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Note

Effect of alkali and of sodium borohydride at alkaline pH on *N*-acetylchondrosine: reduction *versus* cleavage*

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Treatment of blood-group active glycoproteins[1–3] and salivary-gland mucins[4–6] with sodium borohydride at alkaline pH results in the release of the carbohydrate moieties with subsequent reduction of the reducing end-group. This cleavage, a β-elimination reaction, involves the glycosidic linkage of 2-acetamido-2-deoxy-D-galactose to serine and threonine in the peptide chain[4]. 2-Acetamido-2-deoxy-galactitol has been found as a constituent of oligosaccharides isolated from sheep[4,6] and pig[5] submaxillary-gland mucins after treatment with sodium borohydride at alkaline pH. However, reduced oligosaccharides obtained from blood-group active glycoproteins are terminated mainly by D-galactitol or a hexenetetrol[1,2].

D-Galactitol-terminated oligosaccharides are presumed to result from a two-step β-elimination reaction involving a D-galactose moiety linked to C-3 of the 2-acetamido-2-deoxy-D-galactose unit before the aldehyde group of the hexosamine unit can be reduced by sodium borohydride. The oligosaccharide chain then undergoes an alkali-catalyzed "peeling" reaction until an alkali-stable glycosidic linkage is reached[1,2].

The lability under alkaline conditions of linkages to C-3 of *N*-acetylhexosamines has been well documented[7]. However, the rate of the elimination reaction which, in an alkaline sodium borohydride solution, competes with the reduction by borohydride, has not been studied. In this report, the rate of reduction of *N*-acetylchondrosine [*O*-β-D-glucopyranosyluronic acid-(1→3)-2-acetamido-2-deoxy-D-galactopyranose, **1**] has been compared to the rate of cleavage under different experimental conditions. The relationship of these studies on a model disaccharide to the isolation of intact sugar chains from blood-group active glycoproteins and other glycoproteins (mucins) is discussed.

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RESULTS AND DISCUSSION

Treatment of compound **1** with 0.05M sodium hydroxide at 37° completely cleaved the disaccharide within 30 min (Fig. 1A). However, no detectable destruction of **1** occurred for 60 min when the incubation mixture was maintained between 0° and 4°. Increasing the temperature to 37° resulted in rapid cleavage. In a mixture of M sodium borohydride in 0.05M sodium hydroxide at 0–4° the reduction of **1** to *O*-β-D-glucopyranosyluronic acid-(1→3)-2-acetamido-2-deoxy-D-galactitol (**2**) was essentially quantitative within 30 min (Fig. 1B). When **1** was treated with M sodium borohydride in 0.05M sodium hydroxide at 50°, the conditions used to release oligosaccharides from pig submaxillary-gland mucins[5] and H-active blood-group glycoprotein[3], more than 98% of the disaccharide was converted into the glycitol **2** (Fig. 1B). The reduced disaccharide **2** was isolated in a separate experiment, as described previously[5], for identification. The ratio of 2-acetamido-2-deoxy-D-galactitol to D-glucuronic acid in the disaccharide **2** was 1.00:1.09.

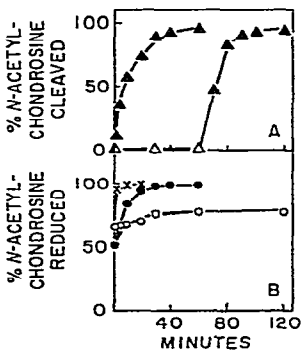


Fig. 1. Effects of alkali and of sodium borohydride at alkaline pH on *N*-acetylchondrosine. (A) Effects of temperature on alkali cleavage of *N*-acetylchondrosine; ^{14}C-*N*-acetylchondrosine was incubated in 0.05M sodium hydroxide and the temperature of the reaction mixture was maintained at 0° (Δ—Δ) or 37° (▲—▲). (B) Effects of sodium borohydride at alkaline pH on *N*-acetylchondrosine; ^{14}C-*N*-acetylchondrosine was incubated with M sodium borohydride in 0.05M sodium hydroxide at 0° (●—●) or 50° (×—×), or with 0.26M sodium borohydride in 0.2M sodium hydroxide at 20° (○—○).

When the reaction conditions reported by other workers[1,2] were used (1% sodium borohydride in 0.2M sodium hydroxide at room temperature) considerable cleavage of **1** was found (Fig. 1B). However, the amount of **1** (70–75%) reduced under these conditions was essentially the same as that reported by Lloyd and Kabat[8] for other model disaccharides. Even the reduction of D-galactose with 1% sodium borohydride in 0.2M sodium hydroxide at 4° proceeds relatively slowly, since more than 20% of the D-galactose had not been reduced after 3 h[8].

