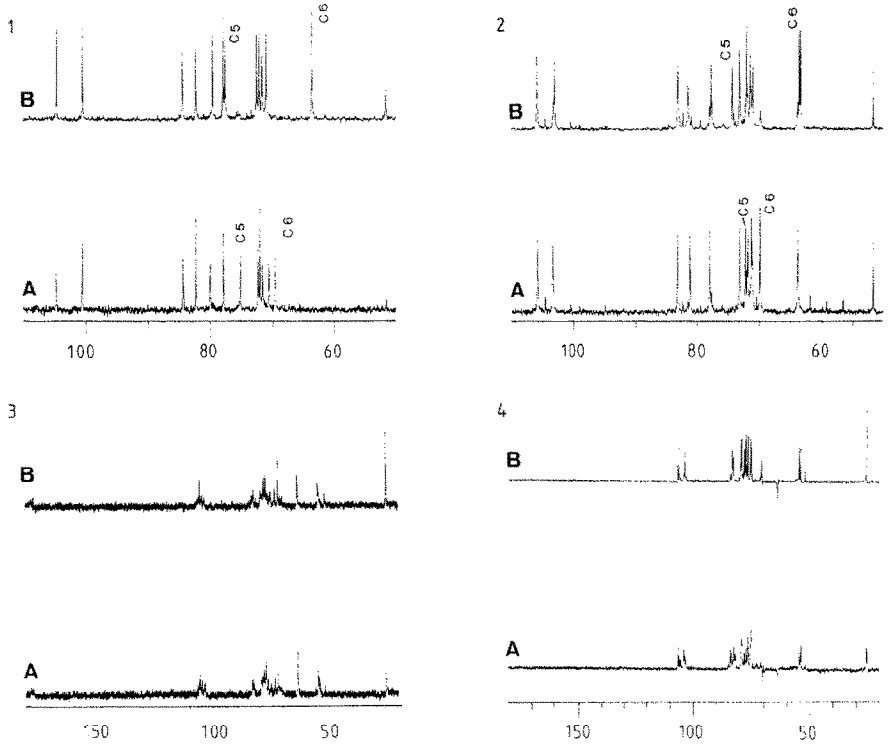

Fig. 1. ^{13}C NMR spectra (75.4 MHz) of solutions at 80°C for 1, funoran; 2, porphyran; 3, dermatan sulfate; and 4, chondroitin sulfate before (A) and after (B) treatment with BTSA.

unaffected. With chondroitin sulfate, which contains the units →4)-β-D-GlcA*p*-(1→3)-β-D-Gal*p*NAc6S-(1→ and →4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc4S-(1→ (see below), ~50% of the sulfates survived the treatment with BTSA. Thus, the loss of sulfate on treatment with BTSA occurred at position 6.

The locations of the sulfate groups in the polysaccharides were determined, before and after treatment with BTSA, by ^{13}C NMR spectroscopy and DEPT (Fig. 1). The spectrum of the BTSA-treated funoran was identical to that of agarose[11], which has the same structure but contains no sulfate. The spectrum of the BTSA-treated porphyran was also identical to that of desulfated porphyran reported[8]. These results confirmed the loss of the 6-sulfate groups. The spectra of dermatan sulfates before and after the treatment with BTSA were essentially identical, indicating that the sulfate groups, mainly at positions 4, were not removed. On treatment of chondroitin sulfate with BTSA, the ^{13}C signal (−70.0 ppm) associated with 6-sulfate groups was shifted upfield (−63.6 ppm) and that corresponding to C-5 (+75.2 ppm) was shifted downfield (+76.5 ppm), thus reflecting[12] the loss of the 6-sulfate group.

Because the dermatan sulfate and chondroitin sulfate were possibly contaminated with other glycosaminoglycans and/or were polydisperse, it is difficult to

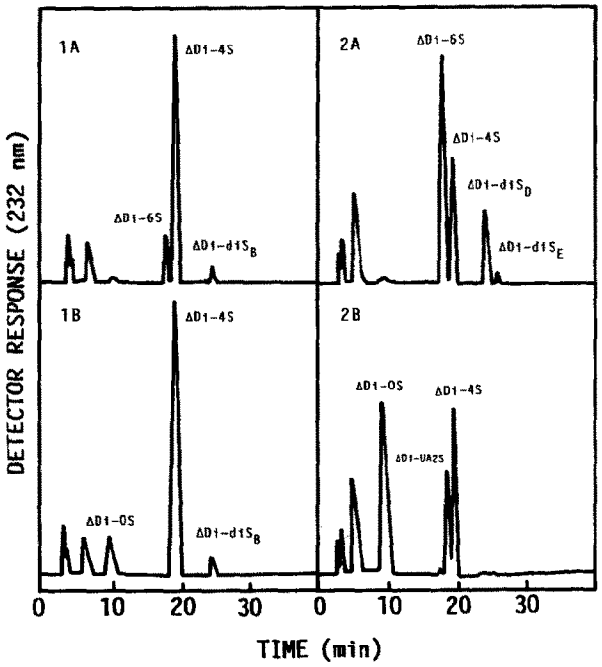

Fig. 2. HPLC on a Shimadzu PNH_2 column (see Experimental) after digestion with chondroitinase ABC of 1, dermatan sulfate; 2, chondroitin sulfate before (A) and after (B) treatment with BTSA: Δ Di-0S, β-HexA-(1 → 3)-GalNAc; Δ Di-4S, β-HexA-(1 → 3)-GalNAc(4S); Δ Di-6S, β-HexA-(1 → 3)-GalNAc(6S); Δ Di-UA2S, β-HexA(2S)-(1 → 3)-GalNAc; Δ Di-diS$_B$, β-HexA(2S)-(1 → 3)-GalNAc(4S); Δ Di-diS$_D$, β-HexA(2S)-(1 → 3)-GalNAc(6S); Δ Di-diS$_E$, β-HexA-(1 → 3)-GalNAc(4,6-diS).

detect the changes in the NMR spectra because of the overlap of signals. Therefore, the changes in structure were confirmed by HPLC of chondroitinase ABC digests (Fig. 2). As shown in Fig. 2-1, a disaccharide arising from the → 3)-Gal*p*NAc4S-(1 → residue and a small amount of that from the → 3)-Gal*p*NAc6S-(1 → residue were detected after digestion of the dermatan sulfate, whereas the latter was absent after digestion of the BTSA-treated polysaccharide. Thus, the structure of dermatan sulfate was little changed on treatment with BTSA except for removal of 6-sulfate group. The 6-sulfate group of chondroitin sulfate is probably eliminated by the treatment with BTSA, because disaccharides arising from → 3)-Gal*p*NAc6S-(1 → disappeared and that from → 3)-Gal*p*NAc(1 → appeared (Fig. 2-2). It is clear that → 3)-Gal*p*NAc4S-(1 → and → 3)-Glc*p*A2S-(1 → were not desulfated, because disaccharides containing a 2- or 4-sulfate group remained after treatment with BTSA. Gel filtration (Fig. 3) indicated that the molecular weights of the dermatan sulfate and chondroitin sulfate were not affected by the treatment with BTSA.

The conditions for the treatment with BTSA are similar to those for the solvolytic desulfation[2] except for the presence of the silylating reagent. However,

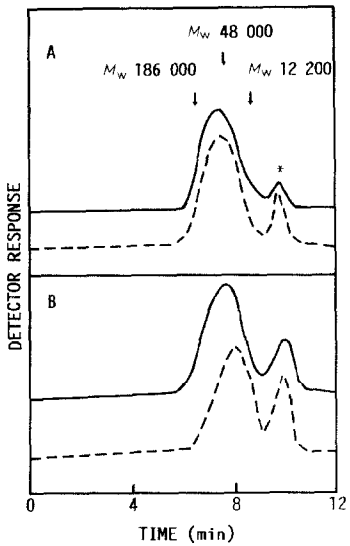

Fig. 3. Gel filtration on TSKgel-G4000PWXL of A, dermatan sulfate; B, chondroitin sulfate before (———) and after (— — —) treatment with BTSA. Arrows indicate the elution positions of pullulan molecular-weight markers, and * indicates inorganic ions.

in a study of the treatment of the pyridinium salt of methyl α-D-galactopyranoside 6-sulfate with several silylating reagents, sulfate was not eliminated unless BTSA was present[6]. Similarly, when the pyridinium salts of the polysaccharide sulfates were treated with TSIM instead of BTSA, there was no loss of sulfate (Table I). Since the pyridinium salt of a polysaccharide sulfate is poorly soluble in pyridine, it is reasonable to infer that, on the addition of TSIM, the hydroxyl groups would be silylated to give a soluble derivative, but no solvolytic desulfation occurred. Dermatan sulfate is desulfated by solvolysis at 80°C for 5 h in methyl sulfoxide containing a small proportion of water[3]. In contrast, in the treatment of dermatan sulfate with BTSA at 80°C for 5 h in methyl sulfoxide, the loss of sulfate did not exceed that achieved by the treatment in pyridine (Table I). Thus, the presence of the silylating reagent did not assist solvolytic desulfation, but prevented it by removing residual moisture in the system. Therefore, it is suggested that BTSA plays a particular role in the specific 6-*O*-desulfation which involved a mechanism other than solvolysis. As the loss of sulfate from funoran after the treatment with BTSA–methyl sulfoxide was far less than that after treatment with BTSA–pyridine, pyridine is likely to enhance the effectiveness of BTSA.

Although the reaction mechanism has not been elucidated, the above results indicate that the desulfation mediated by BTSA in pyridine is specific for 6-sulfates and that glycosidic linkages are not cleaved. Thus, the method has potential application in the correlation of biological activity and structure of the appropriate polysaccharide sulfates.

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