In addition, D-galactose was incubated at 22° with 1% sodium borohydride in 0.2M sodium hydroxide and with M sodium borohydride in 0.05M sodium hydroxide. The "relative reducing efficiencies" differed considerably: with the first procedure,

only 70% of the D-galactose was reduced in 30 min, whereas with the second procedure 100% of the D-galactose was converted into D-galactitol within 5 min.

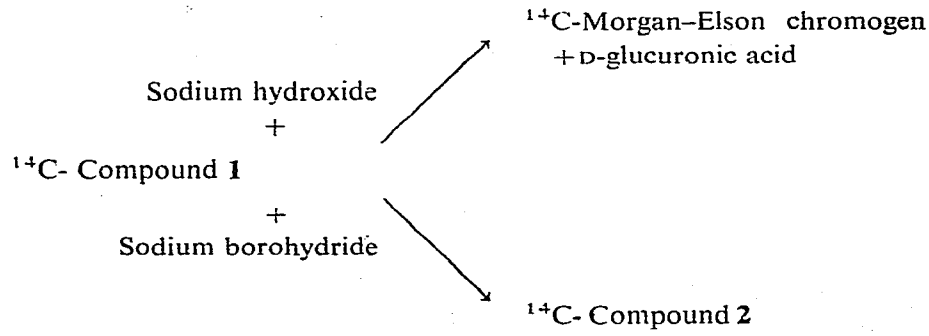
These studies show that under the conditions described previously[5], compound **1**, a disaccharide which contains an alkali-labile linkage, is reduced at a rate much faster than it is cleaved. Thus, the base and borohydride concentrations, and possibly temperature, appear to be critical for the β-elimination and reduction of sugar chains obtained from mucin-type glycoproteins with a minimum of degradation.

Several mucins which contain alkali-labile glycosidic linkages have been studied. Direct evidence for the reduction of 2-acetamido-2-deoxy-D-galactose in oligosaccharides released from pig submaxillary-gland mucins has been obtained[5]. An oligosaccharide which contains 2-acetamido-2-deoxy-D-galactitol was isolated in relatively low yields from Lewis-active blood-group glycoprotein[1]. However, oligosaccharides which contain 2-acetamido-2-deoxy-D-galactitol have been isolated in high yields from H-active blood-group glycoprotein[3,9].

EXPERIMENTAL

General. — Compounds were obtained from commercial sources unless otherwise indicated. [*Acetyl*-^{14}C]-*N*-acetylchondrosine was prepared as described previously[5]. 2-Acetamido-2-deoxy-D-galactitol, following acid hydrolysis, was determined by *N*-acetylation with ^{14}C-acetic anhydride[10]. The carbazole method[11] was used to measure D-glucuronic acid. High-voltage electrophoresis was performed in a Gilson High Voltage Electrophorator. Radioactive areas on paper were detected with a Packard strip scanner and quantitatively determined with a Packard Tri-Carb Liquid Scintillation Counter using a toluene counting system.

Determination of the products of the reaction. — In presence of sodium borohydride under alkaline conditions, *N*-acetylchondrosine (**1**) is susceptible either to alkali cleavage by a β-elimination reaction which releases D-glucuronic acid, or to reduction into **2**:



The reduced product **2** is resistant to mild alkali cleavage. Since a radioactive substrate was employed, and the substrate and proposed products were all separable by electrophoresis, it was possible to measure the rate of each reaction simultaneously.

The reaction mixtures contained 3 μmoles of [*acetyl*-^{14}C]-*N*-acetylchondrosine (37,500 c.p.m./μmole as counted on paper in toluene) in 1.2 ml of (1) 0.05M sodium hydroxide, (2) M sodium borohydride in 0.05M sodium hydroxide, or (3) 1% 0.26M sodium borohydride in 0.2M sodium hydroxide. Aliquots (0.10 ml) were removed at time intervals and neutralized with either 0.1M hydrocloric acid or 4M acetic acid. Separation of the substrate and reaction products was obtained by direct application of the neutralized mixtures to Whatman No. 3MM paper and electrophoresis in 1% sodium tetraborate buffer for 1 h at 50 volts/cm. When the reaction mixtures contained sodium borohydride, the samples were neutralized with acetic acid, treated with a cation exchange resin, and the boric acid was removed on a rotary evaporator as methyl borate. Recoveries of ^{14}C varied from 95 to 100%. A maximum of 3 compounds, which migrated as the substrate or products indicated, was detected.

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